

Variability of airborne cat allergen, Fel d1, in a public place

Abstract Allergen exposure is a risk to develop an IgE-mediated sensitization. The amount of allergen inhaled per unit time should be related to the amount present in the air, i.e. airborne allergen. Thus, measuring allergen levels in the air would be more relevant than measuring allergen levels in dust. Allergens are present in the air in very minute quantities and usually become airborne after disturbance. Large variation of allergen levels have been found in dust. In this study, we measured variability of airborne cat allergen, Fel d1, in a public place using a high-volume air-sampler. We also studied the distribution and relationship between dust and airborne cat allergens in homes and schools. Air samples were collected at three different airflow rates, i.e. 55, 40, and 30 m³ of air per hour. The concentration of airborne Fel d1 in the community gymnastic hall varied from 1 to 10 pg/m³ within a period of 3 weeks, at airflow rates 55–30 m³/h. The coefficient of variation for repeated samplings was 14–43% (day-to-day variation) and 27–38% (within-day variation). As expected, higher levels of airborne cat allergens were found in homes with cats than in cat-free environments. There was a significant relationship between cat allergen levels in dust and air ($r = 0.7$, $P < 0.01$). Our study demonstrates that when measuring airborne cat allergen a large variation is observed within a day and between days. The large variability of measurement may be explained by the disturbance in the environments. We suggest, that when exposure assessment is made the environment in question should be analyzed, if possible in several occasions.

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Practical Implications

Airborne cat allergens are associated with the levels of cat allergen in the dust. Cat allergens are commonly found in the public places where these animals are never kept. Thus, it seems that cat allergens are brought to public places in the clothes of people who have cats at home. The levels of allergens vary both within a day and day-to-day depending on the number of people visit this area. Outdoor clothing and shoes must be avoided in order to achieve allergen-free gymnastic halls.

Introduction

The relationship between exposure to indoor allergens and development of sensitization and asthma has been widely documented (Platts-Mills et al., 1995). The reduction of allergen exposure significantly reduced symptoms of asthma (Harving et al., 1994). Thus, determination of major indoor allergens is an important part of preventive measures to avoid sensitization and to reduce symptoms of allergic asthma.

The risk level of major allergens for sensitization and asthma have been suggested (Platts-Mills et al., 1992, 1995, 2001). These risk levels were based on the concentration of allergen in dust. However, later, it has been shown that children exposed to much lower levels of allergen are at risk for sensitization and

symptoms of asthma (Munir et al., 1997; Wahn 1995; Warner et al., 1996).

It has been suggested that allergen levels in the air would be more relevant than allergen levels in dust (Price et al., 1990). Various indoor allergens have been measured in the air of homes with and without pets, in schools and in day-care centres (Almqvist et al., 1999; Custovic et al., 1997, 1998; de Blay et al., 1991a, 1997; Luczynska et al., 1990; Price et al., 1990; Sakaguchi et al., 1993; Swanson et al., 1989; Warner et al., 1990; Wickman et al., 1999). Allergens are present in the air in minute quantities and usually detectable after disturbance. Cat allergen becomes airborne after minor disturbance and remains airborne for longer time than other airborne allergens, e.g. mites (de Blay et al., 1991b). In recent studies, it has been demonstrated that

a wide range of variation could be found when measuring allergens in the dust (Simpson et al., 1998).

During the past years, several studies pointed out that pet allergens are ubiquitous in the environment (Custovic et al., 1996; Enberg et al., 1993). Studies, including ours, showed that in public places where pet is not present the allergen is carried out there by people who have these animals at home (Almqvist et al., 1999; Berge et al., 1998; D'Amato et al., 1997).

The aim of this study was to investigate the variability of airborne cat allergen in repeated and parallel collections at different airflow rates using a high-volume air-sampling device. Furthermore, we aimed at studying the distribution of airborne cat allergens in various environments of children, i.e. homes and schools. The major cat allergen, Fel d1, was used as a model.

Materials and methods

Air sampling

Air sampling was performed using a high-volume electrostatic air-cleaning device (Munir et al., 1995). It consists of three separate units, a motor, a filter box, and two charged strings for charging metallic plates. The filter box contains 13 metallic filter plates. During airflow, airborne particles are charged and adhere to metallic plates. Three airflow rates of the pump can be adjusted to three different levels, 30, 40, and 55 m³/h. The airborne particles retention capacity of the filter was tested by the manufacturer (ELFI, Lidköping, Sweden) and it was found that the sampler

retains more than 90% of the airborne particles of sizes from 0.1 to 10 µm. In an experimental study in the laboratory, it was found that 72% of the allergen was possible to recover from the filter plates and the variability for repeated analysis was < 10%.

Variability study

The variability study was performed in a community gymnastic hall, approximately 6840 m³ (18 × 38 × 10). Nine air-samplers were used. Always the samplers were placed in the same location within the hall. All air-samplers were placed on a table within an area of about 1 m². The distance from the floor varied from 90 to 110 cm and the distance between samplers was about the width or depth of a sampler, i.e. about 10–20 cm. All samplers were run for a period of 24 h starting in the morning and ending the next morning at the same time. Air sampling was performed on six different days within a period of 3 weeks. The position of the air-sampler was different on different days to avoid influence of position. The hall was used for training purposes and each day about 200 people played sports in the hall. Only sports clothes and indoor shoes were allowed inside the hall. The hall was not cleaned during the study period. The samplers were run at three airflow rates, i.e. 30, 40, and 55 m³/h. Three samplers at each airflow rate, on the same day and also on different days were used to observe the variation of the collection within and between days. Thus, nine samplers were run at each airflow rate for studying the variability within a day and nine samplers for studying the variability between days. A total of 54 samples were

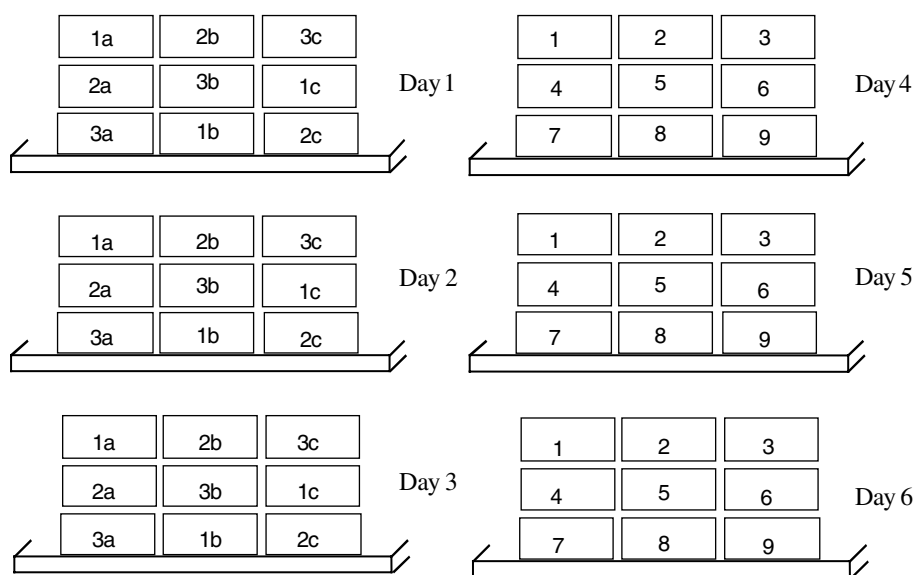


Fig. 1 Arrangements of air-samplers ($n = 9$) used during the collection of airborne allergens to study the variability of air sampling within a day (within day variation) and between days (day-to-day variation). Day 1: 1a-c = 55 m³/h, 2a-c = 40 m³/h, and 3a-c = 30 m³/h. Day 2: 1a-c = 30 m³/h, 2a-c = 55 m³/h, and 3a-c = 40 m³/h. Day 3: 1a-c = 40 m³/h, 2a-c = 30 m³/h, and 3a-c = 55 m³/h. Day 4: 1-9 = 55 m³/h. Day 5: 1-9 = 40 m³/h. Day 6: 1-9 = 30 m³/h

collected to study the variability of collection. The samplers were placed at the front of a table as illustrated in Figure 1.

Dust and air sampling from homes and schools

In addition to the variability study, dust and air samples were also collected from nine homes with cats, nine homes without cats, and seven classrooms from two schools in Linköping, Sweden. Dust samples were collected as described previously by us (Dreborg et al., 1995). In homes, carpets or sofas in the living room were vacuumed for 2 min/m² from an area of 2 m².

In schools, dust was sampled from tables and chairs, floors, curtains, and bookshelves separately. These surfaces were vacuumed for 1 min/m² from a total area of 8 m² (Almqvist et al., 1999). A 24-h air sampling was performed in homes (living rooms) and classrooms. Dust samples were collected on the day after air sampling. The air-samplers were placed in the living room or classroom about 1.5 m above the floor. All samples were collected by one person and one vacuum cleaner was used.

Extraction of dust and air samples

In all, 96 air and 44 dust samples were collected for analysis. Dust and air samples were extracted in ammonium bicarbonate buffer (pH 7.6) with 0.01% bovine serum albumin (BSA), overnight, at +4°C. The extracted air samples were lyophilized and reconstituted in phosphate buffer saline (pH 7.6) containing 0.5% BSA.

Allergen determination using ELISA

The major cat allergen, Fel d1, levels in dust and air samples were determined by ELISA as described previously (Munir, 1994). The ELISA kit was obtained from ALK, Hørsholm, Denmark. The inter-assay variation for repeated assay was <20% and the intra-assay <10%. The detection limit for cat allergen was 0.8 ng/ml of extract. This was equivalent to 8 ng of Fel d1 per gram dust in an extraction ratio of 1 : 10 (w/v) and 0.6–1 pg of Fel d1 per cubic meter air at airflow rate 55–30 m³/h.

Statistical analysis

Non-parametric statistical methods were used to analyze the data. The highest concentration of allergen in dusts obtained from the different locations in the same room was used to study the relationship between settled and airborne allergen using Spearman rank correlation coefficient test. Comparison between various sampling environments was performed using Kruskal–Wallis and Mann–Whitney *U* test.

Table 1 Variability of airborne cat allergen concentrations in repeated collection at various airflow rates. All samples were collected in a community gymnastic hall where no cats were allowed. One day's air sampling

	<i>n</i>	Airflow rates (m ³ /h)		
		30	40	55
Day-to-day				
Fel d1 (pg/m ³) ^a	4	3.9	4.7	4.0
Range (pg/m ³)		1.7–6.1	1.6–6.7	1.0–6.2
Variation (%CV) ^b		43%	32%	14%
Within-day				
Fel d1 (pg/m ³) ^a	9	8.2*	4.4	3.7
Range (pg/m ³)		2.9–10.0	2.8–5.3	2.2–4.4
Variation (%CV) ^b		38%	25%	27%

^a Median Fel d1 concentration.

^b CV, coefficient of variation.

* *P* < 0.05. All other comparisons were statistically non-significant.

Results

The median concentration and range of values of airborne cat allergen in the gymnastic hall at various airflow rates are given in Table 1. During the variability study, a wide range of variation was observed between days (day-to-day) and even in the same day between air-samplers (within day). The concentration of cat allergen in the gymnastic hall varied from 1 to 10 pg/m³ within the study period of 3 weeks. Both day-to-day and within-day variation was lowest, 14 and 27%, respectively, at an airflow rate of 55 m³/h. The highest coefficient of variation (38–43%) was observed at the airflow rate of 30 m³/h (Table 1), but significantly higher concentration of allergen was found using this airflow rate (*P* < 0.05).

The amount of allergen collected by different air-samplers varied but was not influenced by the position of the air-sampler. Thus, in one day the upper samplers contained the highest levels (2–7 pg/m³) of allergen and on other days the lower samplers showed the highest value (1–6 pg/m³).

Dust and airborne cat allergen levels found in home and school environments are given in Table 2. The airborne Fel d1 levels in homes with cats were significantly higher than the levels measured in homes without cats and the levels measured in schools (*P* < 0.01). The classrooms contained significantly higher level of airborne cat allergen than the level in

Table 2 Airborne cat allergen, Fel d1, in various indoor environments (homes and schools). Peak exposure level of allergen exposure in each environment was considered in the analysis. Airborne sampling was performed for 24 hours. Median and range of values are given. Comparisons were made between various environments

Sampling location	<i>n</i>	Settled Fel d1 (ng/g dust)	Airborne Fel d1 (pg/m ³ air)
Homes with cats	9	250,000 (1500–22,800,000)	3333** (5–9,375,000)
Homes without cats	9	250 (14–65,000)	1.2 (0.07–3.1)
Classrooms	7	346 (220–4450)	14* (0.32–42)

***P* < 0.01; **P* < 0.05. All other comparisons were statistically non-significant.

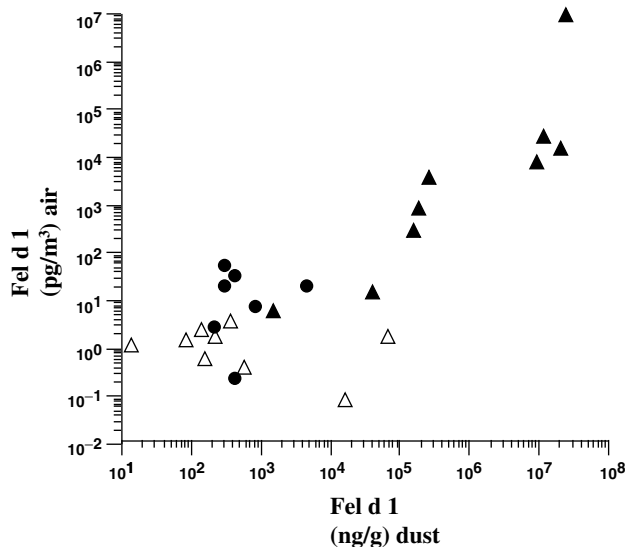


Fig. 2 Relationship between dust and airborne allergens in various environments. Homes with cats ($\blacktriangle$, $n = 9$), homes without cats ($\triangle$, $n = 9$), and classrooms in the schools ($\bullet$, $n = 7$)

homes without cats ($P < 0.05$). This trend was not significant for dust-bound cat allergen.

As expected, homes with cats contained significantly higher ($P < 0.01$) levels of Fel d 1 per gram of dust than did homes without cats and the classrooms. However, the levels of Fel d 1 in dust were similar in homes without cats and in classrooms (n.s.).

There was a significant correlation between the cat allergen levels in the dust and in the air ($r_s = 0.7$, $P < 0.01$) (Figure 2) when all samples from homes and schools were considered together. When the sampling environments were analyzed separately, the relationship was observed only in homes with cats ($r_s = 0.9$, $P < 0.01$).

Discussion

We could measure low levels of airborne allergens ($< 1 \text{ pg/m}^3$) using a high-volume air-sampler. As we used high airflow rates, which collect a large volume of air, it was possible to detect such low levels even using a conventional ELISA.

Several investigators used air-samplers to measure airborne allergens (Custovic et al., 1997, 1998; de Blay et al., 1995, 1997; Luczynska et al., 1990; Sakaguchi et al., 1993; Warner et al., 1990). Few of these samplers were of relatively higher volume. However, none of these measured airborne allergens repeatedly in the same environment. Thus, variability of measuring airborne allergens using air-samplers at different airflow rates is unknown. Our study shows that the variability of measurement of cat allergen in the air was wide and may vary in the range 14–43%. These variabilities were rather lower than the variability of allergens measured in settled dust (Simpson et al., 1998).

The concentration of allergen was almost similar from day-to-day. When studying the within-day variation, the highest level of allergen was found at the lowest airflow rate, although the variation was high (38%). The observed high within-day and day-to-day variation of air sampling could be due to several factors.

First of all, the position of the air-samplers may influence the outcome of the results. We placed all the nine air-samplers on a table within an area of about 1 m^2 . The allergen levels collected varied between the individual samplers and did not systematically contain higher or lower levels depending on the position of the pump. This means that the position of the pump did not influence the amounts of allergen collected or the variation of airborne allergen within a day.

Secondly, the turbulent of airflow close to the samplers may influence the allergen levels passing through the samplers. All samplers were placed in a big gymnastic hall, where hundreds of people played some kind of indoor games everyday. Many of these people may have cats at home. Cat and dog allergens are carried in the clothes of people, especially in the clothes of those who have these animals at home (Berge et al., 1998). Therefore, cat owners passing close to a sampler may cause a sudden increase in the local airborne concentration of cat allergen, which may passed through the sampler.

Thirdly, disturbance of settled allergens is of importance when measuring airborne allergens (de Blay et al., 1995, 1997). The disturbance of the dust in the gymnastic hall, which we used to study the reproducibility of air sampling, was not standardized. Probably, disturbance of settled dust varied between days, since the number of people present in the hall, their contacts with cats, and their activities varied between days. Before the investigation, we assumed that these factors would not influence the repeatability of our sampling to the degree observed. The number of people attending the hall was high. However, at the time of the study we did not consider any other location better suited for the investigation, since a room with a very large volume was needed to run nine air-samplers, each with a capacity of $55 \text{ m}^3/\text{h}$. Our study indicates that the number of persons in a room, the disturbance of the environment, and probably the number of cat owners visiting the room may influence the concentration of airborne allergen indoors and, these factors must therefore be controlled in experiments of this kind.

The variation of the sampling method itself can influence the outcome of the result when measuring variability of allergens. To study the precision of the air-sampler, the environment must be standardized in order to obtain reproducible samples. However, practically this is very difficult. A wide range of variation could be found when measuring allergens in the dust (Simpson et al., 1998). Similarly, airborne allergens also vary widely depending on the disturbances of the

environments. We found <10% variation when analyzing samples repeatedly. The within-day variation ranged from 25 to 38% in our study. Thus, it seems that the variation of airborne allergen observed in a day is more than the variation of the air-sampler. In the USA, Reed et al. (1986) found more than 50% variation when determining cat allergen in the bedroom air in five 24-h samples. However, in a standardized domestic activity in the UK, Price et al. (1990) found similar levels of airborne mite allergens in homes, 1 year apart.

Of course, the variability of airborne levels of allergens in the room depends on the ventilation system but the gymnastic hall we chose had a constant in- and outflow of air during day and night. All ventilation holes were situated close to the roof (10 m from the ground). Thus, it is unlikely that the ventilation system in the gymnastic hall could influence the airborne allergen levels observed in our study.

In general, we found low levels of allergen in our study. Such low levels of allergen may induce sensitization among high allergy risk children, as indicated by several prospective studies (Munir et al., 1997; Wahn et al., 1997). However, a recent study shows that children exposed to cats during the first years of life were less often skin test positive to cats at 12–13 years (Hesselmar et al., 1999). This study was a retrospective study and the other study by the same group in Estonia (prospective study) has shown that prevalence of positive skin test was decreased by age from 7% at age 6 months to 3 years, to 3% at 5 years of age (Julge et al., 2001). Another cross-sectional study in the USA reports that highest exposure to cats was associated with decreased sensitization (Platts-Mills et al., 2001). The development of sensitization is influenced by time at which the exposure takes place, dose of allergen, and the individual variation of sensitivity to a particular allergen. Thus, it seems that the sensitization process is exposure dependent (probably at low exposure level)

and high exposure may induce tolerance. Low levels of allergen unlikely to modify T-helper-2 cells response in order to develop tolerance.

We observed an association between settled and airborne cat allergen. This was also observed in other studies (Custovic et al., 1997; Price et al., 1990; Swanson et al., 1989). However, when carefully analyzing this relationship, an association was only found in homes with cats. The disturbance in the environment is important when measuring airborne allergens (de Blay et al., 1997; Luczynska et al., 1990). The number of cats or the number of persons in homes varied which in turn may influence the disturbance of the settled dust. However, we did not ask these parameters in our study. In homes with cats, disturbance of dust bound cat allergen is probably more pronounced than in other places where cats are not present (Custovic et al., 1998; de Blay et al., 1991a).

Our study shows that when measuring airborne cat allergen in a public place, a high variability is observed within a day and between days. The reason for the observed large variation may be explained by the disturbance in the environment, which remains to be investigated in future experiments. The study suggests that when exploring exposure of allergic/asthmatic patients, the environment in question should be monitored, if possible on several occasions.

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