

Fel d 1 and Can f 1 in settled dust and airborne Fel d 1 in allergen avoidance day-care centres for atopic children in relation to number of pet-owners, ventilation and general cleaning

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Summary

Background Special day-care centres for atopic children have been established in Sweden. **Objective** To study concentrations of cat (Fel d 1) and dog (Can f 1) allergens in settled dust and airborne cat allergen in day-care centres in relation to pet ownership among children and staff, ventilation and general cleaning.

Methods Twelve allergen avoidance day-care centres and 22 conventional day-care centres were included in the study. Settled dust was collected and analysed with ELISA. Airborne cat allergen levels were measured in eight allergen avoidance and seven conventional centres with a personal air sampler and analysed with an amplified ELISA. Air change rate per hour (ACH) was measured. A questionnaire which focused on keeping of cat and dog among staff and children and frequency of general cleaning was used.

Results In the allergen avoidance day-care centres neither children nor staff reported ownership of cats or dogs, compared with 21/22 of the conventional centres in which children and staff kept furred animals. Fel d 1 and Can f 1 were found in settled dust in all day-care centres. In the allergen avoidance compared with the conventional centres the concentrations of Fel d 1 and Can f 1 were lower, Fel d 1: median 0.64 µg/g vs 5.45 µg/g and Can f 1: 0.39 µg/g vs 2.51, both $P < 0.001$, and airborne Fel d 1 was also lower in the allergen avoidance centres compared with the control centres, 1.51 ng/m³ vs 15.8 ng/m³, $P = 0.002$. A correlation was found between airborne and settled Fel d 1, $r_s = 0.75$, $P < 0.001$. Furthermore, a correlation was found between increased ACH and decreased levels of Fel d 1 in the air in the day-care centres with no cat-owners, $r_s = -0.86$, $P = 0.007$. No relation was found between levels of cat or dog allergen and amount of general cleaning. **Conclusion** Not keeping pets seems to reduce children's exposure to pet-allergen in their 'working environment'. Additionally, appropriate ventilation seems to reduce Fel d 1 in the air in day-care centres.

Keywords: allergen, Can f 1, environment, Fel d 1, preschool, transfer

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Introduction

Sensitization to furred pets is prevalent in many western countries and has been reported to increase [1–3]. During the last decades keeping of furred pets, i.e. cats, dogs and

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rodents have been reported to increase in many societies [4,5]. Furthermore, allergens from furred pets have been found in environments where pets are not held [6]. The explanation for these findings has been transportation of pet allergen by pet-keeping individuals and dispersion in environments such as day-care centres and schools where pets are not allowed. Thus, there is reason to believe that exposure to pet allergen in 'pet free' environments may have increased during the same period.

Exposure to furred pets among animal-sensitized individuals is associated with worsening of symptoms and association between sensitization to animals and asthma has been demonstrated [7–9]. Moving of children sensitized to domestic mites and with asthma to mite-free environments have reduced the manifestations of their disease and reduced bronchial hyperreactivity [10,11].

In Sweden, severely atopic preschool children of whom a majority are allergic to furred pets, have problems in attending official day-care due to their disease. Therefore, in some municipalities around Sweden, so-called allergen avoidance day-care centres have been built or established to cope with young allergic children's need. In these special day-care centres, neither children nor staff are allowed to have furred pets at home and the staff are not allowed to be smokers or to use perfumes or other smelling cosmetics at work. General cleaning in the allergen avoidance day-care centres is claimed to be adjusted to the need of the allergic children and therefore more extended than in conventional day-care centres. Furthermore, allergen avoidance day-care centre floors are not fitted with wall-to-wall carpets and furred animals are not allowed indoors.

Only a limited number of studies of airborne allergen levels in pet-free environments have been performed due to technical difficulties in quantifying very low levels of allergens [12]. Analysis of airborne cat allergen, Fel d 1, using portable samplers in cat-free environments, has now been made possible through amplification of an ELISA technique [13]. At the time of this study the technique was not available for Can f 1.

The aim of this investigation was to study concentrations of Fel d 1 and Can f 1 in settled dust and airborne Fel d 1 in day-care centres in relation to pet ownership among children and staff as well as ventilation and general cleaning. Due to a pronounced risk of selection bias, decision was taken not to include health effects of children and staff in the study.

Material and methods

Materials

At the time of the study 12 allergen-avoidance day-care centres in Sweden fulfilled the criteria mentioned above.

They were evenly located over the country. The principal of each identified day-care centre was informed of the study. The local building committee in each district was asked to identify two day-care centres as controls, built and/or renovated the same year as the referent day-care centre. The absolute majority of the day-care centres were built between 1975–1995. For technical reasons, measurements of two differently located conventional centres were not able to be performed. Measurements in matched centres were performed on the same day.

Sampling and extraction of reservoir dust

Dust from one sofa in each day-care centre was collected with a vacuum cleaner belonging to the day-care centres or with a standard vacuum cleaner (Volta Lite U 1840, Volta, Stockholm, Sweden). A small disposable filter bag (Allergy Control Products Inc., Ridgefield CT, USA) was inserted in the front tube of the vacuum cleaner before attachment of a textile mouth piece. The dust sampling was standardized by time (3 min). The samples were extracted in phosphate-buffered saline (PBS) with 0.1% Tween 20[®] and 0.15% Kathon[®] (PBS_{TK}) (1/40 w/v) for 2 h at room temperature, centrifuged (10 min × 1000 g), aliquoted and stored at –20 °C until analysis. All samples of settled dust were analysed for contents of Fel d 1 by the National Institute for Working Life, Solna and Can f 1 by Department of Clinical Immunology, Karolinska Hospital and Institute, Stockholm, Sweden.

Airborne cat allergen

For technical reasons a limited number of 15 day-care centres were selected for measurement of airborne allergen. Exposure to airborne cat allergen, Fel d 1, was examined for 8 h/working day in the breathing zone of the children in eight allergen avoidance and seven conventional day-care centres: one member of the staff carried a personal air sampler connected to a low volume portable pump in a belt during one working day (8 h). Air samples were obtained using IOM inhalable dust samplers (SKC Ltd, Blandford, UK), equipped with 25 mm polycarbonate filters (Millipore AB, Sundbyberg, Sweden; pore size 0.8 µm). An airflow of 2 L/min was obtained with portable pumps (mainly Casella, Casella London Ltd, UK). The flow was measured before and after sampling. Effective sampling time and average airflow were used to calculate total volume of air sampled. The filters with airborne dust were left in the cassettes and kept at –20 °C until extraction. The extraction was done in MinisorpTM vials (Nunc A/S, Roskilde, Denmark) using 1 mL PBS_{TK} under rotation overnight at room temperature. Extracts were then centrifuged (10 min × 1000 g) and stored at –20 °C.

*ELISA assay – Fel d 1**Dust sample assay*

Fel d 1-specific mouse monoclonal antibodies 6F9, biotinylated 3EC4 and cat allergen standard UVA 94/01 were purchased from Indoor Biotechnology (University of Virginia, Charlottesville VA, USA). Dust extracts were run undiluted and if necessary diluted in twofold dilutions with PBS_{TK} to produce absorbance values within the standard concentration range (0.625 – 20 ng Fel d 1/mL). Incubations were performed at ambient temperature (20 – 25 °C).

Briefly, microtitre plates or strips of MaxisorpTM quality (Nunc A/S, Roskilde, Denmark) were coated with 6F9 at 100 µL and 10 µg/mL in 50 mmol/L carbonate/bicarbonate buffer at pH 9.6 overnight. The plates/strips were washed three times with PBS_{TK} in an automated microtitre plate washer/Wellwash 4TM (Denley Ltd, UK). Plates were blocked for 1 h using PBS_{TK} containing 1% bovine serum albumin (BSA). After washing, 100 µL of standards, samples and controls made from in-house cat allergen extract (Fel d 1 at 1.3 ng/mL), were added in duplicate. After 90 min, plates were washed and subsequently incubated with 100 µL biotinylated 3EC4, and diluted 1:1000 for 60 min.

After washing, plates were incubated for 30 min with 100 µL of an in-house streptavidin horseradish peroxidase conjugate diluted 1:500 (100 ng/mL). After final washing, plates were developed by adding 100 µL of KBlueTM (ELISA Technologies Inc., Lexington KI, USA). The blue colour reaction was stopped after 20 min by the addition of 100 µL of 1 mol/L H₂SO₄. End point yellow colour was measured at 450 nm (minus 650 nm reference wavelength) on a ThermoMaxTM reader using SOFTmaxTM vs 2.3 for WindowsTM software (Molecular Devices Inc. CA, USA).

Amplified air sample assay

MaxisorpTM microtitre plates or strips were coated with 6F9 at 100 µL and 5 µg/mL in PBS over night. The coating solution was then discarded and plates/strips were blocked for 1 h using PBS containing 1% BSA. The plates/strips were then washed four times (throughout the assay), with PBS_{TK} in an automated microtitre plate washer (Wellwash 4TM, Denley Ltd, UK). After washing, 100 µL of standards (5 – 160 pg Fel d 1/mL), samples and an in-house control cat allergen extract (Fel d 1 at 40 pg/mL), were added in duplicates. Throughout the assay all dilutions were made in PBS_{TK} containing 1% BSA. After a 90-min incubation, plates were washed and subsequently incubated with 100 µL biotinylated 3EC4 and diluted 1:1000 for 60 min. After washing, plates were incubated for 30 min with 100 µL of an in-house streptavidin horseradish peroxidase conjugate diluted 1:500 (100 ng/mL). The amplifier N-Biotinyl-4-Hydroxybenzohydrazone (13.5 µg/mL, M_w 362 Da; synthesized by

P. H. Larsson), in 0.1 mol/L borate, 0.003% H₂O₂, pH 8.5, was added at 100 µL/well and allowed to react for 15 min according to the CARD (catalysed reporter deposition) concept [14]. Following washing the enzyme conjugate was added and incubated as above. After final washing, plates were developed by adding 100 µL of KBlueTM (ELISA Technologies Inc., USA). The blue colour reaction was stopped after ≈ 10 min by the addition of 100 µL 1 mol/L H₂SO₄. Readings were performed as for the dust sample assay.

The results were expressed as µg Fel d 1/g dust for the sofas and Fel d 1/m³ of air, respectively. Detection limits (as defined by the lowest standard point) of the assays was 25 ng Fel d 1/g dust (nonamplified assay) and 42 pg Fel d 1/m³ of air at 60 min sampling time (amplified assay). Sample values with intrassay coefficients of variation (CVs) above 10% were rejected and re-assayed. Interassay CVs of controls were < 20%.

Radio immunoassay – Can f 1

Allergen content for dog (Can f 1) allergen was analysed with a radio immunoassay. Briefly, purified anti-Can f 1 monoclonal antibodies (kindly provided by Professor Martin D. Chapman, Indoor Biotechnology) 1 µg/disc, was coupled with cyanogen bromide (CNBr)-activated discs (Pharmacia Diagnostics, Uppsala, Sweden). The discs were incubated either with 50 µL of dust sample (1/40 w/v) or with dog allergen standard from 1000 IU/mL to 4.1 IU/mL (purified Can f 1 94/02; Indoor Biotechnology) for 2 h at room temperature. After four washes 50 µL of ¹²⁵I-labelled rabbit antihuman IgE (Phadebas RAST anti-IgE Tracer, Pharmacia Diagnostics) was incubated overnight at room temperature. After final wash the count rate of the discs was measured using a multigamma counter (LKB, Turku, Finland). One IU of the International Dog standard was taken to correspond with ≈ 1 ng of purified Can f 1 (estimated by Prof. R. Aalberse). The detection limit of the assay was 0.08 µg/g dust.

Air change rate

Measurement of the air change rate per hour (ACH) was performed using a passive tracer gas technique, which previously has been described in detail elsewhere [15]. Based on occupancy level, the total air flow rate in day-care centres has been recommended not to be less than 3 ACH. Thus, a specific air flow rate of 3 ACH was used as cut off for statistical analyses.

Questionnaire

A questionnaire which focused on keeping of furred pets among children and staff and methods and frequency of general cleaning was mailed to the day-care centres after the

visit that included technical measurements and collection of dust. The questionnaire was answered by the principal of each day-care centre after discussion with staff and parents of the children.

Statistics

The concentrations of Fel d 1 and Can f 1 are given as median and range when appropriate. A nonparametric test (Mann–Whitney *U*-test) was used for comparative analysis. Correlations between levels and other variables were computed with Spearman Rank correlation (r_s) and two side tests were used and P -value <0.05 was considered significant. Extreme values were included in all statistical tests. All statistical analyses were performed with the aid of the statistical software packages SPSS (SPSS Inc., Chicago IL, USA) and Statistica (StatSoft, Tulsa OK, USA).

Results

None of the staff or children from the allergen avoidance day-care centres reported keeping of cat and/or dog. In the conventional day-care centres keeping of cats was reported in 21 and dogs in 19 of the 22 centres. A correlation was found between number of cat and dog owners and corresponding concentrations of cat and dog allergens in settled dust, $r_s = 0.64$, $P = 0.004$ and $r_s = 0.50$, $P = 0.02$, respectively (data not shown).

Both cat and dog allergens were found in settled dust in all day-care centres. In the allergen avoidance day-care centres the concentrations of Fel d 1 and Can f 1 in the reservoir were significantly lower compared with that of the conventional day-care centres, Fel d 1: $0.64 \mu\text{g/g}$ (0.05 – $6.5 \mu\text{g/g}$) vs $5.45 \mu\text{g/g}$ (0.45 – $46.9 \mu\text{g/g}$), $P < 0.001$, and Can

f 1: $0.39 \mu\text{g/g}$ (0.11 – $2.36 \mu\text{g/g}$) vs $2.51 \mu\text{g/g}$ (0.28 – $10.41 \mu\text{g/g}$), $P < 0.001$, respectively (Fig. 1). One particular allergen avoidance day-care centre with the highest concentrations of Fel d 1 was also found to have the highest levels of Can f 1. This day-care centre was re-contacted to check for pet-ownership among staff or children. However, pet ownership was denied. In one of the conventional day-care centres both Fel d 1 and Can f 1 exhibited the lowest levels of such allergens in dust. In this particular day-care centre neither children, nor staff kept cats or dogs and it was dominated by children with parents from other continents.

In the 15 day-care centres (eight allergen avoidance and seven conventional) where airborne cat allergen was measured, airborne Fel d 1 was significantly lower in the allergen avoidance compared with the control day-care centres, 1.51 ng/m^3 (0.06 – 2.49 ng/m^3) vs 15.8 ng/m^3 (0.28 – 5.58 ng/m^3), $P = 0.005$ (Fig. 2). Furthermore, a significant correlation was found between airborne cat allergen and Fel d 1 in settled dust, $r_s = 0.75$, $P < 0.001$, which seemed to be more pronounced in the lower allergen concentration range, i.e. in the allergen avoidance day-care centres, $r = 0.74$, $P = 0.04$ (Fig. 3).

In all day-care centres there was a significant correlation between the ACH and airborne Fel d 1, $r_s = 0.63$, $P = 0.01$. This correlation was found to be even more pronounced in the day-care centres without cat owners (12 allergen avoidance and one conventional). A tendency towards correlation was also found between Fel d 1 in settled dust and ACH in the day-care centres without cat owners, $r_s = -0.54$, $P = 0.06$. No such correlation was found for Can f 1 in settled dust. In the allergen avoidance day-care centre with the highest Fel d 1 in dust ($6.51 \mu\text{g/g}$), and the second highest Fel d 1 in air (2.49 ng/m^3), the ACH was below 3 changes per hour.

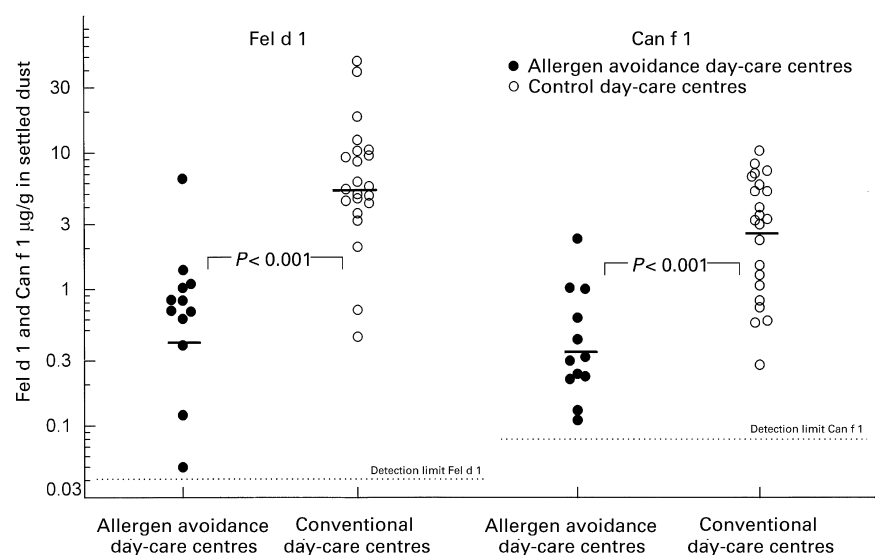


Fig. 1. Cat and dog allergen (Fel d 1 and Can f 1) in 12 allergen avoidance and 22 control day-care centres matched for geographical location and year of construction in Sweden, 1995.

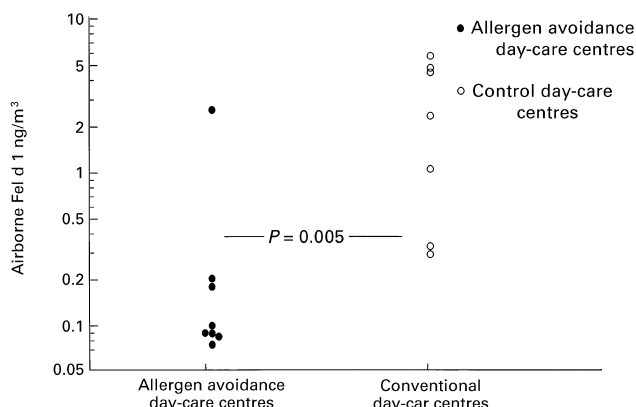


Fig. 2. Airborne Fel d 1 collected for 8 h with personal sampler and portable pump in eight allergen avoidance and seven control day-care centres in Sweden, 1995.

In five of the 13 day-care centres with no cat owners (12 allergen avoidance and one conventional) and an indoor air change rate ≥ 3 ACH per hour, Fel d 1 in settled dust was significantly lower compared with the other eight with lower air turnover, $P < 0.04$ (Fig. 4). This effect of the air change rate of ≥ 3 ACH per hour on the concentrations of Fel d 1 in settled dust in the day-care centres with cat-owners among children and staff could not be demonstrated. These associations could not be demonstrated for Can f 1 either.

In all the day-care centres (allergen avoidance and conventional), general cleaning was estimated to be performed 7 h/week (median); for the allergen avoidance centres 10 h/week (median) and for the conventional centres 6.5 h/week. No significant difference between the amount of cleaning hours and allergen concentrations in settled dust (Fel d 1/Can f 1) or in the air (Fel d 1) was found.

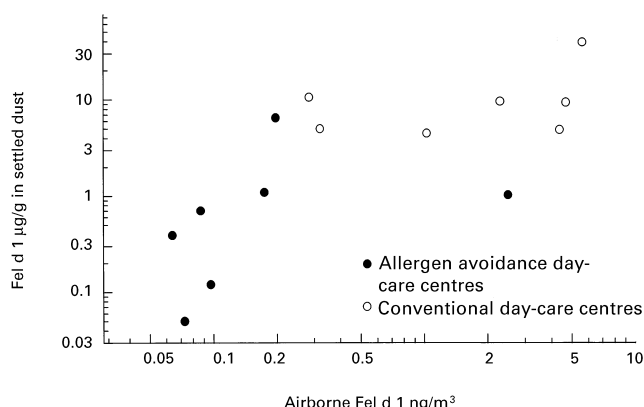


Fig. 3. Correlation between airborne Fel d 1 and Fel d 1 in settled dust in eight allergen avoidance and seven conventional day-care centres in Sweden, 1995. $r_s = 0.75$, $P = 0.001$.

Discussion

In this study we have clearly demonstrated that it is possible to reduce exposure to furred pet allergens, i.e. cat and dog allergens, among atopic children in day-care centres by restrictions of cat and dog keeping among the staff, the children and their families. Such restrictions did not only include a significant reduction in exposure for allergens in settled dust, but also a reduction of exposure to airborne Fel d 1 in the breathing zone of the children. According to our results it is likely that Can f 1 in the air is also reduced. However, as the technique to assess low airborne concentrations of dog allergen was not available at the time of the study, this could not be investigated. In a study on cat allergen avoidance measures in homes with cats, reduction of pet allergens in settled dust has been demonstrated [16]. However, airborne pet allergen concentrations in such homes have failed to show any significant reduction [8]. Hypothetically, this can be explained by the fact that when the allergen source is within the building *per se*, it may be more difficult to reduce the indoor reservoir of such allergen that is continuously produced and built up over time, than to reduce the amount of allergen when the allergen source is not in that building [17]. This difference has to be taken into account when interpreting our data, where the main source of cat and dog allergens must have their origin from contaminated clothes and hair of children and staff.

In all the allergen avoidance day-care centres occurrence of both cat and dog allergens could be demonstrated, which also shows that reducing exposure to zero allergen concentrations seems to be unrealisable. In the allergen avoidance day-care centres, contact with pets outside home or indirect pet contact is likely to have contributed to measured allergen

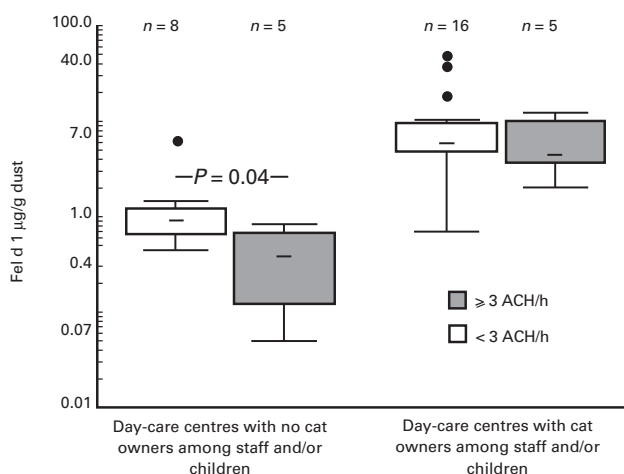


Fig. 4. Fel d 1 in settled dust in day-care centres with and without cat-owners among children and staff in relation to specific air flow in the day-care centres. Filled circles denote to extreme values.

levels of both Fel d 1 and Can f 1 which has been shown in a recent study [17]. Such pet contacts were not asked for in our questionnaire addressed to the principals, but may explain why cat and dog allergen were found in the allergen avoidance day-care centres. However, it cannot be overlooked that some of the children or staff in the allergen avoidance day-care centres with highest Fel d 1 levels in both dust and air, may have kept a cat at home despite their answer in the questionnaire. In the conventional day-care centre, with the lowest concentration of both Fel d 1 and Can f 1, the majority of the children were of immigrant origin which has previously been reported to create an indoor environment in day-care centres with low levels of furred pet allergen, since in many ethnic groups keeping of indoor pets is very uncommon [18].

In public environments where pets are not allowed, such as offices, schools and day-care centres, a way to reduce dispersion of pet allergen after direct or indirect pet contacts could be to let children change to clean clothes just before attending day-care centres. This was done in the particular allergen avoidance day-care centre in our study which exhibited the lowest concentrations of both Fel d 1 and Can f 1 in settled dust. This particular day-care centre had two years earlier participated in another study, in which an extensive allergen avoidance programme had been tested including renovation of floors, instalment of mechanical ventilation and ventilated floors [19]. A change in cleaning routines and frequent change of clothes were also introduced to obtain an 'allergen-free' day-care centre.

A correlation was found between the increase in air change rate and the decrease in airborne cat allergen concentrations. This was most pronounced in the allergen avoidance day-care centres in which an association between an air change > 3 h and reduced cat allergen levels could be demonstrated. Thus, ventilation seemed to have an impact on the allergen levels in the day-care centres, in which the influx of allergens from cats and dogs were considerably lower, compared with the conventional day-care centres. The interpretation of these data is that other measures besides restriction of pet keeping is likely to have a marginal effect on allergen levels from furred pets in indoor public environments with large amounts of transfer and dispersion of pet allergen as a consequence of frequent pet-keeping in the society.

In our study we found weaker and in some occasions no correlations between dog allergen in settled dust and other variables, i.e. Can f 1 in dust vs numbers of pet-owners and ventilation. In the literature, studies on associations between reported exposure and sensitization to dog has been less frequently reported compared with that of cat exposure and subsequent sensitization. Thus, there is reason to believe that dog allergen may be less well characterized. Furthermore, commercially available dog extract has

recently been found to be contaminated with cat allergen (personal communication: R. Aalberse).

Appropriate general cleaning has been claimed to contribute to an indoor climate in which high concentrations of pet allergen can be avoided. From other studies we know that upholstered furniture as well as textiles are reservoirs for allergens [5,20,21]. In the allergen avoidance day-care centres, the cleaning was performed on average three hours more per week than in the conventional centres; and vacuum cleaning of upholstered furniture, dust cleaning of various surfaces and washing of curtains was performed twice as often. Despite the reported increase in cleaning procedures in the allergen avoidance day-care centres, it turned out to have little effect on the concentrations of cat and dog allergens in dust. This is not easily explained, but may be a matter of misclassification since the data on frequency of general cleaning were estimates only asked for retrospectively.

In a recent study, cat and dog allergen in floor dust from homes of families without ownership of cat and dog ever were, on average 0.3 and 1.2 $\mu\text{g/g}$, respectively [22]. In the same study, corresponding allergen concentrations among families with previous ownership of cat and dog were 4.5 and 2.6 $\mu\text{g/g}$. Thus, in many day-care centres the children are exposed to concentrations of both Fel d 1 and Can f 1 in dust which seems to be equal to homes with previous ownership of cat or dog. Furthermore, previous studies have shown that Fel d 1 levels of 8 $\mu\text{g/g}$ dust is a statistical risk level for sensitization to cat and may elicit asthma [23]. In our study the cat allergen levels in the allergen avoidance day-care centres were all below 8 $\mu\text{g/g}$ dust, while the levels in some of the conventional day-care centres, in which a number of atopic children are attending, were well above that. The corresponding airborne levels that may induce or maintain allergic symptoms and asthma are not known. We have shown in another study that the median levels of airborne cat allergen in classes with many cat owners was one third of the airborne concentration of cat allergen in homes with a cat [24]. Considering that children attend day-care centre or school for at least 6 h/day, the total amount of cat allergen inhaled in a week in our study may be significant for children at risk for atopy or for those who have already developed allergic disease.

It is reassuring to conclude that it is possible to significantly reduce the allergen levels by the steps taken in the allergen avoidance day-care centres reported on here.

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