

Determinants of endotoxin levels in carpets in New Zealand homes

Abstract Endotoxin in house dust has been shown to be associated with asthma severity. Little is known about the influence of housing characteristics on endotoxin distribution. Using standardized methods, dust was sampled from a 1 m² site and the whole accessible carpet area in selected Wellington, New Zealand homes ($n = 77$). Endotoxin was measured using a *Limulus* Amoebocyte Lysate assay. Relative humidity and temperature were recorded using sensors placed in carpet bases. Questionnaires were used to collect information on housing characteristics. All analyses were performed for endotoxin units (EU)/mg and EU/m² for each site. Geometric mean endotoxin levels were 22.7 EU/mg [geometric standard deviation (GSD) = 2.4] or 30,544 EU/m² (GSD = 3.2) from the 1 m² site, and 28.4 EU/mg (GSD = 3.4) or 5653 EU/m² (GSD = 6.4) from the whole room. After controlling for confounding, endotoxin was positively associated with dogs inside [geometric mean ratio (GMR): 0.9–2.0], total household occupants (GMR: 1.7–2.0, for 1 m² sample only), vacuum cleaners < 1-year old (GMR: 2.3–2.7), reusing vacuum dust collection bags (GMR: 1.4–3.1), steamcleaning or shampooing the carpet (GMR: 1.4–2.2) and high relative humidity (GMR: 1.4–1.6). Lower endotoxin was associated with floor insulation (GMR: 0.4–0.8), and north-facing living rooms (GMR: 0.4–0.8). This study has identified home characteristics that could be modified to reduce endotoxin exposure.

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Key words: Endotoxin; Relative humidity; Pets; Cleaning; Insulation; Household occupancy.

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Received for review 5 November 2001.

Accepted for publication 25 June 2002.

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Practical Implications

Reducing moisture in the home is important in lowering levels of endotoxin. During construction, homes should be well insulated, particularly against ground moisture, and situated to maximize sunshine hours. Relative humidity in existing housing stock could be lowered by the installation of centralized heating systems.

Introduction

Exposures that occur within the indoor environment have received considerable attention in attempts to understand the increasing prevalence of asthma in Westernized countries (Ninan and Russell, 1992; Peat et al., 1994). Investigations into household dust components have focussed on house dust mite and other indoor allergens. Other components of indoor dust, such as bacterial endotoxin, from the cell walls of gram-negative bacteria (Douwes et al., 2000), and (1 → 3) β -glucan, a marker of mold exposure (Douwes et al., 1999, 2000), are now receiving attention in the hope that they will further contribute to our under-

standing of the influence of the domestic environment on the airway.

The interest in endotoxin arises from its well-established association with respiratory symptoms in the occupational setting (Douwes, 1997; Smid et al., 1994). *In vivo* studies suggest that in the domestic environment endotoxin may interact with Der p 1 in the provocation of airway inflammation (Eldridge and Peden, 2000; Peden et al., 1999; Wan et al., 2000). Epidemiological associations have been reported for endotoxin and asthma severity in mite-sensitive children (Michel et al., 1996; Rizzo et al., 1997) and the risk of any and repeated wheeze in infants of allergic parents (Park et al., 2001). In another study (Douwes et al., 2000), there was no

effect of endotoxin on peak flow variability after adjustment for pets and *Der p 1* concentration.

An additional role for endotoxin has also been proposed. Childhood farm exposures have been associated with a reduced prevalence of atopy and asthma (Braun-Fahrlander et al., 1999; Ernst and Cormier, 2000; Kilpelainen et al., 2000; Riedler et al., 2000; von Ehrenstein et al., 2000) with von Mutius et al. (2000) suggesting that the protective effect may be because of higher environmental endotoxin exposure that occurs in association with animals on farms. In support of this, one small study (Gereda et al., 2000) in a non-rural environment showed that endotoxin levels were significantly lower in the homes of allergen-sensitized infants.

However once asthma has developed the modification of homes to reduce endotoxin exposure may be important for the control of symptoms, but there are few studies (Douwes et al., 1998; Park et al., 2000) that have examined the association between home characteristics and endotoxin levels. Thus the purpose of this study was to investigate the domestic determinants of endotoxin.

Methods

The study sample

The 77 homes surveyed in this study were selected from 355 homes in suburban Wellington with carpeted living room floors from which dust was sampled in 1994 and *Der p 1* measured. Those homes with the highest and the lowest levels of *Der p 1* in 1994 were selected to be part of a follow-up study, conducted between March 1998 and February 1999, primarily to investigate those housing factors that determine *Der p 1* levels (Wickens et al., 2001). Despite these selection criteria, *Der p 1* was log-normally distributed in the follow-up study. This paper investigates determinants of endotoxin levels in these same dust samples. Details of the eligibility criteria have previously been reported (Wickens et al., 2001) but briefly, respondents were eligible if they had lived in the same home for 4 years since 1994 and not changed the carpet in the living room.

Dust collection and extraction

First, 1 m² in the center of the living room was sampled for 1 min using a Hitachi 1100 W vacuum cleaner with the dust collected in a nylon sock inserted onto the furniture attachment of the vacuum. A second sample from the whole accessible floor area required a larger receptacle; thus it was necessary to use a later model vacuum cleaner (Hitachi super 4700, 1300 W, Hitachi Ltd, Tokyo, Japan) and the disposable bag provided by the manufacturers for the dust collection. Between all samples the vacuum floor attachment was cleaned and

the dust collection sock/bag was replaced. The rate of collection for the whole floor sample, at 5 m²/min, was intended to represent normal household cleaning behavior. The area sampled was measured (mean area: 16 m²) so that endotoxin levels could be expressed as endotoxin units (EU)/m², as well as EU/mg. After collection, dust was frozen and later sieved (425 µm) with the fine dust used to estimate endotoxin levels. Dust samples were extracted with 5.0 ml of pyrogen-free water containing 0.05% Tween-20 (Douwes et al., 1995). Endotoxin concentration was determined using a kinetic chromogenic *Limulus* Amoebocyte Lysate (LAL) method (Bio Whittaker, Walkersville, MD, USA; lot no 6L2661) on 1:500 dilution of the extracts. The endotoxin potency of the standard (Bio Whittaker; lot no 5L4100) was 11.5 EU/ng.

Collection of predictor variables

At the time of dust collection, sensor devices (Cunningham, 1998) were placed at carpet bases to record relative humidity and temperature at 15-min intervals for a minimum of 24 h.

The field workers administered questionnaires to collect information on other climatic factors (hours of living room sun, direction living room faced), housing factors (the presence of insulation in living room, numbers and distribution of rooms), cleaning factors (frequency of vacuuming and steam-cleaning/shampooing living room carpet, whether disposable vacuum bags were used, age of vacuum cleaner), the presence of pets and household occupancy (numbers of children and adults). In addition, the field workers checked for visible damp and mold in the living room and the slope of the ground under the house.

Statistical analysis

Statistical analysis was conducted using SAS version 8 (SAS Institute, Cary, NC, USA). Endotoxin and dust weights were log-normally distributed, and analysis was based on log-transformed data. Geometric mean endotoxin units per milligram of dust (EU/mg) and per square meter (EU/m²) [95% confidence intervals (CI)] were calculated for 77 dust samples from the 1 m² and 74 from the whole room site. Geometric mean ratios (GMR) are used to show the associations between the explanatory variables and endotoxin level with *t*-tests used to measure the significance of the differences between subgroups. Mean summer and winter daily hours of sunshine and mean relative humidity and temperature were calculated for each house. The mean across all houses was used to define these variables dichotomously. Associations between endotoxin (EU/mg and EU/m²) from different sites (1 m² and whole room) and between the concentration (EU/mg) and total endotoxin (EU/m²) in each site were assessed

using Pearson's correlation coefficients. Pearson's correlation coefficients were also calculated to show associations between Der p 1 ($\mu\text{g/g}$ and $\mu\text{g/m}^2$) and endotoxin (EU/mg and EU/m²).

Ethical approval for the study was granted by the Wellington Ethics Committee and informed consent was obtained from participants.

Results

After excluding ineligible respondents (Wickens et al., 2001), the overall response rate was 80%.

The concentrations of EU/mg of dust in the 1 m² and the whole room sample were not significantly different ($P = 0.20$), but EU/m² was significantly lower for the whole room sample than the 1m² sample ($P < 0.001$) reflecting the significantly lower dust weight (g/m²) in the whole room sample ($P < 0.0001$) (Table 1).

The correlation coefficient between log endotoxin from the 1m² sample and log endotoxin from the whole room sample was 0.56 ($P < 0.0001$) for EU/mg and 0.74 ($P < 0.0001$) for EU/m². The correlation between log endotoxin expressed as EU/mg and EU/m² was

Table 1 Geometric mean EU/mg and EU/m² from the 1-m² sample and the whole room sample

	Geometric mean EU/mg	Range	GSD
1 m ² ($n = 77$)	22.7	2.9–414.2	2.4
Whole room ($n = 74$)	28.4	1.3–3881.1	3.4
	Geometric mean EU/m ²		
1 m ² ($n = 77$)	30,544	3435–710,984	3.2
Whole room ($n = 74$)	5653	120–1,785,436	6.4
	Geometric mean weight (g/m ²)		
1 m ² ($n = 77$)	1.35	0.21–11.20	1.9
Whole room ($n = 74$)	0.20	0.03–4.69	2.7

GSD, geometric standard deviation.

0.83 ($P < 0.0001$) for the 1m² sample and 0.88 ($P < 0.0001$) for the whole room sample.

Correlation analyses between Der p 1 and endotoxin showed significant associations which were weak when comparing concentrations, $r = 0.23$ ($P = 0.04$) based on the 1m² site and $r = 0.24$ ($P = 0.03$) for the whole room site, but stronger for total Der p 1 and total endotoxin, $r = 0.47$ ($P < 0.0001$) in 1m² and $r = 0.72$ ($P < 0.0001$) in the whole room.

Tables 2–4 show the results of univariate analyses of factors determining endotoxin (expressed as EU/m² and EU/mg) in each site. Variables that were significantly ($P < 0.05$) associated with at least one measure of endotoxin, or for which an *a priori* hypothesis had suggested an effect, were included in a multivariate analysis to assess their independent effects on endotoxin (Table 5). Many of the factors that may influence indoor climate were closely correlated, and thus were added to the model separately. To avoid multicollinearity between these variables only those that best predicted endotoxin level were kept in the final model. For example, the variables for mean and maximum carpet relative humidity were separately included in the model but it was the maximum relative humidity that better predicted endotoxin level. Variables for visible damp and mold and mean summer/winter and daily sunshine hours at dust collection were not associated with endotoxin level after adjustment for the other variables in the model. The variable for insulation contrasted homes with floor insulation, including insulation provided by a room or garage below the living room, with those homes without any form of insulation. The variable for total occupants was used to represent all measures of occupancy.

Pets and people

After adjustment for all other variables in the model (Table 5), the presence of a dog inside the home was

Table 2 Geometric means endotoxin ratios (GMR) for the 1 m² and whole room samples, showing associations with pets and number and concentration of household occupants

	$n = 77$	1 m ² sample		$n = 74$	Whole room sample	
		GMR EU/mg	GMR EU/m ²		GMR EU/mg	GMR EU/m ²
Cat(s) inside ^a						
Yes vs. no	33/35	1.03	1.12	33/33	0.97	1.18
Dog(s) inside ^a						
Yes vs. no	23/48	1.29	1.66	22/47	1.29	1.88
Total occupants						
5 + vs. 2–4	41/36	1.77*	2.28*	37/37	1.19	1.81
Total children						
3 + vs. 1–2	33/44	1.67*	2.06*	29/45	1.16	1.38
Children per room						
> vs. ≤ mean (0.41)	27/50	1.71*	2.51*	24/50	1.41	2.02
People per room						
> vs. ≤ mean (0.76)	31/46	1.73*	2.23*	28/46	1.46	1.82

* $P < 0.01$

^a Numbers do not add up to total n because missing data are excluded from analyses.

Table 3 Geometric means endotoxin ratios (GMR) for the 1 m² and whole room samples for those variables affecting indoor climate that show associations ($P < 0.10$) for any outcome measure

	<i>n</i> = 77	1 m ² sample		<i>n</i> = 74	Whole room sample	
		GMR EU/mg	GMR EU/m ²		GMR EU/mg	GMR EU/m ²
Any floor insulation ^a						
Yes vs. no	24/47	0.72	0.56*	24/44	0.61	0.40
Under living room						
Room, garage, insulation vs. none	31/46	0.62*	0.49**	31/43	0.51*	0.34**
Living room wall insulation						
Yes vs. no	23/54	0.62*	0.53*	24/50	0.72	0.62
Whether ground slopes under house						
Yes vs. no	49/28	1.26	1.73*	46/28	1.71	2.26
Visible damp in living room						
Yes vs. no	8/69	1.37	2.57*	8/66	1.62	2.78
Visible mold in living room						
Yes vs. no	6/71	1.60	2.47	6/68	1.91	2.34
Mean summer/winter daily sunshine hours ^a						
0–6 vs. 6.5+ h	37/38	1.41	1.58	38/36	1.69	1.61
Daily sunshine hours at dust collection ^a						
0–6 vs. 7–12 h	41/34	1.44	1.79*	42/32	1.37	1.78
Direction of living room						
N, NW, NE vs. other	40/37	0.63*	0.48**	39/35	0.50**	0.28**
Season						
Summer vs. Spring	10/24	1.71	2.30	10/25	5.00**	7.36*
Autumn vs. Spring	20/24	1.40	1.56	16/25	2.19**	3.41*
Winter vs. Spring	23/24	1.65*	1.96*	23/25	2.48*	3.64**
Mean carpet RH	<i>n</i> = 73			<i>n</i> = 70		
> vs. ≤ mean (66.2)	38/35	1.52*	1.74*	38/32	1.57	1.71
Maximum carpet RH	<i>n</i> = 73			<i>n</i> = 70		
> vs. ≤ mean (70.8)	39/34	1.44	1.62	39/31	1.64	1.78

* $P < 0.05$, ** $P < 0.01$.^a Numbers do not add up to total *n* because missing data are excluded from analyses.**Table 4** Geometric means endotoxin ratios (GMR) for the 1 m² and whole room samples for those cleaning and carpet variables that showed associations ($P < 0.10$) for any outcome measure

	<i>n</i> = 77	1 m ² sample		<i>n</i> = 74	Whole room sample	
		GMR EU/mg	GMR EU/m ²		GMR EU/mg	GMR EU/m ²
Age of vacuum cleaner ^a						
< 1 vs. 1+ year	8/65	2.55***	2.39*	8/63	2.55	2.69
Vacuum dust bag reused						
Yes vs. no	19/58	1.49	1.85*	18/56	3.18**	5.57***
Steamcleaning or shampooing						
Yes vs. no	13/64	2.18**	2.12*	12/62	2.16*	1.84
Frequency of vacuuming						
< vs. 1+ times a week	3/74	1.72	2.14	3/71	2.62	3.09***
Carpet type ^a						
Tufted vs. woven	58/15	0.74	0.47*	57/13	0.63	0.22**
Depth of pile						
5–12 vs. 0–4 mm	52/25	0.97	0.59	52/22	0.95	0.56
Carpet material ^a						
Wool vs. synthetic	48/10	1.03	0.72	46/10	0.55*	0.34

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$.^a Numbers do not add up to total *n* because missing data are excluded from analyses.

associated with higher endotoxin levels, particularly endotoxin per m². The number of household occupants was also associated with a relative increase in endotoxin of 1.7–2.0 in the 1m² site.

House and climate characteristics

The multivariate model (Table 5) shows that the presence of insulation was consistently protective

Table 5 Geometric means ratios (GMR) for housing factors and endotoxin (EU/mg and EU/m²) in 1 m² and the whole room sample after adjustment for all variables shown in table

	1 m ² sample		Whole room sample	
	GMR EU/mg (95% CI)	GMR EU/m ² (95% CI)	GMR EU/mg (95% CI)	GMR EU/m ² (95% CI)
Dog(s) inside				
Yes vs. no	1.33 (0.88–2.01)	1.95 (1.14–3.33)*	0.91 (0.51–1.65)	1.42 (0.57–3.53)
Total occupants				
5 + vs. 2–4	1.69 (1.18–2.43)**	2.04 (1.25–3.23)**	0.89 (0.53–1.50)	1.08 (0.48–2.39)
Under living room				
Room, garage, insulation vs. none	0.78 (0.52–1.15)	0.65 (0.39–1.09)	0.65 (0.36–1.14)	0.41 (0.17–0.98)*
Sloping under house				
Yes vs. no	1.03 (0.65–1.62)	1.05 (0.59–1.90)	1.33 (0.70–2.52)	1.26 (0.47–3.36)
North facing				
Yes vs. no	0.77 (0.52–1.14)	0.62 (0.37–1.03)	0.58 (0.34–1.00)*	0.36 (0.15–0.85)*
Maximum RH				
> vs. ≤ mean (70.8)	1.62 (1.08–2.42)*	1.35 (0.80–2.27)	1.58 (0.89–2.82)	1.36 (0.56–3.33)
Age of vacuum cleaner				
< vs. 1+ years	2.48 (1.36–4.51)**	2.25 (1.03–4.88)*	2.28 (0.97–5.40)	2.72 (0.72–10.26)
Vacuum dust bag reused				
Yes vs. no	1.41 (0.84–2.17)	1.39 (0.78–2.50)	2.45 (1.30–4.63)**	3.09 (1.16–8.26)*
Carpet type				
Tufted vs. woven	1.20 (0.69–2.09)	1.01 (0.49–2.08)	1.11 (0.49–2.49)	0.45 (0.13–1.59)
Depth of pile				
5–12 vs. 0–4 mm	0.99 (0.64–1.52)	0.62 (0.35–1.10)	0.85 (0.45–1.61)	0.59 (0.22–1.58)
Carpet material				
Wool vs. synthetic	0.95 (0.53–1.68)	0.76 (0.36–1.60)	0.87 (0.38–1.99)	0.73 (0.21–2.61)
Steamcleaning/shampooing				
Yes vs. no	1.68 (1.01–2.79)*	1.40 (0.72–2.70)	2.15 (1.04–4.42)*	1.68 (0.55–5.15)
Frequency of vacuuming				
< vs. 1+ times a week	1.35 (0.49–3.70)	2.08 (0.56–7.69)	1.17 (0.28–4.83)	1.05 (0.12–9.43)
Season				
Summer vs. Spring	1.06 (0.58–1.93)	1.34 (0.62–2.91)	2.40 (1.04–5.54)*	2.89 (0.80–10.52)
Autmun vs. Spring	1.24 (0.78–1.99)	1.26 (0.68–2.31)	2.44 (1.21–4.93)*	3.44 (1.16–10.19)*
Winter vs. Spring	1.29 (0.82–2.03)	1.46 (0.81–2.64)	1.97 (1.05–3.69)*	2.33 (0.88–6.12)

P* < 0.05, *P* < 0.01.

against endotoxin, reducing levels by 20–60%. In north-facing houses endotoxin levels were similarly reduced. In contrast, relative humidities greater than the mean maximum were associated with a 1.4–1.6-fold increase in endotoxin, levels. Other sources of humidity in the home, such as the proximity of the laundry or bathroom to the living room, or the presence of a dehumidifier in the living room, were not related to endotoxin (data not shown). Houses that were sampled during spring had lower levels of endotoxin, compared with houses sampled at other times of the year, with large differences (two to threefold) for the whole room sample only. The addition of mean maximum temperature to the model did not alter this association for season.

Although endotoxin levels tended to be higher in houses built before 1978, none of these associations reached significance. There were also no clear associations for floor or outside wall construction material with endotoxin (not shown in tables).

Cleaning

After modelling, levels of endotoxin associated with new vacuum cleaners (< 1 year) remained more than

twofold greater than in older cleaners. Reusing the dust collection vacuum bag was associated with up to threefold higher levels of endotoxin. Steam-cleaning or shampooing the carpet was also associated with a large increase in the level of endotoxin, 1.4–2.2-fold. Neither the motor size of the vacuum cleaner nor the presence of a high efficiency particulate air (HEPA) filter affected the level of endotoxin (data not shown).

Carpets

The inverse univariate associations that we found for tufted carpets, carpet pile lengths more than 4 mm and wool carpets were no longer significant after adjustment for covariates in the model (Table 5). We found no association for depth of the underlay and the age of the carpet with endotoxin (data not shown).

In subsequent analyses, these same variables were combined to assess their independent effects on the weight of dust/m² collected from each site. The presence of a dog and short pile lengths were each associated with about a 1.5-fold increase in dust weight/m² in each sample. For the whole room sample, lower dust weights/m² were found in the presence of

insulation (40% lower), north-facing living rooms (40% lower), and in tufted carpets (60% lower than woven carpets).

Discussion

Compared with other studies, we found that endotoxin levels in Wellington, New Zealand (22.7 EU/mg) were lower than those found in Boston (75.2 EU/mg) and Denver (178.2 EU/mg), USA (Gereda et al., 2001; Park et al., 2000) and higher than those found in Amsterdam (8.8 EU/mg), The Netherlands (Douwes et al., 2000). However large and significant inter-laboratory variation has been observed with some of the variation related to differences between assay kit types (Chun et al., 2000). Although endotoxin in our study was measured in the same laboratory as the Netherlands' study, standardization between laboratories is necessary before wider international comparisons can be made. On the other hand, intra-laboratory variations are small (Chun et al., 2000).

It is not clear whether airborne or dust endotoxin levels are the best estimate of exposure but most studies of the domestic environment have focussed on dust analyses. Despite the correlation between dust endotoxin level in each site in the present study, some factors were stronger predictors of levels in one site than the other, suggesting that each site may represent qualitatively different types of house dust. Therefore it would be difficult to conclude which site would be most useful in future studies.

The significant correlations found between Der p 1 and endotoxin, particularly strong when expressed per m², raise the possibility that endotoxin may have confounded previously reported associations for Der p 1 and asthma severity (Custovic et al., 1996). However it has been suggested that concomitant exposures to endotoxin and allergens may potentiate allergic airways inflammation (Eldridge and Peden, 2000; Peden et al., 1999; Wan et al., 2000).

The use of a household vacuum cleaner <1-year old strongly predicted high endotoxin levels. There is no obvious explanation for this finding but it was not because of confounding by engine size, age of home or total dust weight. High endotoxin levels were also found where dust collection bags were reused, possibly because of the dispersal of dust back into the room during dust disposal. Socioeconomic status was not collected as part of this follow-up study, but the association between the reuse of bags and endotoxin was not confounded by socioeconomic status measured 4 years previously as part of the original study. High concentrations of endotoxin were also found in the homes of respondents who had steam-cleaned or shampooed the carpet during the last 12 months. It is possible that the water used

during these processes is not completely removed resulting in ongoing dampness in the carpet, likely to be conducive to the build up of bacterial endotoxins.

Although we used different vacuum cleaners for sampling each site, the use of a newer model for the whole room did not appear to substantially inflate endotoxin concentration in the whole room. In fact, the level of endotoxin per m² is significantly lower in the whole room sample, most likely due to the lower intensity of sampling used for the whole room, and is reflected in the much smaller amount of dust per m² collected from this site.

Relative humidity measured using the sensors was also associated with increased levels of endotoxin but there was no temperature effect. On the other hand, inverse associations were found for insulation, including that provided by a room or garage below the living room, and in north-facing homes. To illustrate, based on the 1m² sample, in north-facing homes with insulation and lower than mean maximum relative humidities the geometric mean EU/mg was 15.7 (95% CI, 9.0–27.3) compared with 35.9 (95% CI, 19.7–65.3) in homes with none of these features. The seasonal effect we found occurred independently of temperature and relative humidity and thus is more likely to be due to residual confounding than changes in weather. Factors that affect indoor climate have also been shown to be important determinants of endotoxin levels in other studies. For example, absolute relative humidity has been positively associated with airborne endotoxin but not endotoxin in dust (Park et al., 2000), and central air conditioning has been associated with lower house dust endotoxin levels (Gereda et al., 2001).

The presence of cats (Gereda et al., 2001; Heinrich et al., 2001), dogs (Gereda et al., 2001; Heinrich et al., 2001; Park et al., 2001) and vermin (Gereda et al., 2001; Heinrich et al., 2001; Park et al., 2001) in the home has previously been associated with high endotoxin levels. We found that the presence of dogs (but not cats) was positively associated with increased endotoxin levels, with the association reaching significance for total endotoxin in the 1m² sample only. For each site, the effect was stronger for EU/m², reflecting the positive association we found between dogs and dust weight. The question we asked about pets was limited to cats and dogs that regularly come inside and may have underestimated the effect as outdoor pets may also be a source of endotoxin transported into the home on clothing. In urban New Zealand, it is uncommon to remove shoes before entering the home. As with the presence of dogs inside, the number and concentration of adults and children were also positively associated with endotoxin, but only in the 1m² site. These differences between sites may arise because bacteria from both humans and animals are more likely to end up concentrated around frequently used

areas, such as the center of the living room, from where we sampled (1 m^2) than be distributed around the room. Occupancy levels may also influence endotoxin levels indirectly through raising humidity in the home, as the production and use of water is increased when there are more people. The only other report (Gerada et al., 2001) that we were able to locate (from Denver, USA) that reported the effect of household occupancy on endotoxin levels found no significant associations for household inhabitants or young children.

It is important to note that this analysis involves many comparisons and some will be significant by chance alone.

There is still much research to be carried out before we understand the precise role of home endotoxin levels in asthma development but several studies have indicated an association with asthma exacerbations, and endotoxin in concentrations encountered in the occupational setting has been associated with the development of respiratory health effects (Douwes, 1997). Thus controlling endotoxin in the home may be

important in improving respiratory health. In New Zealand, effective control may need to be through improvements in the heating and structure of homes that lower relative humidity and result in warmer drier homes. This study has contributed towards identifying those factors that are important determinants of domestic endotoxin.

Acknowledgements

The Wellington Asthma Research Group is supported by the Health Research Council of New Zealand and the funding for this study was provided by the University of Otago and the Asthma and Respiratory Foundation of New Zealand. Julian Crane was supported in part by a Health Research Council of New Zealand senior research fellowship and Jeroen Douwes was supported by a research fellowship from the Netherlands Organisation for Scientific Research (NWO). We would like to thank the families that participated in this study.

References

- Braun-Fahrlander, C., Gassner, M., Grize, L., Neu, U., Sennhauser, F.H., Varonier, H.S., Vuille, J.C., Wuthrich, B. and The Scapol Team (1999) Prevalence of hay fever and allergic sensitization in farmer's children and their peers living in the same rural community, *Clin. Exp. Allergy*, **29**, 28–34.
- Chun, D.T., Chew, V., Bartlett, K., Gordon, T., Jacobs, R.R., Larsson, B.M., Larsson, L., Lewis, D.M., Liesivuori, J., Michel, O., Milton, D.K., Rylander, R., Thorne, P.S., White, E.M. and Brown, M.E. (2000) Preliminary report on the results of the second phase of a round-robin endotoxin assay study using cotton dust, *Appl. Occup. Environ. Hygiene*, **15**, 152–157.
- Cunningham, M.J. (1998) Direct measurement of temperature and humidity in dust mite microhabitats, *Clin. Exp. Allergy*, **28**, 1104–1112.
- Custovic, A., Taggart, S.C., Francis, H.C., Chapman, M.D. and Woodcock, A. (1996) Exposure to house dust mite allergens and the clinical activity of asthma, *J. Allergy Clin. Immunol.*, **98**, 64–72.
- Douwes, J., Versloot, P., Hollander, A., Heederik, D. and Doekes, G. (1995) Influence of various dust sampling and extraction methods on the measurement of airborne endotoxin, *Appl. Environ. Microbiol.*, **61**, 1763–1769.
- Douwes, J. (1997) Epidemiological investigations of endotoxins, *Int. J. Occup. Environ. Health*, **3**, S26–S31.
- Douwes, J., Doekes, G., Heinrich, J., Koch, A., Bischof, W. and Brunekreef, B. (1998) Endotoxin and β (1 $\rightarrow$ 3) glucan in house dust and the relation with home characteristics: A pilot study in 25 German houses, *Indoor Air*, **8**, 255–263.
- Douwes, J., van der Sluis, B., Doekes, G., van Leusden, F., Wijnands, L., van Strien, R., Verhoeff, A. and Brunekreef, B. (1999) Fungal extracellular polysaccharides in house dust as a marker for exposure to fungi: relations with culturable fungi, reported home dampness, and respiratory symptoms, *J. Allergy Clin. Immunol.*, **103**, 494–500.
- Douwes, J., Zuidhof, A., Doekes, G., van der Zee, S., Wouters, I., Boezen, M.H. and Brunekreef, B. (2000) (1, 3)- β -D-glucan and endotoxin in house dust and peak flow variability in children, *Am. J. Resp. Crit. Care Med.*, **162**, 1348–1354.
- Eldridge, M.W. and Peden, D.B. (2000) Allergen provocation augments endotoxin-induced nasal inflammation in subjects with atopic asthma, *J. Allergy Clin. Immunol.*, **105**, 475–481.
- Ernst, P. and Cormier, Y. (2000) Relative scarcity of asthma and atopy among rural adolescents raised on a farm, *Am. J. Resp. Crit. Care Med.*, **161**, 1563–1566.
- Gerada, J.E., Leung, D.Y., Thatayatikom, A., Streib, J.E., Price, M.R., Klinnert, M.D. and Liu, A.H. (2000) Relation between house-dust endotoxin exposure, type 1 T-cell development, and allergen sensitisation in infants at high risk of asthma, *Lancet*, **355**, 1680–1683.
- Gerada, J.E., Klinner, M.D., Price, M.R., Leung, D.Y. and Liu, A.H. (2001) Metropolitan home living conditions associated with indoor endotoxin levels, *J. Allergy Clin. Immunol.*, **107**, 790–796.
- Heinrich, J., Gehring, U., Douwes, J., Koch, A., Fahlbusch, B., Bischof, W. and Wichmann, H.E. for the INGA-study group (2001) Pets and vermin are associated with high endotoxin levels in house dust, *Clin. Exp. Allergy*, **31**, 1839–1845.
- Kilpelainen, M., Terho, E.O., Helenius, H. and Koskenvuo M. (2000) Farm environment in childhood prevents the development of allergies, *Clin. Exp. Allergy*, **30**, 201–208.
- Michel, O., Kips, J., Duchateau, J., Vertongan, F., Robert, L., Collet, H., Pauwels, R. and Sergysels, R. (1996) Severity of asthma is related to endotoxin in house dust, *Am. J. Resp. Crit. Care Med.*, **154**, 1641–1646.
- Ninan, T.K. and Russell, G. (1992) Respiratory symptoms and atopy in Aberdeen schoolchildren: evidence from two surveys 25 years apart, *Brit. Med. J.*, **304**, 873–875.
- Park, J.H., Spiegelman, D.L., Burge, H.A., Gold, D.R., Chew, G.L. and Milton, D.K. (2000) Longitudinal study of dust and airborne endotoxin in the home, *Environ. Health Perspectives*, **108**, 1023–1028.
- Park, J.H., Gold, D.R., Spiegelman, D.L., Burge, H.A. and Milton, D.K. (2001) House dust endotoxin and wheeze in the first year of life, *Am. J. Resp. Crit. Care Med.*, **163**, 322–328.
- Peat, J.K., van den Berg, R.H., Green, W.F., Mellis, C.M., Leeder, S.R. and Woolcock, A.J. (1994) Changing prevalence of asthma in Australian children, *Brit. Med. J.*, **308**, 1591–1596.

- Peden, D.B., Tucker, K., Murphy, P., Newlin-Clapp, L., Boehlecke, B., Hazucha, M., Bromberg, P. and Reed, W. (1999) Eosinophil influx to the nasal airway after local, low-level LPS challenge in humans, *J. Allergy Clin. Immunol.*, **104**, 388–394.
- Riedler, J., Eder, W., Oberfeld, G. and Schreuer, M. (2000) Austrian children living on a farm have less hay fever, asthma and allergic sensitization, *Clin. Exp. Allergy*, **30**, 194–200.
- Rizzo, M.C., Naspitz, C.K., Fernandez-Caldas, E., Lockey, R.F., Mimica, I. and Sole, D. (1997) Endotoxin exposure and symptoms in asthmatic children, *Pediatric Allergy Immunol.*, **8**, 121–126.
- Smid, T., Heederik, D., Houba, R. and Quanjer, P.H. (1994) Dust and endotoxin-related acute lung function changes and work-related symptoms in workers in the animal feed industry, *Am. J. Industrial Med.*, **25**, 877–888.
- von Ehrenstein, O.S., von Mutius, E., Illi, S., Baumann, L., Bohm, O. and von Kries, R. (2000) Reduced risk of hay fever and asthma among children of farmers. *Clin. Exp. Allergy*, **30**, 187–193.
- von Mutius, E., Braun-Fahrlander, C., Schierl, R., Riedler, J., Ehlermann, S., Maisch, S., Waser, M. and Nowak, D. (2000) Exposure to endotoxin or other bacterial components might protect against the development of atopy, *Clin. Exp. Allergy*, **30**, 1230–1234.
- Wan, G.H., Li, C.S. and Lin, R.H. (2000) Airborne endotoxin exposure and the development of airway antigen-specific allergic responses, *Clin. Exp. Allergy*, **30**, 426–432.
- Wickens, K., Mason, K., Fitzharris, P., Siebers, R., Hearfield, M., Cunningham, M. and Crane, J. (2001) The importance of housing characteristics in determining Der p 1 levels in carpets in New Zealand homes, *Clin. Exp. Allergy*, **31**, 827–835.