

Short communication

Evaluation of Petri dish sampling for assessment of cat allergen in airborne dust

Background: Dust reservoir sampling is the most commonly used method for assessment of indirect allergen exposure. Because assessment of personal exposure using person-carried pumps is time-consuming and expensive we evaluated the Petri dish sampling method for measurement of airborne cat allergen in classrooms.

Methods: Petri dish sampling was evaluated in three study parts. Part I: by comparison between Petri dish sampling and personal air sampling in 44 classrooms with many ($\geq 20\%$) and few ($\leq 10\%$) cat owners and by additional Petri dish sampling in 40 pet-free homes. Part II: by sampling with duplicate Petri dishes in 28 classrooms. Part III: by sampling in three classrooms at four sampling heights during different sampling times. All samples were analyzed for cat allergen (Fel d 1) content with a monoclonal antibody two-site ELISA (enzyme linked immunosorbent assay), using signal amplification when necessary.

Results: There was a significant correlation between Petri dish sampling and personal air sampling ($r = 0.66$; $P < 0.0001$). Levels were five-fold higher in classes with many cat owners than in classes with few cat owners, regardless of method. A corresponding difference was found in the homes. Duplicate sample values were in fair agreement (Bland-Altman test) and were correlated ($r = 0.77$; $P < 0.0001$). Cumulative levels collected weekly in one Petri dish were lower than using five daily Petri dishes, regardless of sampling height.

Conclusions: Petri dish sampling can be useful as an alternative method to personal air sampling of airborne allergens.

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Key words: airborne; allergens; cats; dust; enzyme-linked immunosorbent assay; Fel d 1; sampling; schools.

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Accepted for publication 18 September 2001

Allergic disease in children has become an increasing problem throughout the world (1–3). Children spend a lot of their time indoors which implies that the indoor environment is of importance from a health perspective. Attention has been focused on the compulsory school environment where children spend a major part of their time apart from home. According to the Swedish Work Environment Act, schoolchildren are considered as employees by law, which gives them the same rights as adult employees (4). Thus, according to legislation, certain standards must be maintained in the working environment, regarding for instance ventilation and indoor temperature and the school has a responsibility to fulfil the needs of all children, including those with allergic and nonallergic hypersensitivity reactions (5). We have shown that the school environment is a site of exposure to pet allergens which is most likely a result of transport on clothing from pet owners (6). This indirect and involuntary exposure may worsen allergic disease (7). A number of different methods are being used today to assess levels of indirect allergen exposure from furred pets in school environments, at home and in public

buildings. To date, analysing allergen levels in dust reservoirs, e.g. upholstery, mattresses and floors has been the most commonly used method to assess allergen exposure (8, 9). However, sampling of airborne allergens on filters using person-carried pumps must be considered as the gold standard method to assess allergen exposure. This method is used in the occupational setting to measure for instance laboratory animal allergen exposure (10). Some years ago, a new method based on collection of house dust mite (Der p 1) allergen in airborne settling dust using Petri dishes was introduced (11). However, the method has not been thoroughly evaluated.

The aim of the present study was thus to improve and evaluate the Petri dish sampling method for collecting airborne settling cat allergen (Fel d 1) in classrooms (children 6–12 years of age), and to compare this method with the personal air sampling method. In addition, the aim was to study whether there was a relationship between the number of cat owners in the classrooms, and cat allergen levels found in the collected dust, as has been shown previously using personal air

sampling (6). Moreover, comparisons were made between allergen levels obtained by Petri dish sampling in classrooms and homes.

Material and methods

Part I. Comparison of Petri dish and personal air sampling

A database with information on cat-ownership among schoolchildren in 284 classes in the Stockholm area was used (7) to randomly select 24 classes with $\geq 20\%$ cat owners and 24 classes with $\leq 10\%$ cat owners. One Petri dish comprising a base and lid was placed with base and lid facing upwards in the classroom during a school week. In addition, within each class a child without furred pets placed a Petri dish at home during the same week. On one occasion two non-pet owning children in every class each carried a pump during whole-class morning lessons. In four classes the rate of cat ownership had changed. They did not fulfill the inclusion criteria, and were therefore, excluded from the study.

Part II. Agreement between Petri dish duplicates

Two Petri dishes were placed in 34 classrooms next to each other during one school week. Three classes were excluded from the study because they did not fulfil the inclusion criteria (at least 15 children in each class) and three classes because of missing information on cat ownership.

Part III. Evaluation of Petri dish sampling height and sampling time

Petri dishes were placed in three classrooms at four different heights: 60, 120 and 165 cm and (on top of a cupboard) 200 cm above floor level. Every day during one school week, Petri dishes were opened in the morning and closed in the afternoon of the same day. They were then replaced by new Petri dishes, which were opened the following morning. Simultaneously to assess the cumulative allergen amount, a single Petri dish was opened and closed every day in the same manner. In addition, Petri dishes were placed during weekends (Friday afternoon to Monday morning) in all three classrooms ($n=19$).

Petri dish preparation, sampling and extraction

The inner surfaces of both the base and lid of Petri dishes were coated with 5 ml 2% teleosten gelatine (Sigma, St. Louis, MO) aqueous solution for 15 min at room temperature, thereafter rinsed three times with distilled water and left to air-dry (11). Sampling was performed continuously during 5 days (Monday morning to Friday afternoon) by placing the dish with base and lid facing upward 150 cm above the floor level (if not stated otherwise) on a desk (Part I) or on aluminium holders (in-house workshop, National Institute for Working Life) attached to the wall (Parts II and III). Petri dishes were stored at room temperature 5–10 days until extraction. The lid and base of the Petri dish were extracted with 3 ml phosphate-buffered saline with 0.1% Tween 20 and 0.15% Kathon CG (Rohmand Haas, Hydrosupra Kemiservice, Helsingborg, Sweden; PBST_K). The extraction method was later enhanced (Parts II and III) with the following alterations: Petri dishes were extracted in 2 ml PBST_K and the extract was then rotated overnight at room temperature and finally centrifuged at 1000 g for 10 min. All samples were stored at -20°C until analysis.

Personal air sampling and filter extraction

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 with the sampling head attached in the breathing zone. Airflow rate was 2 l/min and the total sampling time was approximately 180 min.

After sampling, filters were stored at -20°C until extraction. Filters were thawed and extracted in 2 ml PBST_K during rotation overnight at room temperature. All samples were stored at -20°C until analysis.

Cat allergen enzyme linked immunosorbent assays (ELISA)

All samples from Petri dishes and air filters were initially analyzed neat in a monoclonal two-site sandwich-ELISA (6, 12). Samples near or below the detection limit (0.156 ng/ml) were then analyzed in an amplified ELISA assay. Rare samples with intra-assay coefficient of variation (CV) $> 10\%$ or inter-assay CV $> 20\%$ were re-assayed.

The Fel d 1 monoclonal antibody (mAb) kit including standard was purchased from Indoor Biotechnologies Ltd. (Cardiff, UK). Each incubation step was carried out at room temperature, followed by three washes with phosphate-buffered saline with 0.1% Tween 20 in a microtiter plate washer. All dilutions and blocking were made in PBST_K containing 1% bovine serum albumin (BSA) and 100 μl volumes were used in all wells throughout the assay, unless stated otherwise.

Microtiter plates (Maxisorp[®], Nunc, Roskilde, Denmark) were coated overnight with mAb 6F9, at 10 $\mu\text{g}/\text{ml}$ in 50 mM carbonate/bicarbonate buffer (pH 9.6). After washing, the plate was blocked for 60 min. Standards (range 0.156 to 10 ng Fel d 1/ml), samples and controls were added in duplicate and incubated for 90 min. Biotinylated mAb 3EC4, diluted 1/1000 was added and incubated for 60 min. The conjugate was an in-house streptavidine horseradish peroxidase used at 100 ng/ml and incubated for 30 min. Finally, plates were developed with 100 μl KBlue[™] (ANL-Produkter, Älvsjö, Sweden) and the colour reaction stopped by adding 100 μl 1 M H₂SO₄. Absorbance was measured at 450 nm using a microtiter plate reader (Spectra Max, Sunnyvale, CA) and samples were interpolated from a four-parameter curve using SOFTMAX PRO vs. 2.6.1 for Windows[™] software (GTF AB, Västra Frölunda, Sweden).

Samples below the detection limit were analyzed using a commercial signal amplification kit (Ampak[™], Dakopatts AB, Älvsjö, Sweden) according to the manufacturer's recommendations. The standard curve range was 2.5–160 pg Fel d 1/ml. Samples below this detection limit ($n=4$) were assigned a value of half of the detection limit for statistical calculations.

Statistical methods

All data were analyzed using STATVIEW 5.0 software (SAS Institute Inc., Cary, NC). Comparisons were made using the unpaired Mann–Whitney *U*-test. The Spearman Rank correlation test was used for correlation between methods. Agreement between duplicates was examined with Bland and Altman plot analysis (13). Sample distribution is presented as median and 25–75 percentiles. *P*-values less than 0.05 were regarded as significant. Total coefficient of variation (CV_t) was calculated from the percentages of difference (%D) of a series of duplicate measurements (X_1 and X_2) as follows:

$$\%D = \{ |(X_1 - X_2)| \times [(X_1 + X_2) \times 2^{-1}]^{-1} \} \times 100$$

$CV_t = \Sigma \%D \times (n \times \sqrt{2})^{-1}$, where n = the number of duplicate measurements (14).

Permission for the study was obtained from the Ethics Committee of the Karolinska Institute.

Results

Comparison between Petri dish and personal air sampling and cat allergen levels obtained by Petri dish sampling in classrooms and homes (Part I)

Petri dishes from four homes were not returned. There

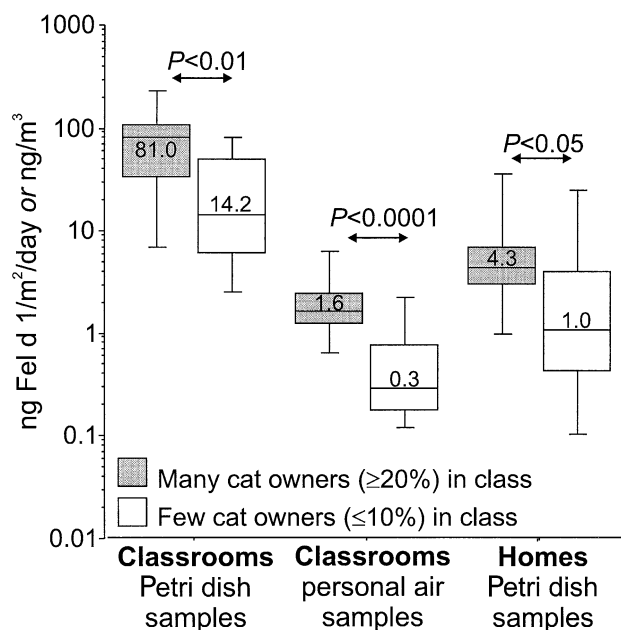


Figure 1. Mann-Whitney *U*-test of analyzed cat allergen levels in classes with many ($\geq 20\%$, $n=22$) and few ($\leq 10\%$, $n=22$) cat owners and in pet-free homes of children attending classes with many ($\geq 20\%$, $n=22$) and few ($\leq 10\%$, $n=18$) cat owners. In both classes and homes, Petri dish sampling of airborne settling dust was performed during the same week (ng Fel d 1/m²/day). In classes, additional personal air sampling with pumps was performed and the mean between two simultaneous samples has been used in the calculations (ng Fel d 1/m³). Box plots with medians are shown in figure where box corresponds to 25–75 percentiles and vertical lines correspond to 10–90 percentiles, respectively.

was a significant correlation between cat allergen levels obtained by Petri dish sampling and personal air sampling ($n=44$, $r=0.66$, $P<0.0001$). Median cat allergen levels in both Petri dishes and personal air samples were approximately five-fold higher in classes with many cat owners ($\geq 20\%$, $n=22$), compared to classes with few cat owners ($\leq 10\%$, $n=22$) ($P<0.01$ and $P<0.0001$, respectively) (Fig. 1). In homes of children without furred pets, levels of cat allergen at home was significantly higher ($P<0.05$) if they attended classes with many cat owners than classes with few cat owners.

Agreement between Petri dish duplicates (Part II)

Duplicate samples of cat allergen collected with Petri dishes were strongly correlated ($n=44$, $r=0.77$, $P<0.0001$). The agreement between duplicate Petri dishes is shown in Fig. 2. The total coefficient of variation between duplicate pairs was 56.5%.

Petri dish sampling height and sampling time (Part III)

Cat allergen could be detected in all 6 h Petri dish samples. The total cumulative amount of cat allergen collected in settled dust with five separate Petri dishes

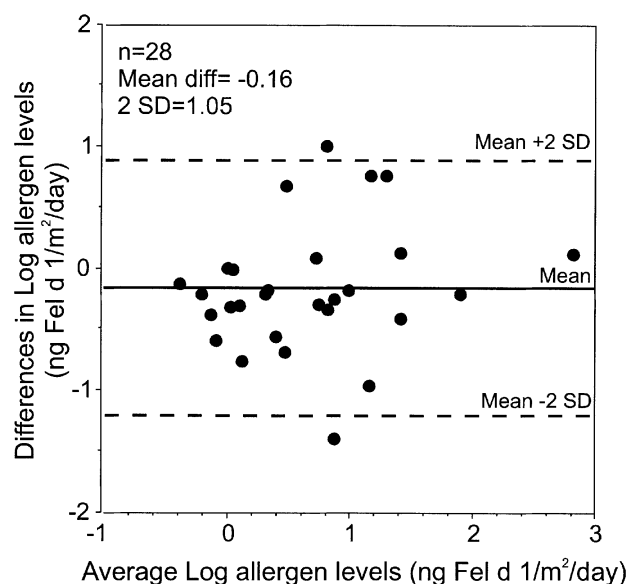


Figure 2. Bland-Altman plot to test the agreement between Petri dish duplicates ('A' and 'B') used for collection of cat allergen in settling dust during one school week in 28 classrooms. The Log₁₀ mean value for the duplicates (X-axis) is plotted against the Log₁₀ difference values between duplicates (Y-axis). The solid line indicates the mean of the difference between values and the dotted lines indicate mean ± 2 SD.

during one week was about 30% higher than the amount collected in settled dust with one single dish during the same time in all three classes, at all heights with one exception. Mean levels of cat allergen collected during weekends were low compared to sampling during school days (Table 1).

Discussion

Previous studies have not presented adequate evaluation of the technique nor comparisons with other techniques to quantify exposure. As a result of our work, we can quite confidently state that Petri dishes can be used as an alternative method to other sampling methods, especially in large-scale studies. In the present study it was shown that the correlation between Petri dish and personal air sampling was fair and cat allergen levels were significantly higher in classes with $\geq 20\%$ cat owners compared with classes with $\leq 10\%$ cat owners, regardless of sampling method.

We have demonstrated a correlation between personal air samples and Petri dish samples in classrooms although measurements were performed at only one occasion per method and classroom. This suggests that Petri dish sampling can be considered a useful method when assessing allergen exposure in environments such as classrooms. Petri dish sampling has several advantages, compared to personal air sampling. Cumulative collection of airborne settling dust with a Petri dish during a week gives an average allergen exposure level per day whereas a 180-minute air sample is more of a

Table 1. Mean daily cat allergen level (ng Fel d 1/m²/day) analyzed in settled dust collected with five daily Petri dishes ('Daily') and one weekly Petri dish ('Weekly') and during weekends in three different classrooms at four different sampling heights. Rate of cat ownership for class A (17%), B (22%) and C (28%)

Class	60 cm height (ng/m ² /day)		120 cm height (ng/m ² /day)		165 cm height (ng/m ² /day)		200 cm height (ng/m ² /day)	
	Daily	Weekly	Daily	Weekly	Daily	Weekly	Daily	Weekly
A	41.3	20.3	33.4*	28.2	18.4	29.3	48.0*	29.0
B	149.0	87.4	145.0	111.0	105.4	72.2	90.6	77.6
C	226.2	53.0	59.8	41.6	29.3	20.0	31.8	14.1
Weekends (all classes)	9.9 (n=5)		13.3 (n=5)		11.5 (n=5)		4.8 (n=4)	

*One Petri dish missing; mean daily allergen level calculated from four dishes instead of five.

point estimate. Hence, unlike cumulative sampling with Petri dishes, rate of pupil presence and movement in the classroom during sampling, which may affect the airborne allergen levels, may not be observed with personal air sampling during 180 min. In spite of these differences in sampling conditions there was a correlation between the two methods, which indicates that cat allergen levels obtained by Petri dish sampling are in proportion to the levels obtained by personal air sampling. Petri dishes are easy to use, inexpensive and more suitable for large-scale exposure assessment studies than personal air sampling. Air sampling requires expensive equipment, is labour intensive and a noisy sampling method, not always suitable for classrooms. Also, long sampling time or access to an expensive amplified analysis method is required in order to achieve detectable allergen levels. On the other hand, personal air sampling is always superior when assessing personal exposure and can be related to a specific time period or work task. Both sampling methods seem to be more suitable than measurement of dust reservoirs from upholstery, mattresses and floors, for estimation of allergen airway exposure in schoolchildren. Airborne cat allergen associated particles remain airborne for a long time and Petri dish sampling may thus be a better predictor for assessment of airborne allergen exposure. In addition, several studies have shown a poor correlation between airborne and dust reservoir Fel d 1 levels, which may indicate that dust reservoir measurement is a less appropriate method to use as an indirect index of personal allergen exposure (15–19).

In a previous study, we found a strong correlation between cat ownership and cat allergen levels in airborne dust in classrooms and that dispersed cat allergen from cat owners' clothes contaminates the clothes of non-cat owners and is subsequently brought to their homes (6). As was shown by Almqvist et al. rate of cat owners among the children in a classroom is an important factor influencing the content of cat allergen in airborne dust collected by pumps with filters. These findings were confirmed in the present study. The levels of allergen were approximately five-fold higher in classes with many cat owners, compared with classes with few cat owners, regardless of the method.

The allergen levels found in pet-free homes, although much lower than in classrooms, suggest a similar

transfer of allergens from cat-owners into noncat-owning homes from school. However, one must take into account the possibility of other exposure situations where allergens can be indirectly brought to the home. The levels were four-fold higher in homes of children attending classes with many cat owners, compared to those attending classes with few cat owners. Similar differences were observed in the classrooms. Airborne cat allergen in settled dust has not previously been detected in Petri dishes from homes without cats (20, 21).

The purpose of using holders attached to the wall was to create more standardized Petri dish sampling conditions and to test the agreement between Petri dish duplicates. The correlation and agreement between Petri dish duplicates in this study were fair, although the total coefficient of variation taking sampling, elution and analysis assay duplicate errors into account, was relatively high (14).

We observed a tendency for increasing cat allergen levels with decreasing height. A significant proportion of cat allergen is associated with small particles (< 5 µm in diameter) which therefore remain airborne for a long time before settling. Larger particles with higher density settle quicker and accumulate at a lower height and may easily be stirred up (21–24). Allergen levels did not differ significantly at 120 and 165 cm, indicating that heights in the breathing zone (sitting or standing position) implicate similar exposure and are suitable sampling heights for detection of cat allergen levels in airborne settling dust.

The mean level of cat allergen analyzed in the cumulative amount of dust collected with one Petri dish continuously during a week was lower than the level found using five consecutive Petri dishes during the same time. One explanation can be that settled dust was accidentally removed from the 5-day dish in the daily opening and closing process, whereas the 1-day dish was handled this way only once. We also showed that levels of cat allergen were low during the weekends which suggests that dust particles are undisturbed and that no additional allergen is brought into the classroom during that time. Hence, the contribution of allergen-associated dust particles settling during night-time is probably negligible. Continuous sampling during a week using Petri dishes takes daily allergen

level variations into account when expressed as average amount of allergen per day and may give a good estimation of indirect allergen exposure.

In conclusion, we have showed that Petri dishes can be considered as a useful and reliable sampling method for allergen exposure assessment. In addition, there was a fair correlation between a standardized method such as personal air sampling and Petri dishes. Rate of cat ownership among children in classes had a clear effect on cat allergen levels, regardless of method, which

further confirms that airborne cat allergen levels collected with Petri dishes is an appropriate index of personal exposure in such environments.

Acknowledgments

The authors would like to thank the participating children, schools and teachers and Siw Siljerud, Luca Perfetti and Hanna Hedrén for help with the distribution of material and information to schools. This study was supported by a grant from the Vårdal Foundation, Sweden.

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