

Original article

Allergen avoidance does not alter airborne cat allergen levels in classrooms

Background: Some schools in Sweden offer allergen avoidance classrooms for allergic children with severe asthma. However, the measures commonly used to achieve a reduction in allergen levels have not been properly evaluated. The aim of the present prospective study was to study whether the levels of airborne cat allergen are altered after introducing feasible intervention measures in classrooms, without interfering with peoples' freedom of choice regarding pet ownership.

Methods: Twenty-five classes, including five established allergy prevention classrooms participated in the study during a school year. After one term, six classes underwent a number of intervention measures recommended by the Swedish National Institute of Public Health. Curtains, upholstery and plants were removed, bookshelves were replaced with cupboards and regular cleaning was increased. Airborne dust was collected weekly (32 weeks) using duplicate Petri dishes ($n = 1574$) and on six occasions using two personal air samplers in each class ($n = 264$).

Results: Airborne cat allergen levels were showing a similar variability throughout the whole study in all classes. Despite extensive measures in order to reduce allergen exposure, cat allergen levels were unaltered in the six classes after intervention. Allergen levels were not significantly lower in the established allergy prevention classes, compared with the other classes. Cat allergen levels differed, however, significantly between classes with few and many cat owners ($P < 0.05$).

Conclusions: We conclude that the recommended allergen avoidance measures used in this study did not reduce airborne cat allergen. It seems plausible that measures that fail to reduce allergen levels also fail to influence health status in allergic children but this remains to be shown.

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Furred animals are not allowed inside schools in Sweden. However, high level of pet allergens can be found in classrooms and other public places, as has been shown in several studies (1–5). There is a strong relationship between cat allergen levels and the number of cat owners in the class, which has been explained by the indirect transfer of allergens between homes with cats and schools or other public places, mainly via clothing (4, 6, 7). However, increased attention has been drawn to the conditions for allergic children at school since the incidence of allergy is increasing, and some schools in Sweden are allocating resources to allergen avoidance measures. This may include increased cleaning, sometimes in combination with removal of upholstery and curtains and the replacement of bookshelves with cupboards in order to minimize dust and allergen load. These measures, recommended by the Swedish National Institute of Public Health and Swedish Asthma and Allergy Association can be difficult to put into

practice and an evaluation of the actual effect of these combined measures on allergen levels and health is therefore necessary. A previous cross-sectional study has shown that allergen levels in reservoir and airborne dust were lower in allergen avoidance day care centres, compared with conventional day care centres (8). The allergen avoidance measures included stringent pet ownership restrictions among staff and children. It is, however, difficult to find school classes or day care centres where no children have pets at home, although some do exist in Sweden. Such classes have occasionally been established to make school attendance at all possible for a few children with severe asthma symptoms against pet allergens.

Most prospective allergen avoidance studies have been conducted in homes of asthmatic individuals using a single measure, like for instance air cleaners or allergen-impermeable mattress covers (9–12). Most commonly, dust samples from floors, bedding and mattresses have

been collected and used as an estimation of allergen exposure assessment in these studies. However, in many cases the desired allergen reduction or clinical effect has not been obtained. Similar intervention studies in schools and day care centres are unusual (13, 14).

Removal or reduction of cat allergen has been reported to be difficult in several studies and it may take a considerable number of months before a substantial reduction can be observed (15–17). The property of the allergen and the association with smaller particles keeps it airborne for a long time, which may explain why reduction of cat allergen is difficult. Chan-Yeung et al. showed that cat sensitization had occurred among residents of the island Tristan da Cunha, many years after cats had been exterminated from the island. The authors conclude that residual cat allergen may be the explanation, since the individuals were born within 5 years of the extermination (18).

The aim of the present study was to evaluate the effect on airborne cat allergen levels after introducing a number of feasible and economically defensible intervention measures in classrooms, without interfering with peoples' freedom of choice regarding keeping pets. Airborne cat allergen levels were measured prospectively during 1 school year in control classes, established allergy prevention classes and in classes where allergen avoidance measures were introduced after half the observation time.

Material and methods

Study design

The study was carried out during a whole school year consisting of two terms (13 and 19 weeks, respectively) in 25 classes of which five were already established allergy prevention classes (see description below). After the first school term (13 weeks), extensive allergen avoidance measures were taken in six classrooms ('intervention classrooms'), all situated in the same building. In the remaining 14 classrooms no intervention was made (control classrooms). Sampling of airborne dust was performed continuously during the whole study using weekly Petri dishes and by using personal air samplers on six occasions (Fig. 1).

Inclusion criteria for intervention and control classes

In order to avoid presence of other children and hence minimize allergen contamination from other sources, classrooms had to be situated in separate one-storey buildings, built approximately the same years. All lessons should exclusively be attended by the same children and be performed in the same classroom. No scheduled leisure activities were allowed inside the classroom after school hours. The ventilation system should be similar in design and capacity and the classrooms otherwise similar (flooring, cleaning routines, room size).

The study was performed in a suburb north of Stockholm, Sweden. Among all compulsory intermediate level schools in the area (grades 1–6, $n = 10$), four schools were chosen according to the inclusion criteria. One of the schools had a separate one-storey building with six classrooms and adjacent cloakrooms and these

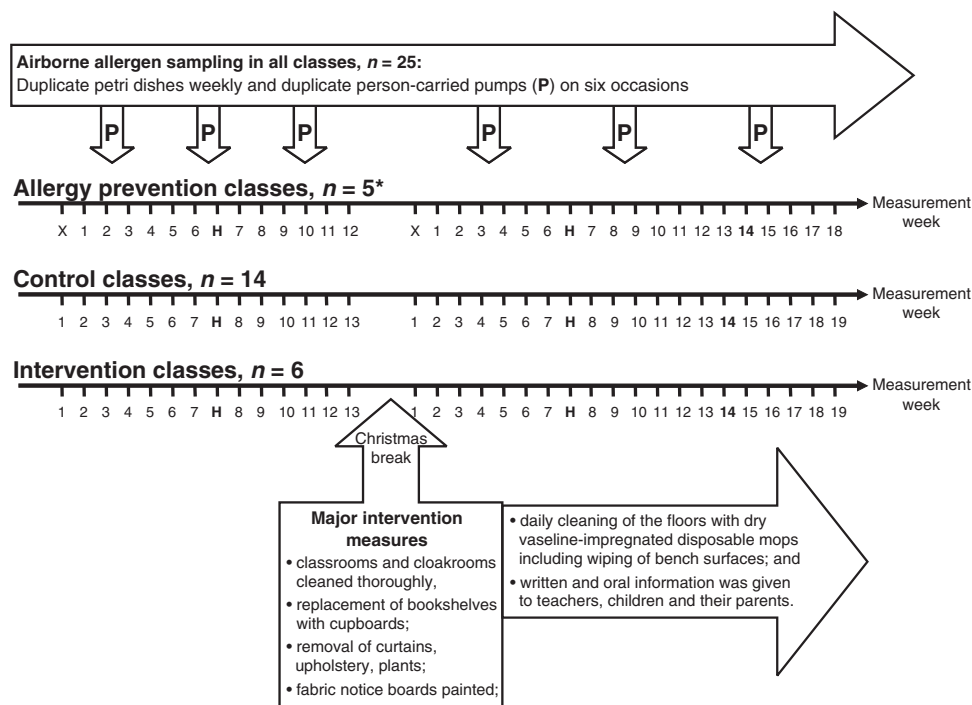


Figure 1. Study design. Allergen sampling during a school year in allergy prevention ($n = 5$), control ($n = 14$) and intervention classes ($n = 6$). Sampling week number in bold, sampling with Petri dishes performed during a school holiday week; H, school holiday week without any sampling; *, personal air samplers used in only two of the allergy prevention classes; X, no sampling due to later school start.

classes were chosen as intervention classes ($n = 6$). In the same school as the intervention classes but in another one-storey building, four classes were included as control classes. The remaining 10 control classes were situated in similar one-storey buildings among the other three schools. All classes had smooth linoleum floors.

Allergy prevention classes

The already established allergy prevention classes ($n = 5$) used for comparison in this study, were situated in a newly purpose-built school building in a neighbouring suburb. In these classes, a number of measures such as cleaning of classrooms twice a day, absence of open shelves, upholstery, carpets, curtains and plants and all school material stored in cupboards had been implemented since several years for the sake of severely allergic children (the first class was established 6 years prior to our study). This was the only available school in the whole Stockholm area, where such extensive measures had been implemented and these classes differed substantially from ordinary classes. However, the children in the allergy prevention classes were allowed to keep pets at home but not allowed to acquire new pets.

In all 25 participating classes, a 'shelf factor' (length of open shelves in relation to room volume) (19, 20) was calculated each term in order to measure the extent and (after intervention) the reduction of dust-collecting surfaces. Mean number of children was 25 (range: 18–30). Children were between 7 and 12 years of age at study start (mean age 10.5 years) and median cat ownership was 21% (Table 1).

Cleaning procedures in 'intervention classes' prior to intervention

Cleaning of classrooms was performed two to three times a week after school hours using a moist mop (TASKI fuktmopp; Orange Clean AB, Solna, Sweden) and when needed, a wet mop. Bench surfaces were wiped once a week using damp paper cloths. Toilets and cloakrooms were cleaned daily. Washing of curtains and other textiles was handled by teachers. Once a year during the summer holiday break, an extended cleaning procedure was performed where walls and other surfaces were cleaned. Cleaning routines were very similar in all classes.

Intervention measures in six classrooms ('intervention classes')

After one term (run in time 13 weeks), during the Christmas break, extensive measures were taken in order to establish allergy prevention classes according to published recommendations from the Swedish National Institute of Public Health and Swedish Asthma and Allergy Association (21). The idea was to introduce measures, which were feasible from an economic point of view and

possible to implement in most schools, without interfering with peoples' freedom of choice, e.g. by introducing pet ownership restrictions in families. All classrooms and adjacent cloakrooms were thoroughly cleaned including floors, walls and other surfaces. Bookshelves were replaced with cupboards with doors in order to avoid accumulation of dust and to make surfaces easy to clean. Curtains, upholstery, cushions and other textiles were removed. Upholstered chairs for teachers were sprayed with a tannic acid (Allersearch ADS, Quorum Allergy Products, Victoria, BC, Canada) in order to neutralize accumulated allergens in the fabric. Some furniture, three wooden sofas with upholstered seats, which were kept for comfort, was re-upholstered and encased with an allergen-impermeable barrier (ACb®, Allergy Control Products, Inc., Ridgefield, CT). Furthermore, plants were removed in order to avoid endotoxin and mould exposure from the earth. Notice boards made of fabric were painted with water-based acrylic paint to make the surface less inclined to accumulate dust. Finally, cleaning was increased to daily cleaning of the floors with dry vaseline-impregnated disposable mops (TASKI novolin golv; Orange Clean AB), including wiping of bench surfaces and vacuuming of the remaining upholstered furniture once a week. In addition, written and oral information was given to teachers, children and their parents. Children were also asked to avoid pet contact in the morning, prior to school start.

Allergen sampling with Petri dishes

The Petri dish sampling method has recently been evaluated and compared with personal air sampling (21). Cumulative sampling of settling dust/allergen is performed continuously over a time period and is then expressed as amount of allergen settling per square metre and day.

Petri dishes (9 cm in diameter) were coated with teleosten gelatine aqueous solution, rinsed with distilled water and finally left to air-dry as described previously (22, 23).

Two Petri dishes were placed side-by-side with base and lid facing upward 150 cm above the floor level on aluminium holders attached to the wall. Placement by nearby windows, doors or air intakes was avoided. The Petri dishes were open during a normal school week from Monday morning to Friday afternoon and were replaced every week by the teacher ($n = 1574$). Any discrepancies from these sampling conditions were noted in a sampling protocol. Petri dishes were closed and placed in plastic zip bags and stored at room temperature 1–2 weeks until extraction of the dust, which was performed as described previously (21).

Allergen sampling with personal air samplers

Personal air sampling was carried out every 5–6 weeks, on three occasions during each term. Three of the allergy prevention classes were excluded from these measurements (younger children unable to handle the equipment). Air sampling took place during whole-class morning lessons. Two pumps (Airchek 52 with IOM sampling head, SKC Inc., Eighty Four, PA) with 25 mm in diameter polytetrafluoroethylene (PTFE) filters, 1 µm pore size (Millipore AB, Sundbyberg, Sweden) were carried by two children (noncat owners) in 22 classes ($n = 264$). The sampling head was attached in the breathing zone. Airflow rate was adjusted to 2 l/min and the total sampling time was approximately 120 min. During breaks (> 10 min), the pumps were switched off. Air filter cassettes were stored at 20°C until extraction.

Filters were thawed and extracted in 2 ml PBST_K rotating over night at room temperature.

Table 1. Rate of cat ownership and the distribution of classes with many and few cat owners

Type of class	Median rate of cat owners (%)		Number of classes ($\leq 21\%$)	Number of classes ($>21\%$)
		Range (%)		
All ($n = 25$)	21	0–36	13	12
Intervention ($n = 6$)	24	11–32	4	2
Allergy prevention ($n = 5$)	15	4–23	4	1
Control ($n = 14$)	20	0–36	7	7

Fel d 1 analysis

All Petri dish samples were analysed for Fel d 1 in a monoclonal sandwich enzyme-linked immunosorbent assay (ELISA) using reagents from Indoor Biotechnologies (Cardiff, UK), as presented previously (21). Rare samples with intra-assay CV > 10% were re-assayed. Samples near or below the detection limit (0.156 ng/ml) and air samples were analysed in an amplified ELISA-assay (using Ampak signal amplification) according to the manufacturer's recommendations (Ampak™, Dakopatts AB, Älvsjö, Sweden). The standard curve range in the amplified assay was 2.5–160 pg Fel d 1/ml. For statistical calculations samples below this detection limit were assigned a value of half of the detection limit.

Statistics

All data were analysed using STATVIEW 5.0 software (SAS Institute, Inc., Cary, NC). Data were log-transformed and further analysed using repeated measures ANOVA. As only left censoring was used values below the detection limit ($n = 10$) were included in the analysis. The mean values of duplicate Petri dishes and simultaneously used personal air samplers were used in the calculations. The total coefficient of variation (CV_t) for duplicate Petri dishes was calculated according to Boleij et al. (24).

Data from Petri dish sampling was stratified for each class into eight measurement periods (four each school term) consisting of the mean value of 3 sampling weeks each. The last 5 weeks (including holiday week) of the second term were not included in order to match the analysis of the first term, which was considerably shorter. Because of missing values, some periods for some classes consist of the mean value of 2 weeks ($n = 17$) and in some cases, the value of 1 week only ($n = 8$). In total, 1130 Petri dish samples were included in the ANOVA analysis. The repeating factor was time (eight measurement periods for Petri dish samples and six repeated measurements for air samples) with the levels before and after. The independent factors were type of measure with the levels control, intervention and allergy prevention and rate of cat owners with the levels high and low. Comparisons of interquartile ranges (IQR) between groups were made using Kruskal-Wallis or Mann-Whitney *U*-test. A *P*-value < 0.05 was considered significant.

Results

Compliance in intervention classrooms

In some classes, teachers had difficulties with compliance regarding keeping material in cupboards. Many visits were required in order to emphasize the importance of maintaining the quality of the introduced intervention measures.

Shelf factor

During the first term, the mean length of open shelves was 7.6, 30.7 and 29.1 m in allergy prevention, intervention and control classrooms, respectively. The calculated shelf factor within each group was 0.064, 0.16 and 0.18 m/m³, respectively. During the second term, the shelf factor was decreased to 0.11 m/m³ (33% reduction) in the intervention classrooms, while it was unchanged in all other classrooms.

Allergen sampling using Petri dishes and personal air samplers

Some Petri dishes were for different reasons never used ($n = 57$), tampered with ($n = 18$), or used for < 0.5 week or more than 1.5 weeks ($n = 40$). These Petri dishes were excluded from the calculations. Because of missing values, the Petri dishes from the first sampling week of each term and the last sampling week of the second term were excluded from the statistical analysis but included in Figs 2 and 4. The mean detection limit was 0.1 ng Fel d 1/m²/day. If Petri dishes mistakenly were left open for sampling over weekends or during school holidays occurring during a sampling week, this time was discarded from the calculation of allergen levels (expressed as Fel d 1/m²/day). We have previously shown that no or a very small amount of cat allergen is sampled in Petri dishes during weekends in empty classrooms (21). A few air samples were discarded due to pump flow rate failures (> 10% drop in flow rate) or other technical problems ($n = 9$). At least one sample was, however, available for each sampling occasion and class and except for these cases, the mean value of two simultaneous air samples was used in the calculations. The mean detection limit was 0.020 ng Fel d 1/m³.

Cat allergen levels in relation to intervention measures and rate of cat owners

There was no significant difference between allergy prevention, intervention and control classes in airborne cat allergen levels during the study period, assessed by Petri dish sampling ($F = 0.015$, $P = 0.98$, Fig. 2), or personal air sampling ($F = 0.041$, $P = 0.96$, Fig. 3). Thus, the introduced allergen avoidance measures did not alter allergen levels.

Airborne cat allergen levels measured with Petri dish sampling were significantly lower in classes with few

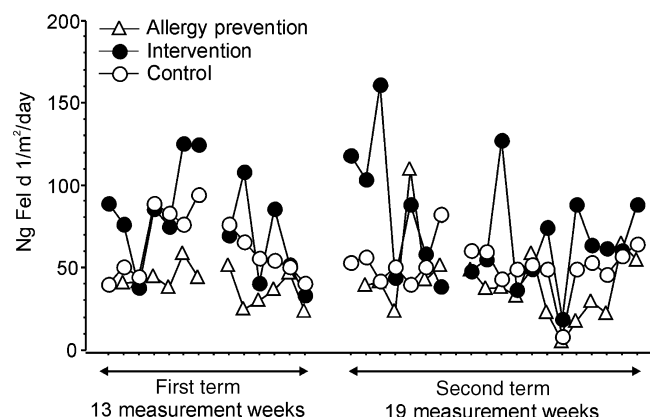


Figure 2. Mean weekly cat allergen levels measured with Petri dishes ($n = 1459$) in allergy prevention ($n = 5$), intervention ($n = 6$) and control classes ($n = 14$) during a school year, including 1 school holiday week (low or undetectable levels).

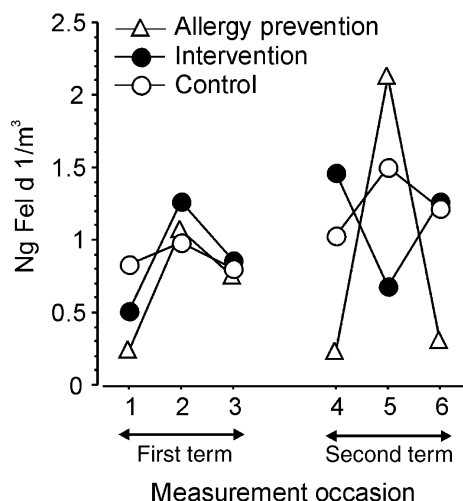


Figure 3. Mean cat allergen levels measured with personal air samplers ($n = 255$) in allergy prevention ($n = 2$), intervention ($n = 6$) and control classes ($n = 14$) on six occasions during a school year.

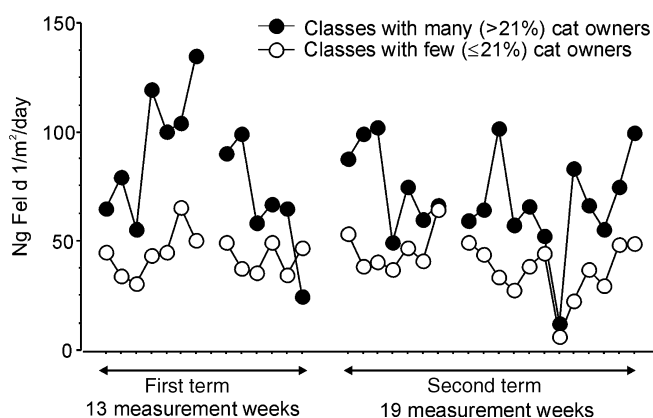


Figure 4. Mean weekly cat allergen levels measured with Petri dishes ($n = 1459$) in classes with many ($>21\%$, $n = 12$) and few ($\leq 21\%$, $n = 13$) cat owners during a school year, including 1 school holiday week (low or undetectable levels).

($\leq 21\%$, $n = 13$) cat owners, compared with classes with many ($>21\%$, $n = 12$) cat owners ($F = 4.68$, $P = 0.044$, Fig. 4). No significant difference was, however, observed from sampling with personal air samplers ($F = 0.84$, $P = 0.37$, data not shown).

Variations in cat allergen levels

The CV_1 between duplicate Petri dishes was 53%. There was a great variation in cat allergen levels within classrooms over time but no consistent pattern could be observed. The variability of IQR was similar in both allergy prevention, intervention and control classes (Kruskal–Wallis test, $P = 0.36$).

The scatter (IQR) was, however, significantly smaller among classes with few cat owners, compared with classes with many cat owners (Mann–Whitney U -test, $P = 0.012$).

Discussion

There was no difference in cat allergen level in the intervention classrooms before and after intervention. Levels were somewhat lower (nonsignificant), in the established allergy prevention classrooms, compared with the control classrooms or classrooms before and after intervention. Overall, a lower rate of cat owners resulted in significantly lower levels of cat allergen sampled with Petri dishes compared with a higher rate of cat owners, which is consistent with previous studies (4, 23). Thus, the tendency towards lower level of airborne cat allergen in the established allergy prevention classes may depend on the lower rate of cat owners in those classes (15%).

The compliance and interest among the teachers in the intervention classes varied. Some teachers found it difficult to keep the school material in cupboards. When material is lying around on tables and benches, cleaning is difficult and hence more dust may accumulate. One teacher initially refused to remove the curtains and a similar attitude was seen regarding the removal of plants. In the established allergy prevention classes, teachers were on the contrary genuinely engaged and kept an irreproachable order. There were considerably fewer shelves in the allergy prevention classrooms compared with all other classrooms. Although we managed to achieve a 33% reduction in shelf factor after intervention, the factor was still considerably higher than in the allergy prevention classes. However, these classes had a 6 year long experience with the implementation of allergen avoidance measures prior to our study. The effect of shelf factor on levels of airborne cat allergen may be difficult to evaluate, partly depending on how surfaces are used and maintained. We found no significant effect on airborne allergen levels, however, in settled dust collected from desks, chairs and floors. Smedje et al. showed that classrooms with more open shelves had higher concentrations of cat and dog allergen (25).

Munir et al. performed a prospective intervention study in a day care centre where a number of extensive measures were introduced, including installation of a new ventilation system (14). Soon after the introduction, the mean cat allergen levels in settled dust decreased by six times. After 1 year, levels were still generally lower compared with before, although the effect was less pronounced. An important part of the combined measures, which was an agreement among personnel and parents of not keeping any pets at home, may have had a great effect on the results.

Our conclusion that the number of cat owners is the predominant factor for the level of cat allergen found in a classroom has now resulted in more stringent restrictions regarding pet ownership in the established allergy prevention classes participating in the present study. From now onwards, new children applying for these classes are not allowed to have furred pets at home. Children already attending the classes are, however, allowed to keep their animals, which will delay the actual effect of this measure.

It has been shown previously that allergens adhere to and are transported on clothing (1, 2, 4). No preventive measures were taken regarding clothes in this study apart from asking the children to avoid pet contact before going to school. Allergen contaminated clothing in combination with rate of cat owners may have a greater impact on allergen levels than the combined measures used in this study. It is, however, important to avoid alienating cat owners and other pet owners. There may also be integrity considerations to take into account when interfering with children's choice regarding clothes for instance. Establishing pet owner free classes is a far more difficult task than taking other allergen avoidance measures, as presented in our study. Some schools in Sweden are now considering introducing special school clothing instead in order to minimize allergen load.

The cleaning method used after intervention was based on a dry cleaning method. It is still under debate whether moist or dry cleaning methods should be used for hard floors (26, 27). It has been shown that pet allergen concentrations are lower in floor dust than in dust from curtains and chairs (2, 28). This suggests that it may be at least as important to wipe chairs and wash curtains, as cleaning the floors regardless of cleaning method used.

Cat allergy is common among school children in Sweden (13.4% of 2149 skin prick tested children in northern Sweden) (29) and we have therefore only studied the effect on airborne cat allergen after intervention. Cat allergen is often associated with small particles and stays airborne for a relatively long period of time before settling (30). Hence, we have chosen to measure the amount of allergen in airborne dust instead of allergen in settled reservoir dust from floors and other surfaces. It has also been suggested that airborne allergen levels may be a better proxy for inhaled pet allergen exposure, than allergen levels measured in reservoir dust (31, 32). We have not measured other allergens, for instance dog or horse allergen. To date, there is no method available to analyse airborne dog allergen at low concentrations. It is, however, important to emphasize that the results in this study only apply to airborne cat allergen levels and the effect on other allergens is not known.

Generally, there was a great variation in allergen levels, both within classrooms and between weeks as well as between classes. The IQR were smaller among classes with few cat owners, indicating that lower levels of cat

allergen lead to less exposure peaks. It has previously been suggested that the peak exposure level of allergens in bakeries contribute substantially to the total exposure level over time (33). We know from experience that a classroom is an extremely dynamic environment and can under no circumstances be considered as 'standardized'; therefore, great fluctuations in allergen levels can be expected, even within a short sampling period of a few hours. Each individual carrying a personal air sampler has a different movement pattern at a given time as well as varying distance from pet owners in the classroom, which can influence the result. The Petri dish sampling method is influenced by similar factors and in order to achieve a good estimate every week, the accumulated allergen level has been calculated as an average level per day.

In conclusion, several simultaneously introduced allergen avoidance measures did not influence airborne cat allergen levels in the present study. Allergen avoidance strategies in schools may differ between countries and hence the outcome of allergen avoidance measures. The introduced measures in the intervention classes were, in the present study, chosen according to recommendations from the Swedish National Institute of Public Health and were very similar to the measures used in the established allergy prevention classrooms. Although the intervention had no effect on airborne cat allergen levels, we cannot conclude, from the present findings that intervention did not have an impact on health status in allergic children. It has, however, previously been shown that asthmatic, cat-sensitized school children deteriorate with increased symptoms after returning from holidays, if they attend a class with many cat owners (34). It has also been shown that 'second-hand' exposure to cat allergen in classrooms and homes without cats contributes to allergic sensitization (35, 36). It is therefore plausible that an intervention that does not alter airborne cat allergen levels probably does not improve symptoms in cat allergic children. Important purposes of the present study were to apply inexpensive measures in existing school buildings and to introduce measures that could be implemented without interfering with peoples' freedom of choice regarding keeping pets. We conclude that in order to help seriously pet allergic children by keeping allergen levels low at school, the rate of pet ownership should be minimized and stringent rules about clothing should be introduced. The clinical effect of such measures remains, however, to be shown.

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