Cat and house dust mite allergen content is stable in frozen dust over time

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Abstract

Background: Dust from indoor environments consists of animal allergens, pollen, endotoxins and other substances which may exacerbate symptoms in sensitive individuals. In prospective cohort studies, dust is often collected from indoor environments in order to assess allergen exposure and possible relationships to health outcomes. Typically, large numbers of samples are collected and kept frozen until further analysis, sometimes several years later. To date, there is insufficient knowledge about what happens to the dust and its contents during storage.

Objectives: In the present study, our aim was to analyse allergen content over a 30 month period frozen dust collected from beds in homes in order to simulate a study design of exposure assessment commonly used in epidemiological studies.

Methods: Thirty-seven dust samples from mattresses in homes were collected using a Duststream dust collector. Each dust sample was subdivided into six aliquots. One tube (baseline) was extracted and analysed for cat and HDM allergen content using ELISA, all other tubes were stored at -80°C until further handling. Approximately every six months (6, 12, 18 and 30 months), dust from one tube was thawed, extracted and analysed the same way. Data was log-transformed and analysed using linear regression.

Results: No trend for decreasing or increasing cat (p=0.606) or house dust mite (p=0.928)allergen levels could be observed over time. Levels of cat allergen were considerably higher in mattresses from homes with cats compared to homes without cats (p<0.001).

Conclusion: It is important to assess the allergen stability in dust before designing costly and labour-intensive studies of allergen exposure and health outcomes, commonly used in environmental epidemiology. Although the present study showed that cat and HDM allergens

remained stable in dust stored at -80°C during a 2.5 year period, analyses of other allergens or

substances in frozen dust is desirable as well as evaluating the effect of longer storage times.

Introduction

Reservoir dust from indoor environments contains a cocktail of animal allergens, pollen, endotoxins and other substances/particles. Exposure to dust may exacerbate symptoms in sensitive individuals [1]. Dust samples are typically collected from surfaces such as floors, chairs and tables but also from beds, soft furniture and even clothes [2], using a vacuum cleaner equipped with a dust collector device. Most samplers are originally intended for sampling of house dust mite (HDM) allergens but are very often used for collection of pet allergens which are, unlike HDM allergens, predominately airborne.

In prospective birth cohort studies within the field of asthma and allergic disease, reservoir dust is often collected from homes or other indoor environments in order to assess allergen exposure and its impact on symptoms and disease development [3-6]. Typically, large numbers of samples are collected during a short time period during the initial phase of the study and neat dust samples are kept frozen until further analysis, which often occurs several years later. Regardless, it is more appropriate to store neat dust samples instead of extracted dust samples since allergen content in extracted, frozen samples (-20°C) may decrease by more than 50% during a 6-year period [7]. To date, there is insufficient knowledge about the stability of the allergens and other health-relevant substances in dust during storage. To collect large numbers of dust samples in birth cohort studies is costly and personnel-intensive and it is of great value to obtain information on how allergen content in dust changes over time.

Our aim was to analyse house dust mite (HDM) and cat allergen content, at regular time intervals, in dust collected from bed mattresses in homes with and without pets. We hypothesise that the allergen content in frozen dust will decrease over time. The study was a pilot study in the LifeGene project where several hundred thousand Swedes will be recruited to study how genes, environment and lifestyle factors affect health [8].

Methods

Collection and handling of dust

Thirty-eight dust samples were collected from bed mattresses in homes (n=29) with and without pets, using a Duststream dust collector (Medeca, Uppsala, Sweden). In some homes, several dust samples were collected but from different beds in different rooms. Approximately 1-2m^2 of the bed mattress, underneath the sheet, was vacuumed in a standardised way for 2-3 minutes [9, 10]. The sampling was performed in May and June (spring) 2008. The collector was sealed and stored at room temperature for approximately two weeks until further handling.

The dust was removed from the collector into a petri dish. Large items such as grit, stones etc. were discarded by using a pair of tweezers before distributing the dust into six pre-weighed 15mL Falcon tubes (approximately 100 mg dust/tube, exact weight recorded on the tube). The tubes were randomized and labelled A-F, representing the different analysis time points. For several dust samples (n=13), dust was not sufficient for all six aliquots (Table I). Three aliquots was considered as an acceptable number for each dust sample and only one dust sample (sufficient for two aliquots) had to be discarded due to insufficient amount of dust to fulfil this criterion and was therefore excluded from further calculations. On average, each dust sample was distributed into 5 aliquots. Each tube was weighed again, randomized and labelled A-F, each representing an analysis time-point (0 months-baseline-unfrozen, 0 months-frozen, 6 months-frozen, 12 months-frozen, 18 months-frozen, 24 months-frozen). Tube A (baseline, 0 months, unfrozen) was extracted and analysed for allergen content straight away using ELISA (see details below), all other tubes were stored at -80°C until further handling. Tube B (0 months-frozen) was extracted and analysed a few days later. Every six months (6, 12 and 18 months), one tube from each dust sample was thawed,

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extracted and analysed the same way. Tube F (24 months-frozen) was analysed at a 30 month time-point, instead of 24 months, and will hereafter be referred to as 30 months-frozen. In total, each dust sample was analysed at on average 6 different time points.

Extraction of dust

Four 3 mm glass beads were added to each tube, followed by PBS-0.05 % Tween (w/v 1:20). The tube was properly mixed on a vortex and then rotated during 2 hours. The tube was then centrifuged 1500xg during 10 minutes and 1 mL of the supernatant was transferred to a 1 mL-Eppendorf tube and centrifuged at 7000xg during 10 minutes. Supernatant was distributed into 2 aliquots and kept frozen at -20°C until analysis a few days later.

Allergen analyses

Cat allergen

Vacuumed dust samples were analysed for cat allergen, Fel d 1, with a monoclonal sandwich ELISA used and described previously using reagents from Indoor Biotechnologies Ltd (Cardiff, UK), as presented previously. However, the standard used in the present study was a multi-allergen standard, termed the universal allergen standard, developed more recently (1000 ng/mL), which consists of eight purified allergens. Conversion factors have been defined in order to facilitate transition between the universal allergen standard and previous standards, as described by Filep et al. [11]. Microtiter plates (Maxisorp™, Nunc, Roskilde, Denmark) were coated over night at 4-8°C with mAb 6F9, at 2 μg/mL in 50 mM carbonate/bicarbonate buffer (pH 9.6). After washing, plates were blocked for 30 minutes. Standards (range 60 to 4000 pg Fel d1/mL), samples and controls were added in duplicate and incubated for 60 minutes. Biotinylated monoclonal antibody 3EC4, diluted 1/1000 was added and incubated for 60 minutes. Streptavidine horseradish peroxidase diluted 1/1000 was incubated for 60 minutes. Finally, plates were developed with 100 μL KBlue[®] (Neogen Corp.,

ANL-Produkter, Älvsjö, Sweden) and the colour reaction was stopped by adding 100 μL

1M H_2SO_4. Absorbance was measured at 450 nm using a microtiter plate reader and

concentration levels were interpolated using a 4-parameter curve fit. The detection limit of the

assay was 60 ng/mL. For statistical calculations samples below this detection limit were

assigned a value of half of the detection limit.

House dust mite allergen

The assay for house dust mite allergen was performed as described above for cat allergen,

with the following exceptions.

Monoclonal antibodies 5H8 and 4C1-biotin and the universal allergen standard (see above)

were purchased from Indoor Biotechnologies Ltd. After coating, the content in the wells was

discarded without washing and plates were blocked for 60 minutes. Standards (range 62.5 to

4000 pg Der p1/mL), samples and controls were incubated for 90 minutes during shaking.

Streptavidine horseradish peroxidase was diluted 1/400.

Statistical calculations

The data was analysed using STATA version 11 (StataCorp LP, College Station, Texas). Data

was log-transformed and analysed for trend using simple linear regression terms. A

comparison between means was also used. A *P*-value < 0.05 was considered significant.

There were 37 observations (one dust sample was discarded due to insufficient amount of

dust) and a total of 203 dust sample aliquots. Variances were calculated with *robust between-*

cluster variance estimator for cluster-correlated data, which in Stata is known as

Huber/White/sandwich estimate of variance [12].

Results

Table II displays characteristics of the 29 homes where dust samples were collected. The majority of the samples (58%) were collected in houses rather than apartments or other type of dwelling. Thirty-two percent of the homes reported pet ownership, and 25% owned a cat.

Allergen levels obtained from sample A (0 months, unfrozen) did not differ significantly from sample B (0 months, frozen), either for cat (P=0.435) or HDM (P=0.390). Hence, sample A was used as baseline value.

Table III shows mean/median values of cat allergen levels (ng/g dust) for each time point, regardless of presence of cat. At baseline (0 months), levels were higher than the following time points but this difference was not significant.

Table IV shows that cat allergen levels at each time point were substantially higher in homes with cats, than homes without cats (p<0.001).

Figure 1 displays cat allergen content for each sample over time, in homes with or without cats. No trend for decreasing or increasing cat allergen levels could be observed over time (p=0.606, adjusted for cat ownership).

Table V displays mean/median values of house dust mite allergen levels (ng/g dust) for each time point.

Figure 2 shows house dust allergen content in each sample over time. No trend for decreasing or increasing house dust mite allergen levels could be observed over time (p=0.341).

Discussion

In the present study, we evaluated cat and HDM allergen content in frozen mattress dust over a time period of 30 months and found that the levels remained stable and no increasing or decreasing trend could be observed for either allergen.

The study design is unique, i.e. sampling of dust at one occasion, keeping the dust frozen in aliquots for over two years at -80ºC and extracting the allergens at each time point, followed by prompt allergen analysis. Fahlbush et al. conducted a similar study but during a considerably shorter time period, 10 months, and storage of dust at -20ºC. They observed no effect on HDM but a decrease of cat allergen levels [13]. Another study has shown that levels of HDM (Der p 1) in house dust remain the same after 47 months of incubation under domestic conditions [14]. For frozen allergen extracts however, allergen content declines over time [7]. This is often due to adsorption of protein onto the surface of the plastic vials, which can be partly prevented by the addition of protein (Bovine serum albumin, BSA) to the allergen extract. Another cause could be the presence of proteases in the extracts. For instance, proteases are present in extracts from fungi, cockroaches and house dust mites [15, 16]. In the present study, the dust extracts consisted of a cocktail of proteins and other substances and it is reasonable to believe that some of them are proteases which can break down the proteins of interest. We used low-absorbent plastic vials during the extraction process but did not add BSA to the extracts. Previously, we conducted a study where extracted and frozen dust samples were stored for six years at -20ºC prior to analysis. The content of horse allergen declined more than 50% during this period [7].

The purpose of the study was to simulate a situation where a large number of dust samples are collected, for instance in epidemiological studies, for later analysis and exposure assessment. It is of paramount importance to have knowledge about the effect of storage of dust on levels

of allergens and other biocontaminants, otherwise the exposure assessment may be misleading and difficult to interpret. In addition, collecting dust from homes is very labor-intensive and expensive, which further emphasizes the importance of correct handling.

Although we observed no significant effect on allergen levels in dust stored at -80ºC during 30 months, we cannot extrapolate the results for longer storage times, which is often the case in epidemiological studies.

As expected, cat allergen levels were much higher in mattresses from homes with cats than homes without cats. Our aim was to include samples with a wide range of allergen content to study if the allergen levels differed over time depending on allergen concentration. This was however not the case in our study.

The allergen levels were overall unchanged but varied between time points, in particular HDM. The handling, extraction and analysis procedures were performed by the same person at all times but there are factors which could explain the variation. For instance, dust was distributed into aliquots of approximately 100 mg each but the dust aliquots ranged from 22 mg to 131 mg (mean 87 mg) depending on total amount of sampled dust. Since an extraction protocol was used (w/v 1:20), different volumes of extraction buffer was added each time depending on weight. This may have affected the extraction efficacy, especially in those samples with small amounts of dust. Although we tried to homogenize the dust prior to distribution into aliquots, there may be some differences between aliquots, within each dust sample.

In conclusion, levels of cat and HDM allergen content in frozen mattress dust seem to be stable over a time period of 30 months. However, it is important to properly assess the allergen stability in dust before designing costly and labour-intensive studies of allergen

exposure, commonly used in environmental epidemiology. In order to do so, analyses of dust stored during a considerably longer period are desired. Regardless, the results from this study are promising for current and future epidemiological studies elucidating the association between early allergen exposure and subsequent allergic disease.

Acknowledgements

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Table I. Number of analysed samples for each time point (among the 37 dust samples).

	0 months unfrozen (A)	0 months frozen (B)	6 months frozen (C)	12 months frozen (D)	18 months frozen (E)	30 months frozen (F)
No of analysed samples (cat and HDM)	37	26*	37	35	36	30

*When dust was not sufficient, we primarily chose to exclude this time point

Table II. Characteristics of the homes (n=29) where dust samples were collected

Home characteristics	
House	58 %
Apartment	31 %
Other type of dwelling	11 %
Homes with any pet	32 %
Homes with cats	25 %
Mean number of occupants (min–max)	3.4 persons (1–5)
Bed sheets changed < 1 week prior to sampling	27 %

Table III. Mean/median values of cat allergen levels (ng/g dust) for each time point

Month	n	Mean	CI	Median	25th percentile	75th percentile
0 (unfrozen)	37	761	281–2064	378	55.5	14328
0 (frozen)	26	587	186–1853	259	47.9	4392
6	37	513	186–1416	169	60.1	12195
12	35	573	203–1618	183	55.4	9526
18	36	491	187–1288	176	49.3	8749
30	30	548	183–1644	193	63.5	7746

CI: Confidence interval

Table IV. Mean/median values of cat allergen levels (ng/ g dust) for each time point, stratified for cat at home or no cat at home.

		No cat				Cat		
Month	**n**	**Mean**	**Median**	**CI**	**n**	**Mean**	**Median**	**CI**
0 (unfrozen)	26	133	108	77.1–230	11	47028	61113	23794–92951
0 (frozen)	19	130	95.2	67.9–249	7	35035	53239	10729–114406
6	26	85.1	85.9	50.7–143	11	35911	42412	18418–70017
12	25	105	95.7	59.1–185	10	40094	51534	17426–92250
18	26	99.7	88.5	61.6–161	10	30956	22852	14072–68099
30	22	117	111	61.0–226	8	38042	46164	15004–96456

CI: Confidence interval

Figure 1. Cat allergen levels (log values) at each time point, for each dust sample (n=37), stratified for cat at home (purple, n=11) or no cat at home (pink, n=26)

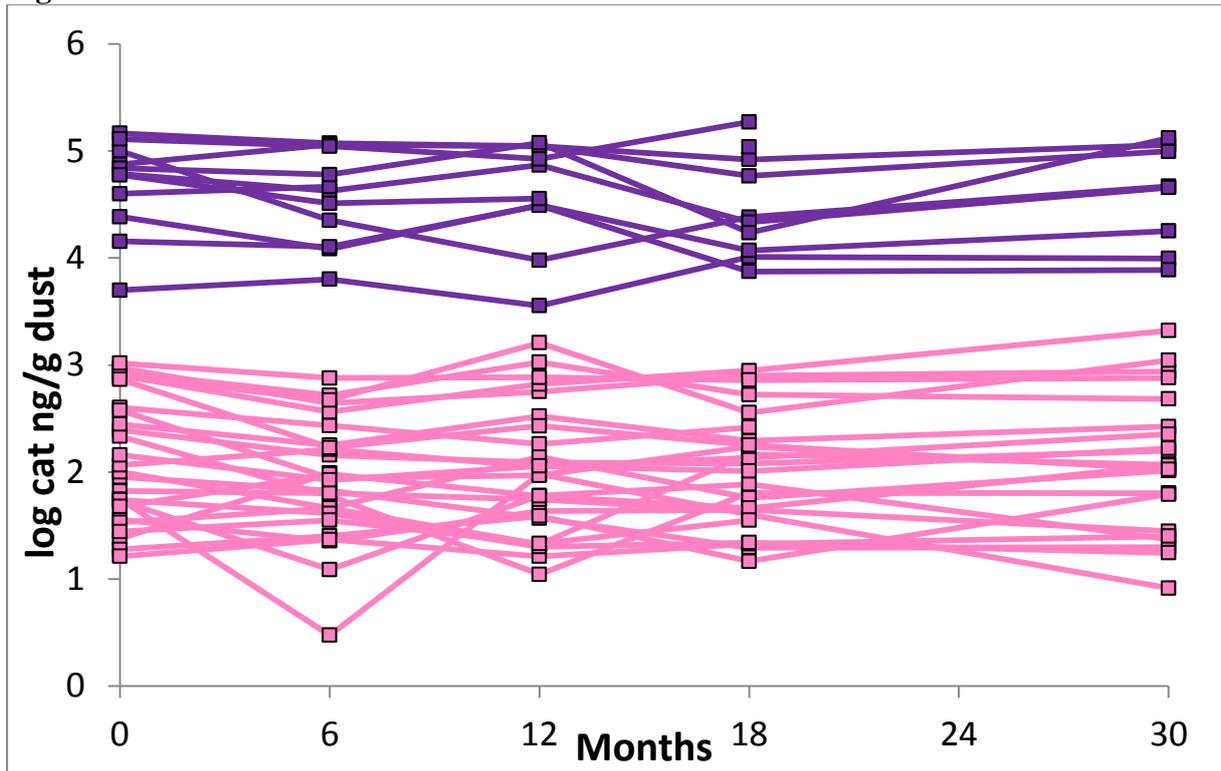**Figure 2. House dust mite allergen levels (log values) at each time point, for each dust sample (n=37)**

Figure 1

Figure 2