

Levels and predictors of endotoxin in mattress dust samples from East and West German homes

Abstract East–West differences in prevalence of asthma and allergies were suggested to be associated with lifestyle factors. To describe endotoxin levels in mattress dust samples from East and West German homes collected approximately 10 years after reunification. To identify factors that may account for an East–West difference. Dust was collected from mattresses of 2157 infants and 2108 mothers living in Leipzig (former East Germany) and Munich (West Germany). Endotoxin was measured using a chromogenic kinetic Limulus amoebocyte lysate test. Data on bedding, dwelling, and housing characteristics, and occupants' behavior were collected using a self-administered questionnaire. Endotoxin levels were significantly higher in Leipzig compared with Munich for the infants' mattresses but not for the mothers' mattresses. Apart from this, predictors for the mothers' and the infants' mattresses were very similar. Pet-ownership and contact with animals outside the home were most influential. Endotoxin levels in mattress dust were highest in summer and increased with the number of persons living in the household. The overall percentage of variability in endotoxin levels explained by these factors was low. Endotoxin levels were associated with lifestyle factors discussed within the framework of the hygiene hypothesis. None of these factors explains the difference in infant's mattress dust endotoxin between Leipzig and Munich or could be used as a surrogate for endotoxin.

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Key words: Endotoxin; Dust; Mattress; Level; Predictor; Hygiene hypothesis.

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Received for review 18 August 2003. Accepted for publication 18 December 2003.

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Practical Implications

Endotoxin levels in mattress dust are associated with a number of factors discussed in the framework of the hygiene hypothesis; among other things they are associated with pet-ownership, contact with pets and number of persons living in the home. However, none of these factors and not even a combination of factors explains the variability of endotoxin levels between homes.

Introduction

Higher prevalence of asthma and allergies in children and adults has been reported for western Germany compared with eastern Germany (von Mutius et al., 1994; Nowak et al., 1996). Only 10 years after reunification, the prevalence of allergic diseases in eastern

Germany has significantly increased and almost caught up with the prevalence in western Germany (Heinrich et al., 1998; von Mutius et al., 1998). Part of the changes in eastern Germany might be caused by a change in diagnostic habits or a change in awareness among study participants (Heinrich et al., 1998), but the increase in atopic sensitization clearly suggests an

association between allergies and (western) lifestyle factors (Wichmann, 1996).

As part of an ongoing birth cohort study ('Influences of Life-style Related Factors on the Immune System and the Development of Allergies in Childhood' – LISA), endotoxin was measured in mattress dust samples from the two German cities of Leipzig (former East Germany) and Munich (West Germany) approximately 10 years after German reunification. The objective of the present work is to compare endotoxin levels in Leipzig with endotoxin levels in Munich. Furthermore, predictors for endotoxin levels will be identified by means of linear regression taking into account a large number of lifestyle factors and housing characteristics that might influence endotoxin levels. Finally, we will investigate whether differences between Leipzig and Munich with regard to lifestyle factors and housing characteristics do account for potential differences in endotoxin levels.

Endotoxins are cell wall components of the outer membrane of gram-negative bacteria. They have strong immune-stimulatory and pro-inflammatory properties. They are more or less ubiquitous in nature and can be found in normal indoor environments as constituents of house dust (Douwes et al., 2000; Michel et al., 1989; Park et al., 2001; Rizzo et al., 1997).

At present, there are a couple of studies on the predictors of house dust endotoxin levels (Bischof et al., 2002; Douwes et al., 1998; Gereda et al., 2001; von Mutius et al., 2000; Park et al., 2000; Wickens et al., 2003) indicating that living on a farm, regular contact with farm animals, presence of pets, number of persons per household, cleaning habits, visible mold and dampness, central air conditioning operating, season, and cigarette smoking are associated with endotoxin levels. Only one study described predictors of endotoxin levels in bed dust samples (Park et al., 2000), whereas the others focused on floor dust endotoxin levels. As it is still unclear, whether exposure to endotoxin from floors or from mattresses is more relevant for peoples' health, more research on mattress dust endotoxin and its predictors is needed.

Materials and methods

The LISA study is an ongoing birth cohort study designed to assess 'Influences of Life-style Related Factors on the Immune System and the Development of Allergies in Childhood'. Study population and methods of the LISA-study have been described elsewhere (Bolte et al., 2003; Gehring et al., 2001; Heinrich et al., 2002).

Study population

In brief, from December 1997 to January 1999, neonates from two large German cities, Munich (West

Germany) and Leipzig (former East Germany) were recruited for this study. Recruitment took place shortly after birth in six obstetrical clinics in Munich and all four obstetrical clinics in Leipzig. The target population of the study was defined as newborns from parents who were born in Germany and have German nationality. Neonates fulfilling at least one of the following criteria were excluded from the study: premature birth (maturity <37 gestational week), low birth weight (<2500 g), congenital malformation, symptomatic neonatal infection; use of antibiotic medication, hospitalization or intensive medical care during neonatal period; immune-related diseases of the mother such as autoimmune disorders, diabetes, hepatitis B, long-term medication use or abuse of drugs and alcohol. The final study population consisted of 2443 neonates, i.e. 55% of all parents who were contacted and met the inclusion criteria.

The study was approved by the ethic committees of the Bayerische Landesärztekammer and of the University of Leipzig and was carried out in accordance with the institutional guidelines for the protection of human subjects. Informed consent was obtained from all parents of the participating infants.

Dust sampling and extraction

The participants' homes were visited from February 1998 through November 1999. During these home visits, dust samples were taken by trained personnel (eight fieldworkers in Leipzig and three fieldworkers in Munich) according to a standardized operating procedure. All fieldworkers were initially trained by the same supervisor. During the first 4 weeks and after 6 months of fieldwork, each fieldworker was accompanied by a supervisor (one person per center) for quality assurance.

Dust samples were taken from the mothers' and the infant's mattresses by vacuuming an area of 1 m² of mattress surface for 2 min using the same type of standard vacuum cleaner (power: 1400 W). Sheets were removed before dust sampling and dust samples were taken from whatever kind of surface that was underneath the sheets, i.e. the mattresses itself or any kind of mattress cover. Dust was collected on cellulose filters using vacuum cleaners equipped with special nozzles (ALK allergen mouthpiece, Hørsholm, Denmark) to collect the dust on paper filters (ALK). Gravimetric measurements of filters were performed before and after vacuuming to determine the total amount of dust. The samples were stored at -20°C until extraction. No dust sample was taken from the mother's mattress, when the child did not sleep at least occasionally in the mother's bed ($n = 58$).

Endotoxin was extracted from dust samples, including filters, under sterile and pyrogen-free conditions with 0.125 M NH₄HCO₃ plus 0.05% Tween-20 (v/v)

for 2 h at room temperature with continuous shaking (Bischof et al., 2002; Fahlbusch et al., 1999). Extraction ratios varied between 1:10 and 1:100 (w/v) depending on the amount of sampled dust. The solution was clarified by centrifugation at 1000 g. Extracts were stored in pyrogen-free glassware in separate aliquots at -20°C .

Analysis of endotoxin in house dust

The endotoxin content of the dust sample extracts was analyzed using a quantitative kinetic chromogenic Limulus Amebocyte Lysate (LAL) method (Kinetic-QCL no. 50-650U; Bio Whittaker (Walkersville, MD, USA); LAL lot no. 0L3900, 0L0520, 6L5520) as described earlier (Bischof et al., 2002) at 37°C . Analyses were performed with an automated microtiter plate reader (anthos ht III, Anthos Labtec Instruments, Wals, Austria) and WinKQCL 1.1 software (Bio Whittaker). *Escherichia coli* endotoxin (Bio Whittaker, lot no. 0L3200, 9L3080, 6L2950) was used as standard endotoxin. The endotoxin potency of this standard varied between 12 and 16 Endotoxin Units (EU)/ng. All measurements were performed in duplicate using positive product control. Analyses were repeated if the difference between sample duplicates exceeded 10%. The limit of detection was 50 EU/g dust. Endotoxin concentrations were expressed as EU per square meter of sampled surface and per gram of dust.

Questionnaire data

Data on presence of pets and contact with animals outside the home; bedding, dwelling, and housing characteristics; as well as occupants' behavior were collected at the same time the dust samples were taken, at 3 months after birth, using a self-administered questionnaire.

Statistical analysis

The distribution of endotoxin levels was right skewed and best described by a log-normal distribution. Therefore, their means were expressed as geometric mean (GM) values with a geometric standard deviation. Correlations were expressed as Pearson correlation coefficients of natural log-transformed values.

In order to select the most influential predictors with regard to their impact endotoxin levels, we first performed simple linear regressions with variables, which were reported to be associated with endotoxin levels by other authors and variables, which we assumed to be associated with endotoxin levels. We subsequently performed multiple linear regression analysis including all variables, which were marginally significantly associated ($P < 0.10$) with either the mother's or the infant's mattress dust endotoxin.

Some building characteristics like age of building and building material are associated with each other. Only one, the most influential one, was included in the model. We asked for the infant's contact with animals outside the home. As we asked this when the infant was 3 months old, it can be assumed that not only the infant but also the mother had contact with animals outside the home.

Simple and multiple linear regressions results are presented as crude and adjusted means ratios with 95% confidence intervals, i.e. as the ratio of the GM in the category of study and the GM in a reference category. Statistical significance was set at a conventional 5% level. All analyses were performed using Statistical Analysis System (SAS for Windows, version 8.2, Cary, NC, USA).

Results

Amount of sampled dust and endotoxin levels

House dust was collected from mattresses of 2157 infants and 2108 mothers. The amount of sampled dust was significantly higher for the mothers' mattresses compared with the infants' mattresses (Figure 1), probably due to a higher percentage of new infants' mattresses than mothers' mattresses (47.6% vs. 15.2%).

Endotoxin was analyzed in all dust samples and concentrations were expressed as EU per square meter of sampled surface and per gram of dust. Correlations between endotoxin levels expressed per square meter and per gram of dust were high ($r = 0.89$ for both the infants' and the mothers' mattresses, $N = 2157$ and 2108 , $P < 0.0001$).

All endotoxin levels were above the limit of detection. Expressing endotoxin levels per square meter, mean endotoxin levels were approximately twice as high for the mothers' mattresses compared with the infants' mattresses in Munich and Leipzig (Figure 1). Expressing endotoxin levels per gram of dust – to take into account the differences between infants' and mothers' mattresses in terms of amount of dust – endotoxin levels in the infants' mattresses dust were approximately twice as high as endotoxin levels in the mothers' mattresses dust (Figure 1). For the infants' mattresses, endotoxin levels in Leipzig were significantly higher than endotoxin levels in Munich.

Endotoxin levels in the mothers' mattresses dust and in the infants' mattress dust were only moderately correlated ($r = 0.48$ and 0.45 for endotoxin levels per square meter and per gram of dust, respectively, $n = 2,099$, $P < 0.0001$, Figure 2). The correlation between the amount of sampled dust from the infant's mattress and the amount of sampled dust from the mother's mattress was weak ($r = 0.27$, $n = 2,099$, $P < 0.0001$). This might also be due to the

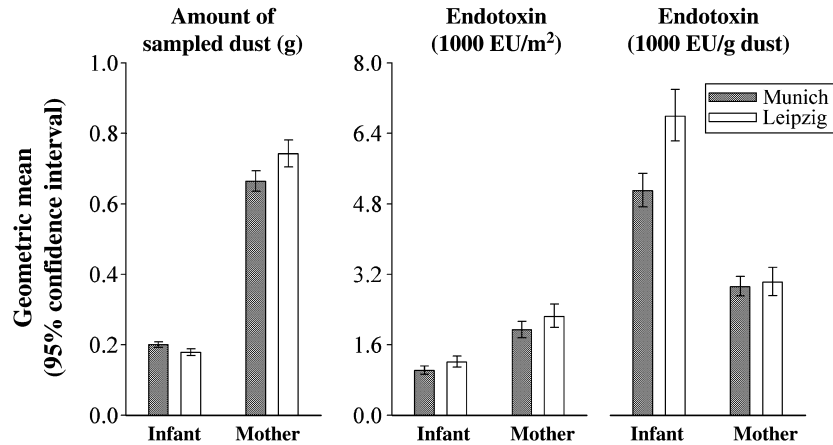


Fig. 1 Mean amount of sampled dust and endotoxin level [geometric mean (95% confidence interval)] for the infants' and the mothers' mattresses related to study region

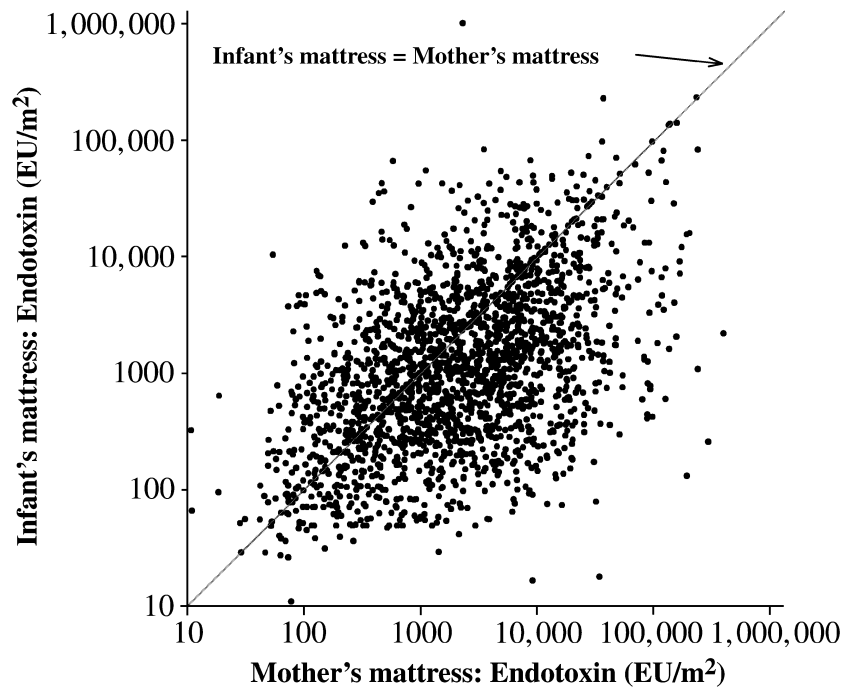


Fig. 2 Scatterplot of endotoxin levels in the infants' mattresses vs. the mothers' mattresses

significantly higher percentage of new infants' mattresses compared to mothers' mattresses. However, excluding new infants' mattresses and new mothers' mattresses did not change the correlations.

Distribution of lifestyle factors and home characteristics

Table 1 shows the distribution of lifestyle factors and home characteristics that were analyzed with regard to their impact on endotoxin levels in mattress dust for Leipzig and Munich. Families living in Leipzig kept pets (except cats) more frequently than families living in Munich, whereas infants living in Munich had contact with cats, dogs, and horses outside the home

more often than infants living in Leipzig. Residency was significantly shorter in Leipzig. The number of persons living in the home was larger in Munich. Mattresses in Leipzig were less old than mattresses in Munich. Furthermore, Munich and Leipzig differed with regard to type of bedding of the infant's bed and the use of pelts sleeping covers and anti-allergic mattress covers as well as cleaning habits. Homes in Munich were larger than homes in Leipzig and more often on located on the first floor and lower. Munich families more often lived in one-family houses, houses built after 1980, and houses built from bricks/clinker. Current or past signs of mold/mildew were reported more often in Munich compared with Leipzig.

Table 1 Distribution of potential predictors for mattress dust endotoxin levels related to place of residence

Variable	Munich		Leipzig	
	n/N	%	n/N	%
Animals				
Current pet-ownership				
Cat	124/1339	9.3	83/806	10.3
Dog	73/1339	5.5	71/806	8.8
Hamster, guinea pig, rabbit	34/1339	2.5	108/806	13.4
Others	106/1339	7.9	154/806	19.1
Contact with animals ^a				
Cat	218/1327	16.4	94/795	11.8
Dog	245/1327	18.5	119/795	15.0
Hamster, guinea pig, rabbit	39/1327	2.9	25/795	3.1
Horses	53/1327	4.0	7/795	0.9
Season of dust sampling				
Jan–Mar	282/1311	21.5	151/761	19.8
Apr–Jun	350/1311	26.7	201/761	26.4
Jul–Sep	305/1311	23.3	251/761	33.0
Oct–Dec	374/1311	28.5	158/761	20.8
Residence utilization				
Occupancy (years)				
≤ 1	524/1316	39.8	496/802	61.9
2–4	508/1316	38.6	222/802	27.7
>4	284/1316	21.6	84/802	10.5
No. of persons living in the home				
2–3	717/1345	53.3	486/805	58.1
4	463/1345	34.4	263/805	32.7
≥5	165/1345	12.3	74/805	9.2
No. of children sharing the infant's bedroom				
≤ 2	1238/1311	94.4	745/785	94.9
>2	73/1311	5.6	40/785	5.1
Bedding characteristics				
Age of infant's mattress (years)				
<1	564/1309	43.1	434/788	55.1
1–5	444/1309	33.9	216/788	27.4
6–10	301/1309	23.0	138/788	17.5
Age of mother's mattress (years)				
<1	184/1338	13.8	143/802	17.8
1–5	669/1338	50.0	478/802	59.6
6–10	485/1338	36.2	181/802	22.6
Type of bedding ^a				
Cotton	327/1340	24.4	120/805	14.9
Feather	674/1340	50.3	449/805	55.8
Synthetic	479/1340	35.8	222/805	27.6
Pelts sleeping cover present (e.g. sheep skin) ^b	871/1347	64.7	327/806	40.6
Synthetic bedding ^b	479/1340	35.8	222/805	27.6
Temperature used for washing the bedding				
<60°C	163/1346	12.1	112/805	13.9
At least 60°C	1005/1346	74.7	615/805	76.4
Alternate	178/1346	13.2	78/805	9.7
Anti-allergic mattress cover ^a	71/1345	5.3	26/806	3.2
Soft toys in the infant's bed	791/1327	59.6	470/799	58.8
Cleaning habits				
Vacuuming/mopping floors several times a week	904/1347	67.1	619/806	76.8
Dust cleaning several times a week	263/1345	19.6	343/805	42.6
Dwelling characteristics				
Storey of the dwelling				
Lower than 2nd floor	906/1331	68.1	343/804	42.7
2nd floor and higher	425/1331	31.9	461/804	57.3
Size of the apartment				
≤ 70 qm	229/1336	17.1	326/799	40.8
70–110 qm	719/1336	53.8	368/799	46.1
>110 qm	338/1336	29.0	105/799	13.1

Table 1 Continued

Variable	Munich		Leipzig	
	n/N	%	n/N	%
Number of rooms				
1–3	181/1338	13.5	147/804	18.3
4–6	1007/1338	75.26	632/804	78.6
≥7	150/1338	11.2	25/804	3.1
Building characteristics				
Type of building				
One-family house	490/1345	36.4	105/806	13.0
Apartment house	749/1345	55.7	666/806	82.6
High-rise building	106/1345	7.9	35/806	4.3
Year of construction				
After 1980	494/1340	36.9	191/786	24.3
1945–80	591/1340	44.1	148/786	18.8
Before 1945	255/1340	19.0	447/786	56.9
Building material				
Brick/clinker	973/1334	72.9	535/798	67.0
Concrete	210/1334	15.7	195/798	24.4
Others/unknown	151/1334	11.3	68/798	8.5
Signs of dampness				
Current signs of dampness	82/1344	6.1	50/805	6.2
Current or past signs of mold/mildew	408/1347	30.3	189/804	23.5
Water damage (past 12 months)	121/1345	9.0	60/806	7.4

^aOutside the home.^bIn infant's bed.

Factors affecting endotoxin levels in house dust

Simple and multiple linear regression models were used to describe the association between endotoxin levels in mattress dust samples and potential predictors. The results of the linear regression analysis for the infants' and the mothers' mattress dust endotoxin levels expressed per square meter and expressed per gram of dust are presented in Table 2.

All in all, associations were very similar for endotoxin levels per square meter and endotoxin levels per gram of dust. However, the effect of contact with hamsters, guinea pigs, or rabbits was not consistent, but showed the same trend. Furthermore, there was an effect of pelts sleeping covers for endotoxin levels per square meter, but not for endotoxin levels per gram of dust, indicating that pelts sleeping covers are associated with higher amounts of dust rather than higher endotoxin concentrations. Finally, older mattresses in the mother's bed contained significantly less endotoxin per gram of dust, but not per square meter.

Endotoxin levels measured in the infants' mattresses dust were significantly higher in Leipzig compared with Munich. This difference remained significant after adjustment for a large number of lifestyle factors and housing characteristics. For the mothers' mattresses, no statistically significant difference between the two cities was found.

With regard to the relevant lifestyle factors and housing characteristics, models for the mothers' and the infants' mattresses were not exactly the same but

Table 2 Simple and multiple linear regression results for natural log-transformed endotoxin levels in the infants' and the mothers' mattresses dust [expressed EU/m² and EU/g dust] presented as crude (MRc) and adjusted means ratios (MRa) with 95% confidence intervals (CI)]

	Endotoxin in infant's mattress dust (<i>n</i> = 1882)						Endotoxin in mother's mattress dust (<i>n</i> = 1922)					
	EU/m ² (<i>R</i> _{adj} ² = 7.3%)			EU/g dust (<i>R</i> _{adj} ² = 9.6%)			EU/m ² (<i>R</i> _{adj} ² = 6.2%)			EU/g dust (<i>R</i> _{adj} ² = 7.3%)		
	MRc	MRa	(95% CI)	MRc	MRa	(95% CI)	MRc	MRa	(95% CI)	MRc	MRa	(95% CI)
Place of residence												
Leipzig vs. Munich	1.20	1.32	(1.11; 1.57)	1.37	1.42	(1.23; 1.64)	1.23	1.11	(0.92; 1.33)	1.08	0.93	(0.80; 1.09)
Current pet-ownership												
Cat (yes vs. no)	1.07	1.06	(0.83; 1.36)	1.05	1.05	(0.85; 1.28)	1.49	1.48	(1.14; 1.91)	1.39	1.39	(1.12; 1.72)
Dog (yes vs. no)	1.68	1.55	(1.15; 2.08)	1.57	1.42	(1.11; 1.81)	1.83	1.64	(1.19; 2.26)	1.82	1.69	(1.29; 2.20)
Hamster, guinea pig, rabbit (yes vs. no)	1.40	1.22	(0.90; 1.65)	1.33	1.08	(0.84; 1.38)	1.58	1.27	(0.92; 1.75)	1.27	1.07	(0.82; 1.40)
Contact with animals^a												
Cat (yes vs. no)	1.20	1.10	(0.89; 1.36)	1.14	1.07	(0.90; 1.27)	1.26	1.13	(0.91; 1.41)	1.15	1.03	(0.86; 1.24)
Dog (yes vs. no)	1.25	1.13	(0.93; 1.38)	1.10	0.99	(0.84; 1.16)	1.26	1.11	(0.90; 1.37)	1.17	1.03	(0.87; 1.22)
Hamster, guinea pig, rabbit (yes vs. no)	1.96	1.43	(0.92; 2.22)	1.95	1.61	(1.12; 2.31)	2.45	1.79	(1.14; 2.82)	1.82	1.39	(0.96; 2.03)
Horses (yes vs. no)	2.09	1.59	(1.00; 2.52)	1.58	1.41	(0.96; 2.05)	1.92	1.54	(0.94; 2.52)	1.76	1.46	(0.97; 2.20)
Season of dust sampling												
Spring vs. winter	0.92	0.94	(0.76; 1.16)	0.86	0.86	(0.72; 1.02)	0.87	0.87	(0.69; 1.09)	0.83	0.82	(0.68; 0.98)
Summer vs. winter	1.40	1.44	(1.17; 1.79)	1.53	1.49	(1.25; 1.77)	1.33	1.30	(1.04; 1.62)	1.23	1.21	(1.01; 1.46)
Autumn vs. winter	1.17	1.25	(1.01; 1.54)	1.34	1.44	(1.21; 1.72)	1.22	1.26	(1.00; 1.57)	1.16	1.20	(1.00; 1.44)
Residence utilization												
No. of persons in household												
4 vs. <4 persons	1.64	1.47	(1.24; 1.75)	1.43	1.33	(1.16; 1.54)	1.55	1.67	(1.41; 1.99)	1.41	1.54	(1.33; 1.77)
≥5 vs. <4 persons	2.22	2.01	(1.54; 2.61)	1.79	1.65	(1.33; 2.05)	2.07	2.17	(1.66; 2.84)	1.86	1.98	(1.58; 2.48)
No. of children sharing the infant's bedroom												
>2 vs. ≤2	1.68	1.23	(0.89; 1.71)	1.41	1.06	(0.81; 1.39)	—	—	—	—	—	—
Bedding characteristics												
Age of mattress (years)												
1–5 vs. <1	1.63	1.41	(1.18; 1.68)	1.31	1.16	(1.01; 1.35)	1.02	1.01	(0.83; 1.24)	0.86	0.83	(0.71; 0.98)
>5 vs. <1	1.74	1.52	(1.25; 1.86)	1.39	1.22	(1.04; 1.44)	0.98	0.99	(0.80; 1.22)	0.85	0.81	(0.68; 0.97)
Pelts sleeping cover in the bed (yes vs. no)	1.19	1.34	(1.16; 1.56)	0.79	1.09	(0.96; 1.23)	—	—	—	—	—	—
Dwelling characteristics												
Storey of the dwelling												
<2nd floor vs. ≥2nd floor	1.10	1.01	(0.85; 1.20)	1.01	0.99	(0.86; 1.14)	0.82	1.16	(0.97; 1.39)	1.05	1.10	(0.95; 1.28)
Number of rooms												
4–6 vs. 6	0.88	1.14	(0.86; 1.52)	0.93	1.11	(0.88; 1.41)	1.03	0.98	(0.73; 1.33)	0.89	1.05	(0.82; 1.35)
<4 vs. 6	0.89	1.44	(1.01; 2.04)	0.97	1.32	(0.99; 1.76)	1.08	1.43	(0.99; 2.07)	1.08	1.44	(1.06; 1.95)
Building characteristics												
Type of building												
Apartment house vs. one-family house	0.78	0.87	(0.72; 1.07)	0.88	0.91	(0.77; 1.07)	0.94	1.09	(0.88; 1.35)	0.98	1.12	(0.94; 1.34)
High rise building vs. one-family house	0.79	0.89	(0.63; 1.27)	0.80	0.84	(0.63; 1.11)	1.03	1.30	(0.90; 1.87)	0.77	1.12	(0.83; 1.52)
Year of construction												
Before 1945 vs. after 1980	1.05	1.05	(0.88; 1.26)	1.03	1.06	(0.91; 1.22)	1.26	1.20	(1.00; 1.45)	1.26	1.22	(1.04; 1.42)
1945–80 vs. after 1980	1.14	1.07	(0.88; 1.29)	1.18	1.07	(0.92; 1.25)	1.52	1.50	(1.23; 1.83)	1.46	1.46	(1.24; 1.71)
Amount of dust (per gram)	—	—	—	2.46	2.23	(1.71; 2.91)	—	—	—	1.23	1.19	(1.09; 1.30)

^aOutside the home.

very similar. Pet-ownership and contact with animals outside the home were most influential. Keeping cats or dogs increases endotoxin levels in the mother's mattress dust, whereas the effect of pet-keeping for the infant's mattress was limited to dogs. Endotoxin levels in mattress dust show some seasonal variability with highest levels from July to September and lowest levels from April to June. In addition, endotoxin levels in mattress dust increased with the number of persons living in the household. Furthermore, dust from infants' mattresses aged 1 year and older contained

more endotoxin. In contrast, age of the mattress was negatively associated with endotoxin levels (per gram of dust) in dust samples from the mothers' mattresses. An additional predictor for high levels of endotoxin in the mothers' mattresses dust was living in buildings constructed in 1980 and earlier. For both, infants' and mothers' mattresses, endotoxin levels per gram of dust were found to increase with increasing amounts of sampled dust indicating that endotoxin levels per square meter are not simply the product of endotoxin levels per gram of dust and amount of dust. The overall

R^2 for the infants' mattresses is slightly higher than for the mothers' mattresses, but still low.

Discussion

Levels of house dust endotoxin

Only few studies describing levels of endotoxin in house dust have been published so far, and the comparison of endotoxin levels between studies is limited due to differences in sampling, extraction, and storage procedures (Douwes et al., 1995). Another difficulty in comparing dust samples from different studies is that some authors express endotoxin levels as EU per gram of dust, whereas others use microgram per gram of dust or EU per square meter of sampled surface. Only if the potency of the standard endotoxin is known, can endotoxin levels in nanograms be calculated from EU. However, the comparison of levels per gram of dust with levels per square meter of sampled surface is not possible, as the amount of sampled dust is [in particular for floor dust (Douwes et al., 2000) and maybe to a lesser extent for mattress dust] highly dependent on the type of sampling surface.

East–West differences in endotoxin levels

Endotoxin levels in dust from the infants' mattresses differed between study locations. The difference between Leipzig remained statistically significant after adjustment for a large set of lifestyle factors. The reason for the significantly higher levels in Leipzig compared with Munich is not clear. One possible explanation may be that there is an underlying difference in lifestyle factors between Leipzig and Munich, which were not considered in our questionnaire.

Predictors of house dust endotoxin levels

The positive association between presence of pets and endotoxin levels is in agreement with the findings of other studies (Bischof et al., 2002; Gereda et al., 2001; Heinrich et al., 2001; Park et al., 2001; Wickens et al., 2003) who found increased endotoxin levels in homes where pets, and in particular dogs, were kept. Furthermore, contact with pets outside the home was found to be associated with endotoxin levels in mattress dust. No other study on contact with pets outside the home and endotoxin levels was found in the literature. Contact with pets could be considered as a direct source of endotoxin, but contact with pets, in particular horses, could also be a proxy for living in more suburban or rural areas.

The association between endotoxin levels and the number of persons per household supports the hygiene hypothesis. However, only two small studies conducted by Gereda et al. (2001) and Wickens et al. (2003)

analyzed the association between endotoxin levels and number of persons per household. A positive association of endotoxin levels with number of persons per household was confirmed by Wickens et al. (2003), but not by Gereda et al. (2001).

The association between endotoxin levels and cleaning habits is not clear. Bischof et al. (2002) found lower frequencies of vacuum cleaning associated with higher levels of endotoxin in living room floor dust. In contrast, Gereda et al. (2001) did not find any association with cleaning habits, which is in agreement with the findings of the present study. However, in the present study, we did not ask any questions about cleaning or vacuuming of the mattresses.

Furthermore, the present data suggest that endotoxin levels increase with increasing age of the infants' mattresses. Possibly, differences between the amount of dust sampled from 'old' and 'new' mattresses contribute to the observed endotoxin differences. However, the positive association between endotoxin levels and age of the infants' mattress was also present when endotoxin levels were expressed per gram of dust instead of square meter, to take differences in the amount of sampled dust into account (even when we adjusted for the amount of sampled dust). Interestingly, for the mothers' mattress a negative association of endotoxin levels with age of the mattress was found. The reason for this is not clear.

Moreover, there was an effect of pelts sleeping covers on endotoxin levels per square meter, but not on endotoxin levels per gram of dust, indicating that pelts sleeping covers are associated with higher amounts of dust rather than higher endotoxin concentrations.

Increased endotoxin levels were measured in 'old' houses (constructed before 1945). One interpretation of this finding might be that endotoxin levels are associated with indoor climate, in particular with humidity. However, (marginal) significant associations with visible mold and dampness reported by other authors (Bischof et al., 2002; Wickens et al., 2003) could not be confirmed. Another interpretation might be that endotoxin simply accumulates over time. Central air conditioning and operating humidifiers as reported by Gereda et al. (2001) and Park et al. (2000) are not relevant in studies of house dust endotoxin levels in German homes, because they are rare.

Seasonal variation of endotoxin levels in settled house dust was found by some authors (Rizzo et al., 1997; Wickens et al., 2003) but not by others (Bischof et al., 2002; Park et al., 2000). In accordance with Rizzo et al. (1997) and Wickens et al. (2003) endotoxin levels the LISA mattresses dust were found to be highest in summer (July–September). Heinrich et al. (2003) showed higher levels of endotoxin in ambient particle mass in summer compared with spring. Thus, one potential explanation for the elevated levels of endotoxin in mattress dust sampled in summer might

be penetration of endotoxin from outdoors to indoors. This is supported by the fact that frequent ventilation significantly increased endotoxin levels in the mothers' mattress dust samples taken in summer by a factor of 1.37 (data not shown). However, for the infants' mattresses, no significant association was found between endotoxin levels and ventilation. This lack of an association between ventilation and endotoxin levels in the infant's mattress dust might be due to the fact that the bedding of a 3-month-old infant is usually washed very often.

Although the amount of dust was already taken into account when endotoxin levels were expressed per gram of dust, there was still a positive association between the amount of sampled dust and endotoxin levels per gram of dust. This indicates that endotoxin level per square meter of sampling surface is not simply a product of endotoxin level per gram of dust and the amount of sampled dust. Thus, for the total amount of endotoxin present in the infants' mattress dust [which has been shown to be more relevant with respect to the infant's health (Braun-Fahrlander et al., 2002; Douwes et al., 2000; Gehring et al., 2002)] the difference between mattresses is much bigger than for endotoxin levels per gram of dust.

Although endotoxin has been shown to be an active component of cigarette smoke, no significant difference was found between endotoxin levels in smokers' and non-smokers' homes, which was in agreement with the results of Park et al. (2000).

Percentage of variability of endotoxin levels explained by the model

The overall percentage of variability explained by the predictor variables that were included in the model was low compared with the R^2 of 22% reported by Bischof et al. (2002). One explanation with regard to the infants' mattresses may be that approximately half the children's mattresses were new and consequently the amount of dust sampled was very low. Furthermore, dust sampling could not be carried out as highly standardized as necessary as in many cases the mattress surface area was less than 1 m², and in some cases dust was taken from other locations than the child's bed where the infant slept most frequently, e.g. from baby carriages. The reason for the low R^2 for the mothers' mattresses is not clear. Maybe the questionnaire information on bedding, dwelling, and housing characteristics that was collected in the present study, is not specific enough for mattress dust endotoxin. More specific information on bedding characteristics and cleaning of mattresses might improve the model. Thus,

none of the predictors analyzed and not even a combination of predictors can be used as a surrogate for high or low mattress dust endotoxin levels in epidemiological studies.

Conclusion

Endotoxin was found to be present in all mattress dust samples. Among others, endotoxin levels were associated with a number of factors discussed within the framework of the hygiene hypothesis, e.g. pet-ownership, contact with pets outside the home, number of persons living in the home. However, the percentage of variability in endotoxin levels explained by these factors was low. Therefore, we conclude that none of the predictors analyzed and not even a combination of predictors can be used as a surrogate for high or low mattress dust endotoxin levels in epidemiological studies.

Acknowledgements

The authors would like to thank all families for their participation in the LISA study. The LISA study was supported by the Federal Ministry for Education, Science, Research and Technology, grants no. 01 EG 9732 and 01 EG 9705/2.

Appendix

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