



Endotoxin and indoor allergen levels in kindergartens and daycare centres in Wellington, New Zealand

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Abstract

Aims A large majority of children in New Zealand attend daycare centres and kindergartens early in life. Overseas studies have demonstrated a possible protective effect of daycare attendance against asthma and allergy later in life. One hypothesised agent for this protection is high levels of endotoxin, which have not previously been measured in New Zealand childcare facilities. The purpose of this study was to measure endotoxin and indoor allergens in kindergartens and daycare centres in the Wellington region.

Methods Dust samples were collected from 18 kindergartens and 18 daycare centres and analysed for endotoxin by the kinetic limulus amebocyte lysate assay and for indoor allergens by double monoclonal/polyclonal antibody ELISA.

Results The geometric mean level (95% CI) was 29,206 EU/g (19,410–43,950) for endotoxin, 0.25 µg/g (0.04–2.28) for *Der p 1*, 1.24 µg/g (0.80–1.90) for *Fel d 1*, 0.43 µg/g (0.26–0.71) for *Can f 1*, and 0.028 µg/g (0.020–0.039) for *Bla g 2*.

Conclusions Endotoxin levels in daycare centres and kindergartens in Wellington, New Zealand are similar to domestic dwellings in Wellington, however indoor allergen levels are much lower. The low indoor allergens in the daycare centres and kindergartens are unlikely to be problematic for sensitised infants, although some individual childcare facilities had very high *Der p 1* levels.

In New Zealand, a large majority of children attend daycare centres and kindergartens early in life. New Zealand statistics in 1996 show that 93.4% of 4-year-old children are involved in some form of early education.¹ This is often due to the work commitments of the parents or guardians of the children.

Recent studies have shown that children who attend daycare centres may be protected from asthma and atopy later in life.^{2,3} Celédon et al followed 453 children from birth to 6 years and found that daycare attendance decreased the prevalence of wheezing at age 6 years.² This was only associated with daycare in the first 6 months of life. If the child had a maternal history of asthma, no significant protection was found. The authors hypothesised those infants in daycare had an increased exposure to infectious illnesses, thus leading to a decreased incidence of atopy and asthma later in life.

In Tucson, USA, Ball et al followed 1035 children and studied the relationship between asthma and daycare attendance.³ They found that children who attended daycare in the first 6 months of life had a decreased prevalence of wheeze at age 6 years, especially those with an increased number of older siblings. This study elicited an important finding that, at age 2, there were many children who wheeze when attending daycare centres. However, this was not always because of asthma. Instead it

was attributed to the many infectious respiratory infections that children of that age are exposed to.

One hypothesised protective agent that is thought to cause a decrease in atopy and asthma if exposed to it early in life is endotoxin. Endotoxin is a lipopolysaccharide that is found in the cell walls of Gram-negative bacteria and is able to modulate the immune system strongly,⁴ favouring the Th1 immune response that produces cytokines IL-12 and IF- γ . These cytokines, in turn, down-regulate the Th2 immune response that is thought to be responsible for asthma and atopy. This concept (the hygiene hypothesis) proposes that the increase in allergic disease is a result of decreased exposure to microbes in the environment.⁵

To our knowledge there has only been one study that examined levels of endotoxin in childcare centres and schools. A study in San Paolo, Brazil found that daycare centres and preschools had higher levels of endotoxin in comparison to elementary schools as well as all these sites studied having a significantly greater amount of endotoxin than local homes.⁶

Whilst early childhood exposure to high levels of endotoxin is thought to be protective against the later development of atopy and asthma in children, high levels of indoor allergens, such as those from house dust mites and animals, when found together with high levels of endotoxin, are able to exacerbate asthma symptoms.^{7,8} It is therefore important to examine levels of these indoor biocontaminants in the daycare environment as high levels may exacerbate symptoms in sensitised children.

As levels of endotoxin in New Zealand daycare centres or kindergartens have not previously been determined—and there has been only one study that examined levels of Der p 1 in childcare centres in New Zealand,⁹ but not of other indoor allergens—we measured levels of endotoxin and *Der p 1* (house dust mite), *Fel d 1* (cat), *Can f 1* (dog), and *Bla g 2* (cockroach) indoor allergens in daycare centres and kindergartens in the Wellington region and compared these to previously measured levels in Wellington homes.

We hypothesised that there would be higher levels of endotoxin in daycare centres, due to the increased amount of nappy changing in children under the age of 3—as was originally proposed by Russo et al.⁶

Methods

Twenty kindergartens identified in the Wellington city region were contacted by phone and asked to participate in the study, which involved a visit from the researcher with collection of dust samples and answering a simple questionnaire. Eighteen of the kindergartens contacted agreed to participate. Due to the high numbers of daycare centres in the region (120), 36 were randomly selected through random number generation and then contacted by phone. Eighteen of these agreed to participate in the study. The age range of children at the kindergartens was 3–5 years and the age range for children at daycare centres was 0–6 years.

At the visit, the head teacher or manager was asked a series of questions about the building (building characteristics, floor coverings, cleaning habits, heating type, damp and mould, bedding and location, shoes and pets) and its population (number of children enrolled, age-range, adults present, and number of children less than 3 years old).

Dust samples were collected from both high-use areas and sleeping areas. On floors covered with mats or carpets, a 1 m² area was selected and dust collected with an 1100W Hitachi vacuum cleaner (Hitachi Ltd., Singapore) for 1 minute in a nylon dust sock placed over the vacuum cleaner head.

For smooth floors, a 2 m² area was selected and vacuumed for 2 minutes. Dust samples were sifted through a 425 µm steel mesh sieve; total dust weight recorded, and stored at –20°C until analysis. For endotoxin levels, 200 mg of sifted dust was extracted with 5 ml of endotoxin-free water containing 0.05% Tween 20, shaken for 30 minutes at 250 rpm at room temperature and then centrifuged for 10 minutes at 1000g.

Aliquots of supernatants were stored in endotoxin-free glass tubes at –20° until analysis. Endotoxin activity was measured by a kinetic amebocyte lysate assay on 1:500 dilutions of the supernatants (BioWhittaker Inc., Walkersville, MD, USA) and analysed by four-parameter curve fitting.

For indoor allergen levels, 100 mg of sifted dust was extracted with 1 ml of phosphate-buffered saline for 30 minutes at room temperature, centrifuged for 10 minutes at 3,000g and supernatants stored at –4°C. *Der p 1* and *Fel d 1* were measured by double monoclonal ELISAs, and *Can f 1* and *Bla g 2* by monoclonal/polyclonal antibody ELISA (Indoor Biotechnologies, Cardiff, UK).

Our research group has used these analytical methods for the last decade and between-batch precision for endotoxin and allergens are about 20% and 10% respectively.^{10,11}

As endotoxin and indoor allergen data were skewed, they were log-transformed and results expressed as geometric means with 95% CI as in previous studies from our research group.⁹⁻¹¹ Differences between types of childcare centres and measured parameters were analysed by two-tailed Student t-test. Statistical significance was set at the p<0.05 level. Due to the relatively small study size we did not conduct multivariate analyses.

The Central Regional Ethics Committee approved the study and informed written consent was obtained from all participating childcare centres.

Results

On average there were 39 (range 25–45) children per session in kindergartens, and 25 (range 16–50) children per session in daycare centres. In daycare centres there were on average 12 (range 2–23) children per session under the age of 3 years.

Of the buildings visited, 35 were wooden and 1 was brick; 35 centres had carpet or mats on their floor, while 1 had a wooden floor. There were 9 synthetic carpets, 16 wool carpets, and 6 mixed carpets (carpet type was unknown in 3 centres).

Twenty-six centres had pets that were kept in cages, and 25 reported that they commonly had cats coming onto the property. All centres were cleaned daily. This involved mopping and vacuuming the entire area used by the children.

All 60 dust samples collected were analysed for endotoxin and 57 samples were analysed for indoor allergens (3 samples had sufficient dust only for endotoxin determination). Table 1 shows the results for endotoxin and indoor allergen levels.

Table 1. Endotoxin and indoor allergens in kindergartens and daycare centres in the Wellington region (n=36)

Allergen	Geometric mean	Range of values	95% CI
<i>Der p 1</i> µg/g	0.25	0.01–103.8	0.04–2.28
<i>Fel d 1</i> µg/g	1.24	0.06–13.9	0.80–1.90
<i>Can f 1</i> µg/g	0.43	0.01–4.09	0.26–0.71
<i>Bla g 2</i> µg/g	0.028	0.006–0.203	0.020–0.039
Endotoxin EU/g	29,206	2364–1,398,709	19,410–43,950

There was no significant difference in endotoxin levels between kindergartens and the other daycare centres (p=0.38), nor for *Fel d 1* (p=0.35), *Can f 1* (p=0.51), or *Bla g 2*

($p=0.78$). However, there were significant differences in *Der p 1* with daycare centres having higher *Der p 1* levels than kindergartens; geometric means were $0.30 \mu\text{g/g}$ (95% CI: 0.09–2.33) and $0.03 \mu\text{g/g}$ (95% CI: 0.008–0.22) respectively; $p=0.0055$).

Discussion

This study found that levels of endotoxin in kindergartens and daycare centres are of similar levels to those found in Wellington homes.¹⁰ Wickens et al studied 77 Wellington homes in New Zealand where the geometric mean floor endotoxin was 22,700 EU/g, comparable with a geometric mean of 29,206 EU/g in this study.

Our results differ from Brazil, where the endotoxin levels (EU/g) in daycare centres and preschools were three-fold higher than in local homes.⁶ However, this comparison with Brazil must be treated with caution as extraction and analytical methods were different from the current study and may also be due to batch differences in LAL reagents.¹²

Endotoxin is thought to be protective for the development of atopy and asthma if children are exposed in the first year of life.⁴ This is one of the reasons why attendance at daycare centres may be protective against atopy and asthma. However, our study shows that there is little difference in endotoxin levels between daycare centres and kindergartens, and homes in New Zealand, unlike Brazil. Also, a previous study in New Zealand has shown that daycare attendance is not protective for the development of asthma.¹³ However, exposure to higher endotoxin levels than found in our study early in life could be protective against the development of asthma and atopy in childhood.

Exposure to high endotoxin in infancy leads to increased wheezing in childhood¹⁴ and also causes wheezing in house dust mite sensitised asthmatics.¹⁵ Thus, the levels of endotoxin in our study may be of relevance to wheezing in children attending kindergartens and child-care centres. Wheezing is a hallmark of asthma and New Zealand has a high prevalence of asthma.

Indoor allergens levels in kindergartens and daycare centres were much lower than in Wellington homes.¹¹ We have previously measured *Der p 1* from carpets in Wellington homes with a geometric mean of $25.5 \mu\text{g/g}$.¹¹ This is much higher than the levels found in kindergartens and daycare centres, where the levels were $0.03 \mu\text{g/g}$ and $0.30 \mu\text{g/g}$ respectively. These results are similar to those found in public places in New Zealand results.⁹ In that study, 17 childcare centres were included where the geometric mean of *Der p 1* was $0.22 \mu\text{g/g}$. In comparison, geometric *Der p 1* levels in daycare centres and preschools in Brazil were much higher at $2.6 \mu\text{g/g}$ and $6.3 \mu\text{g/g}$ respectively.⁶

It is important to note that in some of the daycare centres and kindergartens in our study there were high levels of *Der p 1*, with the highest recorded level of $103.8 \mu\text{g/g}$. *Der p 1* levels greater than $10 \mu\text{g/g}$ can exacerbate symptoms in atopic patients,⁸ thus in these childcare centres an increase in the occurrence of symptoms in house dust mite sensitised children may be expected.

The geometric mean of *Fel d 1* ($1.24 \mu\text{g/g}$) found in kindergartens and childcare centres is lower than the proposed exacerbation threshold of $8 \mu\text{g/g}$.⁸ In one study, levels found in 224 Wellington homes varied between those with or without cats

present.⁷ However, in those homes with a cat (40.8 µg/g) and those without (3.3 µg/g), *Fel d 1* levels were higher than in kindergartens and daycare centres.

In another study we found that *Fel d 1* levels in public places were lower than in homes, equal to those found in our study.¹⁶ Cat allergen is most likely transferred into places such as kindergartens on the clothes of children who own pets,¹⁷ but it also important to note that 25 of the centres in our study reported that they had cats straying onto the property, which may account for some of the higher levels found (highest: 13.9 µg/g).

The *Can f 1* and *Bla g 2* levels in kindergartens and daycare centres (geometric means: 0.43 µg/g and 0.028 µg/g respectively) are similar to levels found in a study of Tokelauan family homes in Wellington, New Zealand.¹⁸ In that study geometric mean levels of *Can f 1* and *Bla g 2* were 0.62 µg/g and 0.03 µg/g respectively.¹⁶

The low levels of indoor allergens found in the kindergartens and daycare centres may be attributed to the rigorous daily cleaning regime that the centres undergo. Each centre was mopped and vacuumed at least one a day, with some also being cleaned in the weekend. A previous study found that daily vacuuming for five weeks consecutively reduces *Der p 1* in carpet dust samples by about 60% and returns to initial levels when switched to weekly vacuuming.¹⁹

Study limitations include the small number of kindergarten and daycare centres in the study, which may not be representative of all childcare facilities in New Zealand. For instance, domestic *Der p 1* levels are about four-fold lower in Dunedin than in Wellington.²⁰ Also, only a small area was sampled for endotoxin and indoor allergens. However, we have previously shown that a central area is representative for the whole room for *Der p 1* and *Fel d 1*.²¹

In conclusion, daycare centres and kindergartens in the Wellington region have similar levels of endotoxin as homes in the region, and much lower levels of indoor allergens. However, due to similar levels of endotoxin in daycare centres and domestic dwellings in Wellington, it would be interesting to see if higher levels of endotoxin would be protective against the development of atopy and asthma later in life, as has been shown in overseas studies.

Another implication is that those children who are already sensitised to allergens before attending a daycare centre or kindergarten are unlikely to be at risk from exacerbation of symptoms, except in some centres where there were high levels of *Der p 1* and *Fel d 1*.

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