

Allergens and endotoxin in settled dust from day-care centers and schools in Oslo, Norway

Abstract Allergy to indoor allergens can cause frequent and severe health problems in children. Because little is known about the content of allergens in the indoor environments in Norway, we wanted to assess the levels of cat, dog and mite allergens in schools and day-care centers in Oslo. Allergen levels in dust samples from 155 classrooms and 81 day-care units were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits. Additionally, we measured the levels of endotoxin in 31 day-care units, using the limulus amoebocyte lysate test. Most of the dust samples contained detectable amounts of cat and dog allergens. In mattress and floor dust (day-care centers), and curtain and floor dust (schools) the median *Fel d 1* levels were 0.17, 0.002, 0.02 and 0.079 $\mu\text{g}/\text{m}^2$, while the median *Can f 1* levels were 1.7, 0.03, 0.1 and 0.69 $\mu\text{g}/\text{m}^2$, respectively. Levels of cat and dog allergens in school floor dust were associated with the number of pupils with animals at home. In contrast, <1% of the samples had measurable levels of the mite allergen *Der p 1*. Moreover, the levels of endotoxin tended to be higher in dust from floors (1.4 ng/m^2) compared with that from mattresses (0.9 ng/m^2).

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Practical Implications

To reduce allergen exposure, allergic individuals should be placed in the classes/rooms with the fewest pet owners. Moreover, mattresses in day-care centers are major reservoirs of cat and dog allergens and should be cleaned frequently.

Introduction

Allergy is a common disorder causing frequent and sometimes severe health problems in children. In addition to homes, schools and day-care centers are important indoor environments in which children may experience adverse health effects from exposure to allergens. This view is supported by findings from Sweden indicating that allergic children experience worsening of allergic symptoms after indirect exposure to cats at school (Almqvist et al., 2001). Moreover, it has been found that pupils without regular contact

with cats were twice as likely to test positive for cat allergen when the proportion of schoolmates with cat contact was high (Ritz et al., 2002). Allergens are most likely transported into day-care centers and schools on clothes by children with animals at home (D'Amato et al., 1997; De Lucca et al., 2000; Karlsson et al., 2004). A study from Sweden reported that levels of allergens in Swedish school dust were most likely high enough to cause asthmatic symptoms in children sensitized to cats and dogs (Munir et al., 1993). However, relatively little is known about exposure to allergens and other environmental factors in schools

and day-care centers in Norway. A study of 10 public schools in Norway showed that dust samples contained more dog allergen than dust in homes without dogs. A similar tendency was observed for levels of cat allergen as well (Dybendal and Elsayed, 1994).

Endotoxin is a component of the outer membrane of gram-negative bacteria and is ubiquitous in the environment. Exposure to endotoxin has been found to correlate with an increase in asthma symptoms (Michel et al., 1991; Rizzo et al., 1997). In contrast, endotoxin has also been found to protect against development of atopic eczema (Gehring et al., 2001) and allergic sensitization (Gehring et al., 2002, 2004). Endotoxin levels were higher in homes with pets than in those without pets (Douwes et al., 2000; Gereda et al., 2001). The levels of endotoxin in day-care centers and schools in Norway have not been examined previously. This further underscores the need for studying allergen and endotoxin exposure in school and day-care environments. The aim of the present study was to assess the allergen and endotoxin content in settled dust in day-care centers and schools in Oslo.

Materials and methods

Study population

School environment As part of a 10-year follow-up of the Oslo Birth Cohort (Nafstad et al., 1997), 155 classrooms for fourth-form pupils (born 1992) were inspected in the period March 5 to June 17, 2002 at 81 randomly selected schools in Oslo. For schools with more than two parallel fourth-form classes, two classrooms were chosen at random. Dust was sampled from floors and curtains by vacuum cleaning. Furthermore, children in each class were asked whether they kept pets at home and the number of children in the class was recorded.

Day-care environment Dust was collected from floor and mattresses in 81 units in 24 day-care centers owned by Oslo City authorities during the winter and spring (February–May) of 1999 and in the winter of 2000. The centers were in need of improved ventilation and had been included in a study of ventilation and health in day-care children.

Sampling and preparation of dust from day-care centers and schools in Oslo

A special dust collector from ALK Laboratories (Copenhagen, Denmark) with a Millipore filter (pore size 6 μm) was connected to a 500-W vacuum cleaner and used to sample settled dust. Dust sampled in the day-care centers was sieved by vacuuming through a filter (porosity of 300 μm) to collect the fine dust. Dust from the classroom floor was not sieved because it contained

too much sand to obtain the fine dust only. Moreover, samples from classroom curtains were unsieved. All dust samples were weighed and the allergens extracted by incubation in phosphate-buffered saline with 0.02% NaN_3 and agitation at room temperature overnight. Then they were centrifuged and the supernatants stored at -20°C until analysis. Dust was collected from smooth floors in playrooms and from mattresses in resting rooms (vacuuming of 2.25 m^2 for 4 min) in day-care centers. In the classrooms, dust samples were collected for 4 min from 1.5 m^2 of smooth floor [two corners (0.25 m^2) plus in the center (1 m^2)] and, separately from 2 m^2 of curtains [two curtains (1 m^2)].

Analyses

All samples were analysed for the major allergens from cat, *Felis domesticus* (Fel d 1), dog, *Canis familiaris* (Can f 1) and from mite, *Dermatophagoides pteronyssinus* (Der p 1) by using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Quantitative ELISA Kits; Indoor Biotechnologies, Charlottesville, VA, USA). The lower detection limits for Fel d 1, Can f 1, and Der p 1 assays differed between the individual ELISA plates dependent on the standard curve. Dust samples from eight of the day-care centers (31 units) were also analysed for endotoxin with the limulus amebocyte lysate test (Pyrogen Plus®; Bio Whittaker, Walkersville, MD, USA). Five dust samples were, in addition, analyzed for endotoxin with the chromogenic LAL assay (QCL-1000®; Bio Whittaker).

Statistical analysis

ANOVA on ranks was used to compare all groups, followed by a pair-wise Dunns' method to compare differences between groups. To correlate data, the Spearman's correlation test was used. Values obtained by ELISA below the detection limits were, for statistical purposes, set at 0.3 mU/ml for Fel d 1 (1.2 ng/ml) and 0.3 U/ml for Can f 1 (3.0 $\mu\text{g}/\text{ml}$) measurements before calculations. Statistical analyses were performed with SigmaStat® (Jandel Scientific, Erkrath, Germany). The limit for statistical significance was set at $P = 0.05$.

Results

Cat allergen (Fel d 1)

Cat allergen was detected in dust from floors and mattresses in 52% and 100% of the day-care centers, respectively. In the schools, 66% and 83% of the dust samples from the floors and curtains, respectively, contained detectable amounts of cat allergen. The levels of Fel d 1 in samples from smooth floors in schools were approximately 30 times higher than in samples from day-care centers ($P < 0.001$) (Figure 1). The median levels

($\mu\text{g/g}$) in mattresses and floors (day-care center) and curtains (school) were 1.5, 0.2 and 1.7, respectively.

Dog allergen (Can f 1)

In the schools, 87 and 84% of samples from curtains and floors, respectively, contained detectable levels of Can f 1. In the day-care centers 100% and 87% of samples from the mattresses and floors, respectively, contained detectable levels of Can f 1. The levels of Can f 1 are shown in Figure 2. The Can f 1 level in samples from smooth floors in schools was approximately 25 times higher than that in day-care centers ($P < 0.001$). The median levels ($\mu\text{g/g}$) in mattresses and floors (day-care centers) and curtains (schools) were 10.0, 1.7 and 7.0, respectively. Regardless of whether allergen levels are given as $\mu\text{g/m}^2$ or $\mu\text{g/g}$, the Can f 1 levels were five to 10 times higher than Fel d 1 levels in dust samples from all locations examined ($P < 0.001$). There was no relationship between the levels of allergen (Can f 1 or Fel d 1) in dust from floors and mattresses in day-care centers and between dust from floors and curtains in schools.

Mite allergen (Der p 1)

Mite allergen was only detectable in four dust samples from the same day-care center and in two samples from

two different schools. The highest value measured was 0.2 $\mu\text{g/g}$ dust.

Allergen content in classroom dust in relation to number of pet owners

In our study, 12.8% of the pupils had a cat and 11.4% had a dog at home. The number of cat owners relative to the Fel d 1 levels is shown in Figure 3. The number of dog owners relative to the Can f 1 levels is shown in Figure 4. There was a weak, but statistically significant correlation between the amount of allergen and the number of pupils having an animal at home ($\rho \sim 0.3$). Data on pet ownership in day-care centers are not available.

Endotoxin levels in dust samples from day-care centers

The level of endotoxin was measured in dust collected from floors and mattresses in eight day-care centers (31 units). The median endotoxin levels in samples from floors and mattresses were 1.4 ng/m^2 (50 ng/g , 0.5 EU/mg) and 0.9 ng/m^2 (5.0 ng/g , 0.05 EU/mg), respectively. There was no relationship between levels of cat or dog allergen and endotoxin (data not shown).

Discussion

The allergen concentration ($\mu\text{g/g}$) was negatively correlated ($\rho = -0.5$) with the weight of dust, whereas the

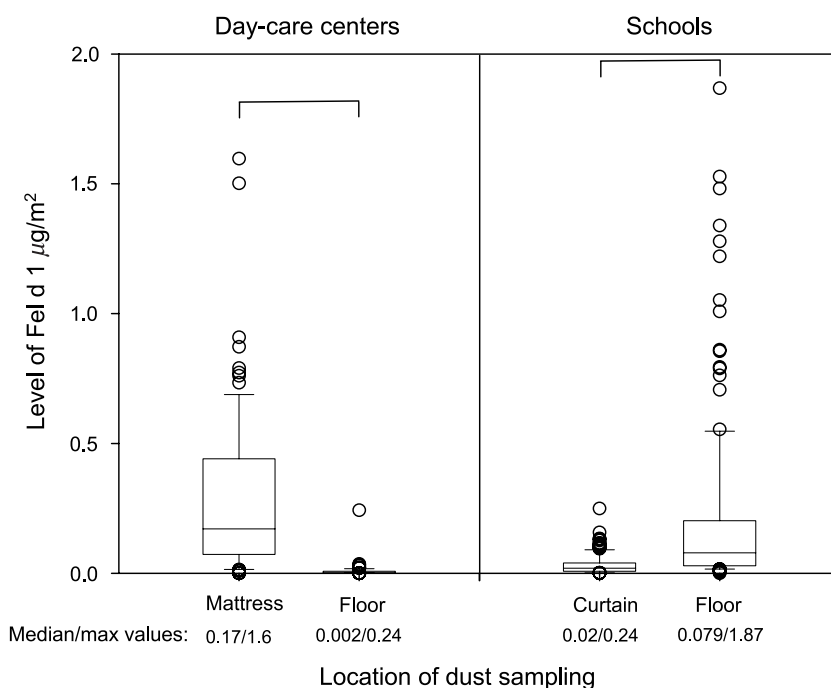


Fig. 1 Levels of Fel d 1 given as $\mu\text{g/m}^2$ measured in dust samples from mattresses and floors in 81 units in 24 day-care centers and in samples from curtains and floors in 155 classrooms in 81 schools in Oslo, Norway. Boxes comprise the 25th and 75th percentiles and the median (horizontal line). Vertical bars show the 10th and 90th percentiles, and circles represent values outside of the given percentiles. The brackets indicate a statistically significant difference between groups ($P < 0.001$). The detection limits were set according to the standard curve for each ELISA plate. The lower detection limits varied between 0.0004 and 0.0047 $\mu\text{g/m}^2$

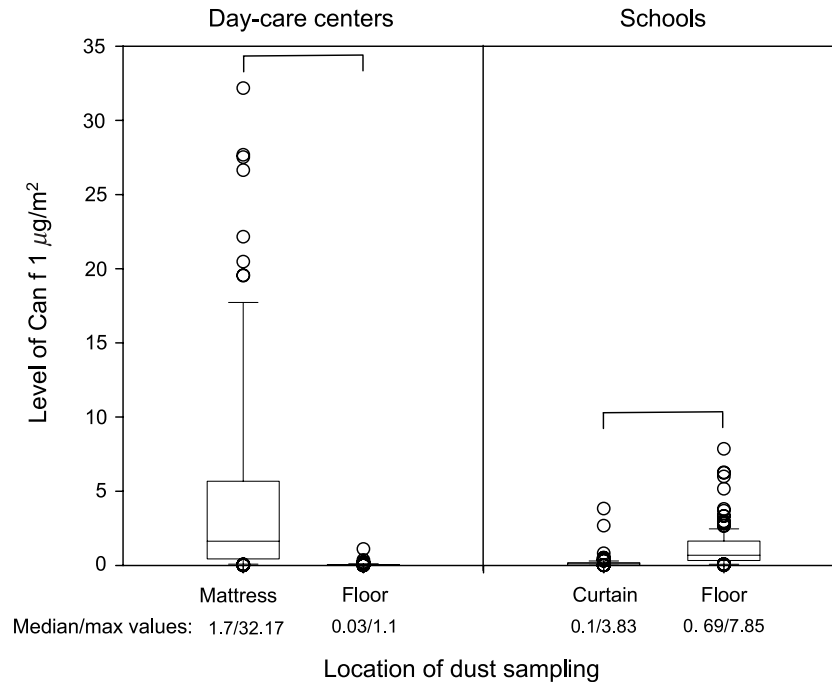


Fig. 2 Levels of Can f 1 given as $\mu\text{g}/\text{m}^2$ measured in dust samples from mattresses and floors in 81 units in 24 day-care centers and in curtains and floors in 155 classrooms in 81 schools in Oslo, Norway. Boxes comprise the 25th and 75th percentiles and the median (horizontal line). Vertical bars show the 10th and 90th percentiles, and circles represent values outside of the given percentiles. The brackets indicate a statistically significant difference between groups ($P < 0.001$). The detection limits were set according to the standard curve for each ELISA plate and the lower detection limits varied between 0.001 and $0.127 \mu\text{g}/\text{m}^2$

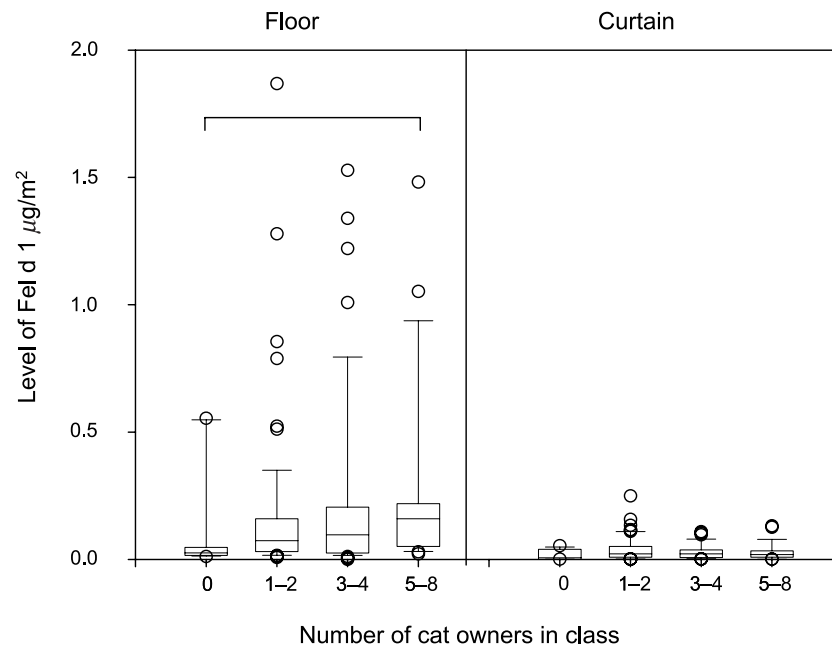


Fig. 3 Levels of Fel d 1 given as $\mu\text{g}/\text{m}^2$ in dust samples from floors and curtains in 155 classrooms in 81 schools in Oslo with 0, 1-2, 3-4 and 5-8 pupils with a cat at home. Boxes comprise the 25th and 75th percentiles and the median (horizontal line). Vertical bars show the 10th and 90th percentiles, and circles represent values outside of the given percentiles. The bracket indicates a statistically significant difference between groups ($P < 0.05$). The detection limits were set according to the standard curve for each ELISA plate and the lower detection limits varied between 0.0004 and $0.0047 \mu\text{g}/\text{m}^2$

absolute amount of allergen ($\mu\text{g}/\text{m}^2$) was not. This indicates that cat and dog allergens bound to dust will become diluted in dust without allergens. Moreover, as

discussed by Gaarder (1998), when the allergen levels and the weights of dust are low, the μg allergen/g dust value becomes a highly unreliable measurement com-

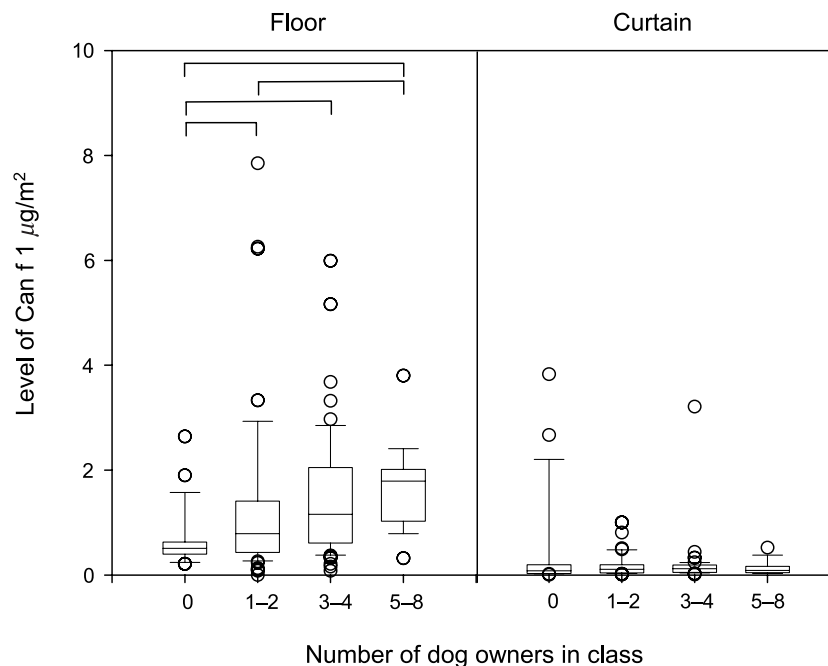


Fig. 4 Levels of Can f 1 given as $\mu\text{g}/\text{m}^2$ in dust samples from floors and curtains in 155 classrooms in 81 schools in Oslo with 0, 1–2, 3–4, 5–8 pupils with a dog at home. Boxes comprise the 25th and 75th percentiles, and the median (horizontal line). Vertical bars show the 10th and 90th percentiles, and circles represent values outside of the given percentiles. Brackets indicate statistically significant differences between groups ($P < 0.05$). The detection limits were set according to the standard curve for each ELISA plate and the lower detection limits varied between 0.001 and 0.127 $\mu\text{g}/\text{m}^2$

pared with μg allergen/ m^2 . Hence, the use of area unit (m^2) seems to be a more adequate indicator for allergen exposure than dust weight. On the contrary, the allergen content ($\mu\text{g}/\text{m}^2$) is highly dependent on the dust-collecting procedure, which is influenced by sampling equipment and routine. In this study, a strict sampling routine was followed and the results in the figures in the present study are expressed as $\mu\text{g}/\text{m}^2$. The most relevant expression for personal exposure to allergens is the amount of allergen inhaled. However, collecting indoor ambient air particulate matter is a time-consuming method and the amount of allergen is often below the detection limits (Custovic et al., 1999).

In agreement with other studies (Munir et al., 1993, 1995), levels of cat and dog allergens varied with the location of sampling. The allergen levels were higher in mattresses than in curtains and on floors. Possible explanations are that mattresses have a larger volume compared with floors and curtains. This indicates that mattresses are important sources of allergen exposure in day-care centers.

The levels of Can f 1 were far higher than those of Fel d 1, even if the frequency of dog and cat ownership in our study was approximately the same. This finding is in accordance with other studies (Egmar et al., 1998; Munir et al., 1993, 1995). A closer contact with dogs than cats may be the explanation. In a previous review article, the levels of indoor allergens in settled school dust were evaluated (Tranter, 2005). The content of

indoor allergens in different studies varies largely with the experimental design, dust sampling equipment, and the analytic methods used. Reports on cat and dog allergen levels in day-care centers, expressed as $\mu\text{g}/\text{m}^2$ and comparable with the present study in design, were not found. However, in the present study of day-care centers, the levels (expressed as $\mu\text{g}/\text{g}$) of cat allergens were in the same range, whereas the levels of dog allergens were higher than previous comparable studies (Dornelas de Andrade et al., 1995; Munir et al., 1995). The levels of cat allergen, but not those of dog allergen, expressed as $\mu\text{g}/\text{m}^2$ in comparable school studies, showed that the present levels were higher than in a previous study from northern Norway, but lower than those in a study from New Zealand, where 50% of the homes had cats (Dotterud et al., 1997; Patchett et al., 1997). In the present study, the day-care centers were selected on the basis of a need for new ventilation systems, which may have resulted in higher allergen content than in the general day-care center in Oslo. The levels of dog allergens measured in the curtains in schools contained more allergens than in similar previous studies (Munir et al., 1993; Smedje and Norback, 2001). Overall, the levels of dog allergens in schools and day-care centers seemed to be higher, while those of cat allergens were similar to findings in previous studies.

We found that cat and dog allergen levels were higher in floor dust from schools than from day-care centers, even if daily cleaning was the standard in both

environments. A possible explanation is that more school children than pre-school children have pets at home. Different procedures in preparation of the dust could also have contributed to these differences. Only fine dust was used from day-care centers in contrast to the whole sample from schools. If allergens distribute evenly in the fine and coarse dust fractions, as we found in a preliminary study for Der p 1 (data not shown), the allergen content in the dust samples from day-care centers would be expected to be half of the levels found in school samples. However, the levels in school samples were more than 25 times higher than the levels in day-care center samples. Taken together, the exposure to allergens in schools seems to be higher than in day-care centers.

The present study confirms the fact that mite infestation in Oslo is rare, which is in accordance with previous findings from Norway and some other Scandinavian locations (Berge et al., 1998; Dybendal and Elsayed, 1992; Munir et al., 1995; Nafstad et al., 1998). Cold and dry climate is most likely the explanation. Mite allergen exposure in school and day-care center environments appears to be a minor problem in places with a climate similar to that in Oslo. We should however, not generalize the findings to all parts of Norway, because of variations in climate.

For the first time in Norway, we measured endotoxin levels in indoor dust samples. Endotoxin levels in dust samples from floors in 31 day-care units were approximately 30 times lower than the median level measured in German homes without animals (1500 ng/g) (Heinrich et al., 2001), and in Swedish homes (14 EU/mg) (Böttcher et al., 2003), and 5–60 times lower than in day-care centers and schools in Finland (0.2–0.3 ng/mg) (Andersson et al., 1999) and Brazil (1–3 ng/mg) (Rullo et al., 2002). As the endotoxin levels were remarkable low, we tested some of the dust samples by a chromogenic LAL assay, but got similar results. The

literature shows that there are large differences between studies in the endotoxin levels measured, and differences in study design and analytical methods could have contributed to this. This warrants carefulness when comparing results from separate studies. Instead of pyrogen-free water that is commonly used, we extracted the dust samples with PBS containing 0.02% azid, which might have affected the results. However, endotoxin levels in the present study tended to be higher in dust from floors compared with dust from mattresses, as shown previously by Douwes et al. (2000).

In the present study, we found that mattresses in day-care centers are most likely a major reservoir for cat and dog allergens. Moreover, in cases where children experience worsening of symptoms related to cat and dog allergy, more thorough cleaning routines should be considered for the mattresses and also removal of old ones, as well as placing allergic children in classes/rooms with as few children as possible with pets at home. The content of cat and dog allergens in some day-care centers and schools in Oslo is probably high enough to cause sensitization and triggering of allergic symptoms in sensitized individuals. On the contrary, exposure to mite allergens in these environments do not seem to be a problem.

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