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ENDOTOXIN, (1→3)-β-D-GLUCANS AND FUNGAL EXTRA-CELLULAR POLYSACCHARIDES IN NEW ZEALAND HOMES: A PILOT STUDY

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Abstract: Bacterial endotoxin, fungal (1→3)-β-D-glucans, and extracellular polysaccharides from *Aspergillus* and *Penicillium* (EPS-*Asp*/*Pen*) have been suggested to be stable markers of microbial exposure. This paper describes a pilot study in which we measured endotoxin, (1→3)-β-D-glucans, EPS-*Asp*/*Pen* and mite allergen in house dust collected in 32 homes in Wellington, New Zealand. Endotoxin (GM 60,295 EU/g; GSD 2.4) and glucan (GM 2,687 µg/g; GSD 1.5) levels were higher in comparison to previous international studies, whereas EPS-*Asp*/*Pen* levels (37,347 Units/g; GSD 1.9) appeared comparable. Concentrations expressed per square meter were highly correlated among the measured components ($p<0.05$). When expressed per gram of dust only (1→3)-β-D-glucans and EPS-*Asp*/*Pen* were correlated ($r=0.55$, $p<0.01$; $n=32$). Endotoxin and glucan levels were higher (borderline statistically significant; $p<0.10$) in homes with self-reported water damage. A positive association ($p<0.10$) was also found for dust mite and a combination of self-reported mould, dampness and water damage. EPS levels were higher in homes where residents indicated the presence of mould spots on the wall, but this did not reach statistical significance. In conclusion, levels of microbial contaminants in a small random sample of New Zealand homes were high and weakly associated with water damage.

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INTRODUCTION

Exposure to indoor microorganisms and particularly fungi has been widely recognized as a plausible cause of dampness-related respiratory morbidity. However, the evidence that microorganisms play a causal role is limited, mainly due to the lack of valid methods to assess exposure accurately in epidemiological studies [8]. Exposure to microorganisms in the indoor environment is most frequently studied by counting culturable spores in settled dust or in the air, but this approach has serious drawbacks [8]. Measuring markers of microbial biomass in house dust such as bacterial endotoxin, fungal (1→3)-β-D-glucans, and extracellular polysaccharides from *Aspergillus* and *Penicillium* (EPS-*Asp*/*Pen*) has been suggested to be a valid alternative [3, 5]. EPS-*Asp*/*Pen* is a general marker of indoor fungi, whereas endotoxin and (1→3)-β-D-glucan may have direct relevance to health effects because of their pathogenic properties [7, 9].

In New Zealand, house dust mite allergen levels are very high [18] and have often been suggested to be related to the high prevalence of asthma. Little information is available regarding indoor microbial levels. This paper describes a pilot study in which we assessed endotoxin,

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(1→3)-β-D-glucan, EPS-*Asp*/*Pen* and house dust mite allergen levels in 32 Wellington (New Zealand) homes. We also determined the correlation among them and assessed the association between these exposure indicators and self-reported signs of indoor mould, dampness and water damage.

MATERIALS AND METHODS

Dust sampling. Settled dust was sampled in 32 Wellington homes using a Hitachi CV-2500 vacuum cleaner (1100 W) equipped with a nylon dust sampling sock. Homes were selected from a previous study [12] where there was at least one 6- to 14-year-old child present. Households were otherwise randomly selected. Floor dust was sampled from one square meter from the centre of the living room for one minute. All living rooms had wall-to-wall carpet. We chose to focus on living room floor samples (rather than bedroom floor or mattress samples) since previous studies conducted in the Netherlands had shown that house dust glucan and EPS levels on living room floors were most consistently associated with symptoms and peak flow variability in (asthmatic) children [5, 6].

Samples were sieved through a 425 µm mesh steel filter and stored at -20ºC until extraction. Endotoxin, EPS-*Asp*/*Pen* and (1→3)-β-D-glucan were extracted sequentially, as described previously [15]. Endotoxin was extracted in pyrogen free water with 0.05% Tween-20 at room temperature [2], and glucan in pyrogen free water with 0.05% Tween-20 at 120ºC [3]. Dust mite allergen (Der p1) and EPS-*Asp*/*Pen* were extracted in phosphate-buffered saline with 0.05% Tween-20 at room temperature [15, 16].

Endotoxin, EPS-Asp/Pen, (1→3)-β-D-glucan and Der p1 analysis. Endotoxin was assayed with a quantitative kinetic chromogenic Limulus Amebocyte Lysate (LAL) method (Kinetic QCL no- 50-650 U, LAL lot No. 6L2661, and LPS standard No. 5L4100; Bio Whittaker) at 37ºC [2]. Samples did not show inhibition or enhancement of the LAL assay at the 1:500 dilution, tested as described earlier [11]. EPS-*Asp*/*Pen* and (1→3)-β-D-glucan were analysed with a sandwich enzyme immunoassay (EIA) [5] and an inhibition EIA [3], respectively. The glucan assay is not entirely specific for fungal (1→3)-β-D-glucan and may also detect plant and bacterial (1→3)-β-D-glucan [3]. Der p1 was analysed using a commercial sandwich EIA (Indoor Biotechnologies, Cardiff, UK).

Indoor dampness and mould. Residents of the home were asked 3 questions to assess indoor dampness or mould problems: (1) In the past 12 months have you ever noticed patches of mould or mildew on any surface in the living room? (2) In the past 12 months have you ever noticed damp spots on any surface in the living room? (3) Since you have lived in this house has it ever been affected by water damage? We did not collect information on other home characteristics.

Statistical analysis. The data were analysed using SAS statistical software (SAS Institute, Cary, NC). Concentrations approximated a log-normal distribution, hence exposure measurements were log transformed and geometric means (GM) were presented with geometric standard deviations (GSD). Pearson correlation coefficients were calculated to assess the correlation among several exposure indices. Due to the relatively small study size we did not conduct multivariate analyses. We performed t-tests to determine statistical significance. A p-value of 0.05 or less was considered statistically significant. Since the study size was relatively small, we also considered "Borderline statistical significance ($0.05< p<0.10$)" to identify general trends.

RESULTS

Microbial agent and dust mite allergen levels were readily detectable in all samples with relatively high mean concentrations (Tab. 1). Concentrations expressed per square meter were highly correlated among the measured components (Tab. 2). Expressed per g of dust, only the fungal components (i.e. EPS-*Asp*/*Pen* and (1→3)-β-D-glucan) were significantly correlated ($r=0.55$; $p<0.01$).

Residents of approximately 20% (n=6) of the studied homes reported the presence of surface mould in the living room; only 2 homes had damp spots, and more than 40% (n=14) of the homes had previously been affected by water damage. EPS and Der p1 levels were somewhat higher in living rooms with mould spots, but this was not statistically significant (Tab. 3). All measured components were higher in homes that had ever been affected by water damage, borderline statistically significant ($p<0.10$) only for (1→3)-β-D-glucan and endotoxin. When combining several indicators of dampness (i.e. mould spots, damp spots, and water damage) a similar picture was seen, except that differences for endotoxin and (1→3)-β-

Table 1. Living room floor levels of dust and microbial contaminant levels.

	N	GM	GSD	Min	Max
Level/g of dust					
Glucan µg/g	32	2,686.5	1.5	865.9	7,705.6
Endotoxin EU/g	32	60,294.7	2.4	8,305.9	228,435.5
EPS U/g	32	37,346.7	1.9	9,383.2	121,156.8
Der p1 µg/g	30	33.5	3.0	2.4	175.5
Level/m^2					
Glucan µg/m^2	32	6,105.8	2.2	483.4	18,729.4
Endotoxin EU/m^2	32	137,036.1	3.5	6,290.1	1,274,272.4
EPS U/m^2	32	84,795.7	2.3	10,112.8	329,402.6
Der p1 µg/m^2	30	77.8	3.4	4.1	598.4
Dust g/m^2	32	2.3	1.9	0.6	7.2

Table 2. Pearson correlations among levels of endotoxin, (1→3)-β-D-glucan, EPS-*Asp*/*Pen* and Der p1 (*p<0.05; **p<0.01).

	Glucan	Endotoxin	EPS	Der p1	Dust
Levels/m^2 dust					
Glucan	1.00	0.71**	0.78**	0.47**	0.84**
Endotoxin		1.00	0.59**	0.47**	0.76**
EPS			1.00	0.50**	0.64**
Der p1				1.00	0.42*
Dust					1.00
Levels/g dust					
Glucan	1.00	0.22	0.55**	0.23	-
Endotoxin		1.00	0.12	0.19	-
EPS			1.00	0.35	-
Der p1				1.00	-

D-glucan were less significant whereas the difference for Der p1 became borderline significant (Tab. 3).

DISCUSSION

This was a small study including only 32 houses and results should therefore be considered preliminary. However, despite the small size we found some interesting and novel results. Firstly, microbial contaminant levels in New Zealand homes were relatively high. Secondly, microbial levels (particularly endotoxin and (1→3)-β-D-glucan) appeared to be associated with previous water damage.

Despite using the same laboratory and analytical methods for dust extractions and analyses, endotoxin and (1→3)-β-D-glucan levels were higher in the present study compared to previous studies in Europe. The geometric mean endotoxin concentration in our study was approximately 60,000 EU/g compared to 11,000-17,000 EU/g in the Netherlands and Germany [4, 6]. In a previous study in New Zealand using the same laboratory methods, we found a geometric mean concentration of approximately 23,000 EU/g [4, 19], which is considerably lower than the currently measured levels. The reasons for these differences are not clear, but it may be due to batch differences in LAL reagents [13]. Glucan levels were approximately 1.5-3.5 times higher than previously measured in the Netherlands and Germany [1, 4, 6, 10, 20]. EPS-*Asp*/*Pen* levels were substantially higher compared to a previous study in the Netherlands [20], but comparable to more recent studies in the Netherlands and elsewhere in Europe [1, 15]. Other studies focusing on house dust endotoxin levels in Europe, Brazil, US and Asia have also been conducted, but these will not be discussed here since extraction and analytical methods were different from the current study, thereby precluding a valid comparison.

New Zealand homes generally do not have central heating, are poorly insulated, and dampness/water damage problems are relatively common (as also suggested by the fact that 14 out of 32 houses had been affected by water damage). In addition, New Zealand (including Wellington) has a temperate and relatively humid climate. These conditions are likely to favour indoor microbial growth possibly explaining the relatively high indoor levels of (1→3)-β-D-glucan and endotoxin. Alternatively, the differences might be explained by the fact that in the present study we sieved our dust samples whereas most of the European studies did not. However, when we compared our results with the only other study in Europe

Table 3. Indoor microbial contaminant levels in living room floor dust and mould, dampness and water damage.

	No			Yes			
	n	GM	GSD	n	GM	GSD	p-values
Mould spots in the past 12 months							
Glucan µg/g	26	2,657.1	1.6	6	2,824.3	1.5	0.76
Endotoxin EU/g	26	60,114.1	2.4	6	61,267.2	2.5	0.96
EPS U/g	26	35,101.5	1.8	6	48,825.1	2.1	0.26
Der p1 µg/g	24	29.9	3.3	6	52.8	1.9	0.27
Water damage in the house ever							
Glucan µg/g	18	2,397.1	1.4	14	3,111.9	1.7	0.09
Endotoxin EU/g	18	47,906.2	2.5	14	81,145.6	2.1	0.09
EPS U/g	18	35,066.4	2.1	14	40,457.2	1.6	0.54
Der p1 µg/g	17	25.4	3.2	13	48.2	2.6	0.12
Mould, dampness in living room (in past 12 months) or water damage in the house (ever)							
Glucan µg/g	13	2,340.2	1.4	19	2,951.3	1.6	0.14
Endotoxin EU/g	13	46,073.8	2.6	19	72,475.2	2.2	0.15
EPS U/g	13	32,435.2	2.1	19	41,109.7	1.7	0.31
Der p1 µg/g	13	22.2	3.7	17	46.0	2.3	0.07

that sieved the dust [1] we still found approximately 50% higher glucan concentrations in the present study (endotoxin data were not available), suggesting that the observed differences in microbial levels cannot be fully explained by differences in sample processing. Interestingly, a recent multicentre in farming children, Rudolf Steiner school children and reference children, also showed substantial differences in endotoxin and glucan (and to a lesser extent EPS-*Asp*/*Pen*) concentrations in house dust within Europe (shown only for mattress levels; [15]). The reasons for these differences are not clear since they were not related to differences in analytical methods (all samples were analysed by the same laboratory) or home characteristics [15].

The fact that EPS-*Asp*/*Pen* and (1→3)-β-D-glucan were significantly correlated, as previously shown in another small study in the Netherlands [1], suggests that these measures may indeed be valid markers of fungal exposure. In addition, these markers have previously been shown to be (weakly) correlated with culturable fungi in dust [1, 5, 10]. Levels per square meter were highly correlated, but this correlation was mainly driven by the amount of dust sampled, since, with exception of EPS-*Asp*/*Pen* and (1→3)-β-D-glucan, none of the correlations remained after adjusting for sample weight (table 2). Despite the significant correlation between EPS-*Asp*/*Pen* and (1→3)-β-D-glucan it cannot be excluded, however, that glucan as measured in our study also contained some non-fungal (1→3)-β-D-glucan from plant and/or bacterial sources.

Our preliminary finding that endoxin and (1→3)-β-D-glucan (and to a lesser extent EPS-*Asp*/*Pen*) in house dust was associated with indoor dampness/water damage problems is in agreement with previous studies [10, 14, 19], although other studies found no such association [4, 1]. Using self-reports of water damage and mould spots may have resulted in exposure misclassification and subsequent bias. Furthermore, we only collected 1 sample in each home, whereas temporal and spatial variation in indoor microbial levels may be substantial which may also have resulted in exposure misclassification. However, these types of non-differential misclassification would most likely have resulted in a bias towards the null and is therefore unlikely to explain our positive findings. The reason that microbial levels were more strongly associated with water damage than with the presence of mould spots may be because water damage was more broadly defined, relating to the whole house rather than only the living room (as was the case for “mould spots”). Alternatively, water damage may be a better indicator of microbial growth (including microbial growth in areas that were not immediately visible to the residents). Several previous studies [17, 18] have shown a positive association between Der p1 levels and indoor dampness similar to those reported here.

In conclusion, levels of microbial contaminants in a small random sample of New Zealand homes appeared to be high compared to previous international studies. In addition, microbial levels were associated with water damage (borderline statistically significant). Further studies to confirm these preliminary findings and to assess whether these microbial exposures are associated with the high prevalence of respiratory disease in New Zealand are needed, particularly since in recent years the focus has almost exclusively been on high mite allergen levels.

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