Can Increased Endotoxin Levels in House Dust be used as an Indicator of a Mould Problem?

Helena Mussalo-Rauhamaa[1,2,*], Peter Elg[3], Lea Paloheimo[3] and Pirkko Koukila-Kähkölä[4]

[1]*Department of Dermatology, Allergology and Venerial Diseases, Helsinki University Central Hospital, PO Box 160, FI-00029 Helsinki, Finland*

[2]*Institute Clinicum, Department of Public Health, University of Helsinki, Finland*

[3]*HUCH-Laboratorydiagnostics, Helsinki University Central Hospital*

[4]*Mycology Laboratory, Helsinki University Central Hospital*

Abstract: Endotoxins are common indoor biocontaminants. Our purpose was to investigate the association of endotoxin content with mould problems in house dust in buildings, to determine whether endotoxins can be used as an indicator of the presence of moulds in buildings. The participants were selected from the Clinic for Indoor Air Health Problems on the basis of symptoms attributed to indoor air problems. We examined their health status. House dust was collected in new vacuum cleaner bags from surfaces in the rooms of their homes or workplaces. We measured the endotoxin concentrations in the house dust of 13 homes and 9 workplaces of the 19 participants, using a quantitative limulus test. The median endotoxin concentrations in the house dust of their homes was 9.9, and the range was 1.9–103 EU/mg; and in the house dust of workplaces it was 4.5, and the range 0.5–81 EU/mg. We found higher house dust endotoxin concentrations in rooms in which moisture problems had been identified or in dwellings in which pets were kept. Our pilot study suggests that endotoxins may be a useful additional tool for estimating possible mould contamination at homes or workplaces.

Keywords: Mould, pets, endotoxin, health.

1. INTRODUCTION

Endotoxins are composed of lipopolysaccharides (LPS) and are a non-allergenic cell wall component of Gram-negative bacteria, showing strong pro-inflammatory properties. They may initiate a proinflammatory response from innate immunity [1]. Their toxicity is related to the chemical structure of the lipid A portion of LPS, which varies among bacterial species. Endotoxins are common indoor bio-contaminants. Homes contain low but measurable concentrations of endotoxins, and their concentrations are linked to conditions such as the presence of animals, smoking, crowding, and farm life [2]. Endotoxins are especially found in farm buildings, for example, in hog houses [3-5].

For this study we used an endotoxin assay reported in the literature as being able to detect cell components of Gram-negative bacteria in house dust. Our purpose was to investigate the association of endotoxin content with mould problems in buildings to determine whether endotoxins can be used to indicate the presence of moulds in buildings.

*Address correspondence to this author at the Institute Clinicum, Department of Public Health, University of Helsinki, Finland; Tel: +358505476795;
E-mail: helena.mussalo-rauhamaa@helsinki.fi

2. PARTICIPANTS AND METHODS

We selected our participants from the Clinic for Indoor Air Health Problems, from the Departments of Dermatology, Allergology and Venerial Diseases at the University Central Hospital, on the basis of symptoms attributed to indoor air problems. A trained environmental inspector from the clinic visited the homes of these participants and collected material samples from water-damaged structures and surfaces. The Mycological Laboratory of Helsinki University Central Hospital isolated and identified various mould genera from these samples. Certificates issued by indoor air inspection companies were also obtained from the water-damaged workplaces.

We selected 19 participants for the study. Of these adult participants, 15 were female and 4 male. We found mould problems in the homes or workplaces of seven participants. The commonly found moulds in the moisture-damaged homes and workplaces were *Penicillium spp, Aspergillus ustus, Aspergillus versicolor, Aspergillus sydowii, Stachybotrys* atra, *Chaetomium* globosum, *Acremonium* sp, *Ulocladium* sp, *Peacilomyces variotii*, *Trichoderma viride*, *Mucor racemosus.* In addition, the samples often contained *Rhodotorula rubra yeast* and *Streptomyces* bacteria.

We collected house dust in new vacuum cleaner bags from the surfaces in the rooms of the homes or

E-ISSN: 2312-5470/18

Table 1: Endotoxins in House-Dust

Sampling Place /Participant no.	Number of dust Samples	Mould Problem	Smoker	Pets	Endotoxin Concentration EU/mg Dust	Endotoxin Concentration EU/mg Dust, Range
Home/ 1	2	yes	no	no	11.5	9.4–13.5
Home/ 2	2	no	no	no	9.4	9.3–9.5
Home/ 3	2	no (a	yes	no	6.5	6.5
Home/ 4	2	yes	no	no (b	103.1	81.3–125
Home/ 5	1	no	no	dogs	78.8	
Home/ 6	1	no	no	cats	23.4	
Home/ 7	3	no	no	dog	2.9	1.5–3.8
Home/ 8	1	no	no	no	1.9	
Home/ 9	1	yes	no	no	6.3	
Home/ 10	1	no	no	no	8.8	
Home/ 11	1	yes	no (c	dog	74.4	
Home/ 12	1	yes	no	no	12.8	
Home/ 13	1	yes	no	no	9.9	
Workplace/ 1	1	yes	*	no	45.0	
Workplace/ 4	1	yes	*	no	81	
Workplace/ 5	1	no	*	no	12.5	
Workplace/ 14	1	yes	*	no	4.5	
Workplace/ 15	3	no	*	no	4.3	3.3–5.0
Workplace/ 16	1	no	*	no	8.8	
Workplace/ 17	1	no	*	no	1.0	
Workplace/ 18	1	no	*	no	0.5	
Workplace/ 19	1	no	*	no	1.5	

a) Earlier mould problem, repaired,

b) Earlier dog owner,

c) Ex-smoker,

* Smoking is forbidden in enclosed workplaces in Finland.

workplaces. This dust was emptied into clean polyvinylchloride (PVC) bags and kept frozen until analyses. After thawing, the dust was sieved through a 200-μm steel filter to remove sand and large particles.

3. ENDOTOXIN ASSAY

We extracted fine dust using a physiological NaCl (0.9%) phosphate buffer (pH 7.4) at a concentration of 5 g/l. The eluted endotoxin was assayed with a quantitative Limulus test (Limulus Amebocyte Lysate QCL-1000 Quantitative Chromogenic LAL method no. 50-650 U; Bio Whittaker, USA) at 37°C, as described [6]. The *Escherichia coli* O55:B5 endotoxin (Bio Whittaker) was used as the standard endotoxin. We analysed serially diluted samples in duplicate in two independent test runs. The endotoxin concentrations were expressed per gram of house dust. The coefficient of variation (CV) for the method was 6.6%. Nearly half of the participants brought several samples from their homes and/or workplaces; the endotoxin contents of these parallel dust samples varied only slightly (Table **1**).

4. HEALTH EXAMINATION

The general health of the participants of this study was examined by the staff physician (HMR). The clinical assessment included eliciting medical history and current symptoms, a physical examination, pulmonary function tests, chest and maxillary sinus radiographs, a complete blood cell count, skin prick tests to common allergens and moulds, sputum tests (concentration of eosinophil cationic protein (ECP) and myeloperoxidase (MPO), as an indicator of an inflammation process in the mucous membranes) and

determinations of IgG and IgE antibodies to moulds. In some cases, we also performed examinations such as high-resolution computer tomography, flexible bronchoscopy and/or diffusing capacity determination.

4.1. Statistics

We calculated the results using the IBM SPSS Statistic for Windows (IBM Corporation, USA). They are represented as means, and we tested the significance of the differences between the means using the non-parametric Mann-Whitney U-test. Alpha levels of less than 0.05 were considered statistically significant.

4.2. Results

The median and mean concentrations of endotoxins in house dust in the participants' homes were 9.9 and 26.9 EU/mg, respectively. The range was 1.9-103 EU/mg (Table **1**). At the workplaces, the corresponding median and mean were 4.5 and 17.7 EU/mg, range 0.5-81 EU/mg (Mann-Whitney test $p > 0.05$). Higher endotoxin concentrations in house dust were found in the sampling dwellings in which mould problems were identified (mean 38.7, median 12.8 (range 4.5-103) EU/mg vs. not identified 12.3 and 6.5 (range 0.5-78.8) EU/ mg, Mann-Witney U-test, $p = 0.02$. We found no statistically significant difference between the endotoxin concentrations of dwellings with and without pets.

4.3. Health Effects

Most of the participants whose houses we examined reported eye and nose irritation, shortness of breath, and coughing (Table **2**). Only one person experienced a pulmonary disease such as allergic alveolitis (hypersensitivity pneumonitis) or ODTS (organic dust toxic syndrome), i.e., 'flu-like' episodes with chills, fever, malaise, dyspnoea, coughing and chest tightness. Asthma was diagnosed in three cases, but nearly all the participants often had asthma-like symptoms. Shortness of breath was slightly prevalent even among those whose homes or workplace environment contained more than 20 EU/mg of endotoxins.

Increased MPO concentrations were found in the sputum of 75% of the participants (reference value < 1200 μg/L), whereas the ECP levels were within the reference value (< 2500 μg/L) in all but two samples. ECP is released from eosinophilic as well as neutrophilic white cells during inflammation [7]. We have previously shown that high MPO contents in sputum may also suggest exposure to indoor air pollutants [8].

5. DISCUSSION

Douwes *et al*. [6] showed that the endotoxin levels in Central European houses were 4.2–48.6 EU/mg in living rooms and 1.2-19.5 EU/mg in bedrooms, comparable to the levels found in our study. The geometric mean of the living room floor content of 454 German homes with children aged 5-10 years were reported to be higher, averaging at 1973 ng/g dust (1807 $\mu g/m^2$) [9]. Having a dog or a cat at home significantly increased exposure to endotoxins, whereas no major statistically significant association was found for other animals such as ants and mice. In all these studies, endotoxins were assayed using the LAL method also used in the present study. Elevated endotoxin concentrations in house dust were also found in the present study in homes in which pets (dogs or cats) were kept.

Table 2: Occurrence of Illnesses and Participants' Symptoms

Symptoms/ illnesses	All (N= 19) participants %	Participants (N= 6) endotoxin > 20 EU/mg %
Flu	65	33
Sinusitis	24	17
Headache	83	65
Eye irritation	91	83
Nasal irritation	75	67
Coughing	65	67
Skin symptoms	47	50
Wheezing	35	33
Shortness of breath	77	83

Endotoxins are also released from cigarettes during smoking [10]. Finns very seldom smoke at home; if they are smokers they generally smoke on balconies or near open windows. Thus, we suggest that smoking did not bias the result of our study.

To examine stability over time and seasonal variation, measured concentrations of mite and cat allergens, endotoxins and mould spores in the living room floor dust of 745 German homes were collected biannually during two different seasons [11]. The median interval between the two dust samplings was approximately seven months. Endotoxins were measured in settled house dust using the LAL method, and total spore counts using cultural methods. Crude Pearson's correlation coefficients between log-transformed concentrations in the first and second dust samples ranged between 0.59 for endotoxins and only 0.06 for total spore counts. Heinrich *et al*. [11] concluded that although repeated measurements of endotoxins in settled house dust improve the estimate of annual mean concentrations, even a single observation of these biocontaminants may be a good proxy for an exposure, as the repeated measures correlated highly. We observed only a single case of endotoxins in this study, and the endotoxin contents of the parallel dust samples varied only slightly.

Endotoxin concentrations in house dust have shown to remain constant over a storage period of one year at room temperature and at -20^{o}C [12]. High endotoxin concentrations in settled dust may be an indicator of deficient hygienic conditions in homes [13]. This was also reported by Ownby *et al*. [14], who found that cats and dogs are not the major endotoxin source in homes, but that the more dominant factors are the density of human occupation and poor cleaning.

The results of the Gehring *et al*. [15], Huang *et al*. [16] and Bischof *et al*. [13] suggest that mould growth may be associated with an increased endotoxin level. Huang *et al*. [16] found that the mean endotoxin concentration was 773 ng/mg dust in homes with prior water damage, and 25.4 ng/mg dust in homes without prior water damage.

Our participants suffered from shortage of breath, which is commonly connected to endotoxin exposure [17]. A limitation of this study is that the number of participants was too low to draw any conclusions regarding health status or illnesses connected to endotoxin exposure. Severe symptoms and illnesses were associated in our study with mould exposure [18-20].

Michel *et al*. [21] showed that in comparison to saline, inhaling a purified derivative of LPS induces a significant rise in both sputum MPO and ECP. The response was dose-dependent, and the no-response threshold for an acute inhalation of LPS was < 0.5μg. This may be one explanation for the increased levels of MPO observed in the sputum of our participants.

From the early 2000s, the relationship between exposure to bacterial endotoxins and several illnesses such as asthma and atopy has been widely studied to understand the origins of these illnesses. Endotoxins are a protective factor against asthma in children [22-24], but contradictory results have also been reported. Inverse associations between domestic exposure to endotoxins, and doctor-diagnosed asthma were found among German, but not among Dutch school children [25]. Among inner-city children with asthma who were exposed to high levels of airborne endotoxins at school, endotoxin exposure resulted in increased asthma symptoms among children with non-atopic asthma [26-27].

Among adults, for example, among an occupational cohort of laboratory animal workers, the atopic workers seemed to be more susceptible to, and non-atopic workers protected from, endotoxin-associated upper and lower respiratory symptoms [28]. In the US farming population, house dust endotoxins were associated with current atopic and non-atopic asthma. The degree of association with lower pulmonary function depended on early-life exposure [29]. In a study of a US nationwide representative sample, higher endotoxin exposure was significantly associated with measures of wheezing, but no protective effects were observed, regardless of sensitization status [30].

Protective effects have also been reported among adults: a European study supported the potentially protective effect of Gram-positive bacteria and endotoxin exposure [30]. Douwes *et al*. [31] concluded that endotoxin exposure may prevent the primary causation of allergic asthma, but that it may be both a primary and secondary cause of non-allergic asthma.

The WHO review concluded in 2009 that there is sufficient evidence of an association between moisture damage, mould and dampness in dwellings and the development of asthma among children [32]. A subsequent extensive review on asthma development among children and adults agreed with this conclusion [20]. The effect of moulds as a confounding factor, however, has rarely been investigated in studies on endotoxins.

CONCLUSION

Based on the results of the present study, we suggest that endotoxins may be a useful additional tool for estimating possible mould contamination in homes without pets, or at workplaces.

REFERENCES

[1] Zielen S, Trischler J and Schubert R. Lipopolysaccharide challenge: immunological effects and safety in human. Expert Rev Clin Immunol 2015; 11: 409-418. https://doi.org/10.1586/1744666X.2015.1012158

[2] Rennie DC, Lawson JA, Senthilselvan A, Wilson PJ and Dosman JA. Domestic entoxin exposure and asthma in children: epidemiological studies. Front. Biosci. /Elite ED) 2012; 1: 56-73. https://doi.org/10.2741/e360

[3] Duchaine C, Grimard Y and Cormier Y. Influence of building maintenance, environmental factors, and seasons on airborne contaminations of swine confinement buildings. AIHAJ 2000; 61: 56-63. https://doi.org/10.1202/0002-8894(2000)061<0056:IOBMEF>2.0.CO;2

[4] Hyvärinen A, Roponen M, Tiittanen P, Laitinen S, Nevalainen A and Pekkanen J. Dust sampling methods for endotoxin – an essential, but underestimated issue. Indoor Air 2006; 16: 20-27. https://doi.org/10.1111/j.1600-0668.2005.00392.x

[5] Barnig C, Reboux G, Roussel S, Casset A, Sohy C, Dalphin JC, deBlay F. Indoor dust and air concentrations of endotoxin in urban and rural environments. Lett Appl Microbiol 2013; 56: 161-167. https://doi.org/10.1111/lam.12024

[6] Douwes J, Doekes G, Heinrich J, Koch A, Bischof W and Brunekreef B. Endotoxin and (β1→3)-glucan in house dust and the relation with home characteristics: A pilot study in 25 German houses. Indoor Air 1998; 255-263. https://doi.org/10.1111/j.1600-0668.1998.00006.x

[7] Venge P, Byström L, Håkansson L and Moberg L. Eosinophil cationic protein (ECP) is taken up by and stirred in neutrophil granulocytes. Allergy Clin Immunol. Int 1997; Suppl 4:116.

[8] Mussalo-Rauhamaa H, Hakala K, Kiviranta K, Metso T and Haahtela T. Increased myeloperoxidase (MPO) in induced sputum in patients with a history of indoor air health problems. In: Proceedings of Healthy Buildings 2000, Exposure, Human Responses and Building Investigations, vol. 1., Eds. O. Seppänen, J. Säteri, SIY Indoor Air, Information Oy, Helsinki, Finland/ Gummerus Kirjapaino Oy, Jyväskylä, pp. 263- 266.

[9] Heinrich J, Gehring U, Douvest J, Koch A, Fahlbusch B, *et al*. Pets and vermin are associated with high endotoxin levels in house dust. Clin Exp Allergy 2001; 31: 1839-1845. https://doi.org/10.1046/j.1365-2222.2001.01220.x

[10] Larsson L, Szponar B, Pehrson C. Tobacco smoking increases dramatically air concentrations of endotoxin. Indoor Air 2004; 14: 421-424. https://doi.org/10.1111/j.1600-0668.2004.00290.x

[11] Heinrich J, Holscher B, Douwes J, Richter K, Koch A, *et al*. INGA Study Group. Reproducibility of allergen, endotoxin and fungi measurements in the indoor environment. J Exp Anal Environ Epidemiol 2003; 13: 152-60. https://doi.org/10.1038/sj.jea.7500267

[12] Koch A, Heilemann KJ and Bischof W. Storage stability of viable mould spores and endotoxin in house dust samples. Proceedings of the Indoor Air, Monterey, California 2002; 46-51.

[13] Bischof W, Kock A, Gehring U, Fahlbusch B and Heinrich J. Predictors of high endotoxin concentrations in the settled dust of German homes. Indoor Air 2002; 12: 2-9. https://doi.org/10.1034/j.1600-0668.2002.120102.x

[14] Ownby DR, Peterson EL, Wegienka G, Woodecroft KJ, Nicholas C, *et al*. Are cats and dogs the major source of endotoxin in homes? Indoor Air 2013; 23: 219-226. https://doi.org/10.1111/ina.12016

[15] Gehring U, Douwes J, Diekes G, Koch A, Bischof W, *et al*. INGA Study Group. Indoor Factors and Genetics in Asthma. Beta (1-3)-glucan in house dust of German homes: housing characteristics, occupant behaviour, and relations with endotoxins. Allergens and molds. Environ Health Perspect 2001; 109: 139-144.

[16] Huang YJ, Huang JY, Lin LL, Wu PC, Ma YP and Su HJ. Environmental endotoxin concentraions are associated with home characteristics. In: Proceedings of the Indoor Air 2002, Monterey, California, p. 764-769.

[17] Rylander R. Endotoxin in the environment - exposure and effects. J Endotoxin Res 2002; 8: 241-252. https://doi.org/10.1179/096805102125000452

[18] Bornehag CG, Blomquist G, Gyntelberg F, Jarvholm B, Malmberg P, *et al*. Dampness in buildings and health. Nordic interdisciplinary review of the scientific evidence on association between exposure to 'dampness' in buildings and health effects. Indoor Air 2001; 11: 72-86. https://doi.org/10.1034/j.1600-0668.2001.110202.x

[19] Mussalo-Rauhamaa H, Nikulin M, Koukila-Kähkölä P, Hintikka EL, Malmberg M and Haahtela T. Health effects of residents exposed to Stachybotrys in water-damaged houses in Finland. Indoor and Built Environment 2010; 19: 476-485. https://doi.org/10.1177/1420326X10367290

[20] Mendell MJ, Mirer AG, Cheung K, Tong M and Douwes J. Respiratory and allergic health effects of dampness, mold, and dampness-related agents: a review of the epidemiologic evidence. Environ Health Perspect 2011; 119: 748-756. https://doi.org/10.1289/ehp.1002410

[21] Michel O, Nagy AM, Schroven M, Duchateau J, Neve J, *et al*. Dose-response relationship to inhaled endotoxin in normal subjects. Am J Respir Crit Care Med 1997; 156: 1157-1164. https://doi.org/10.1164/ajrccm.156.4.97-02002

[22] Von Mutius E and Radon K. Living on a farm: impact on asthma induction and clinical course. Immunol Allergy Clin North Am 2008; 28: 631-634. https://doi.org/10.1016/j.iac.2008.03.010

[23] Mendy A, Gasana J, Vieira ER, Forno E, Patel J, *et al*. Endotoxin exposiure and childhood wheeze and asthma: a meta-analaysis of observational srudies. J Asthma 2011; 48: 685-693. https://doi.org/10.3109/02770903.2011.594140

[24] Carlsten C, Ferguson A, Dimich-Ward H, Chan H, DyBuncio A, *et al*. Association between endotoxin and mite allergen exposure with asthma and specific sensitization at age 7 in high-risk children. Pediatr Allergy Immunol 2011; 22: 320-325. https://doi.org/10.1111/j.1399-3038.2010.01123.x

[25] Thischer C, Gehring U, Chen CM, Kerkhof M, Koppelman G, *et al*. Respiratory health in children , and indoor exposure to (1,3)- β--D-glucan, EPS mould components and endotoxin. Eur Respir J 2011; 37: 1050-1059. https://doi.org/10.1183/09031936.00091210

[26] Lai PS, Sheehan WJ, Gaffin JM, Petty CR, Coull BA, *et al*. School endotoxin exposure and asthma morbidity in inner-city children. Chest 2015; 148: 1251-1258. https://doi.org/10.1378/chest.15-0098

[27] Jacobs JH, Krop EJ, de Wind S, Spithoven J and Heederik DJ. Endotoxin levels in homes and classrooms of Dutch school children and respiratory health. Eur Respir J 2013; 42: 314-322. https://doi.org/10.1183/09031936.00084612

[28] Newton AN, Davis M, Koehler K, Shreffler W, Ahluwalia S, *et al*. Atopy as a modifier of the relationships between endotoxin exposure and symptoms among laboratory animal workers. Work Expo Health 2017; 61: 1024-1028. https://doi.org/10.1093/annweh/wxx061

[29] Thorne PS, Mendy A, Metwali N, Salo P, Jaramillo R, *et al*. Endotoxin exposure: predictors and prevalence of associated asthma outcomes in the United States. Am J Respir Crit Care Med 2015; 1287-1297. https://doi.org/10.1164/rccm.201502-0251OC

[30] Carnes MU, Hoppin JA, Metwali N, Wyss AB, Hankinson JL, *et al*. House dust endotoxin levels are associated with adult asthma in a U.S. Farming population. Ann Am Thorac Soc 2017; 14: 324-331. https://doi.org/10.1513/AnnalsATS.201611-861OC

[31] Douwes J, Pearce N and Heederik D. Does environmental endotoxin exposure prevent asthma? Thorav 2002; 57: 86-90. https://doi.org/10.1136/thorax.57.1.86

[32] World Health Organisation. WHO guidelines for indoor air quality: dampness and mould. WHO Regional Office for Europe 2009; 228 p.

Received on 26-02-2018 Accepted on 28-03-2018 Published on 30-06-2018

DOI: https://doi.org/10.12974/2312-5470.2018.05.1