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RESEARCH LETTER

WILEY

Individual airborne characteristics of dog allergens

To the Editor,

Exposure to dog allergens is almost impossible to avoid, as dogs are part of our society and frequently encountered both outdoors and indoors. This poses problems to individuals allergic to dogs, a common condition with reported sensitization rates around 20%.[1] It is therefore crucial to increase our understanding on how dog allergens spread in the environment and on exposure by inhalation. Previously, the major dog allergen Can f 1 has been quantified using reservoir dust samples, air sampling on filters or electrostatic dust collectors.[2–4] The results are affected by extraction method and deployment time, which might be up to weeks. When sampling over such prolonged periods in homes or public areas, the origin of the collected material may be questioned.

The size of airborne particles carrying the major dog allergen Can f 1 has been investigated using an eight-stage Andersen cascade impactor. The major part of Can f 1 was found to be associated with sizes larger than 9 μm in aerodynamic diameter, while a lesser part (20%) was found in particles smaller than 4.7 μm.[5] No data have been reported on the size of airborne particles carrying other dog allergens, or on samples collected under controlled experimental conditions.

In this study, we aimed for the first time to characterize the five dog allergens Can f 1, 2, 3, 4 and 6 in airborne particles, in terms of concentrations and aerodynamic diameters. Sampling was performed in a controlled laboratory environment, with a defined source of allergens in a sealed air chamber with adjustable airflow rate and minimal background contamination.

Four dogs were included in the study. Before sampling of aerosol particles, fur and saliva samples were collected using a brush sampling kit (Medi-Tec Research and Development Stockholm) according to the manufacturer's manual. Dog allergens were extracted and quantified by ELISA as previously described.[6] The four dogs revealed individual allergen profiles (Table S1). Higher levels of Can f 1 than Can f 4 were found in fur. Can f 2 was exclusively detected in saliva, while Can f 6 was abundant in both fur and saliva samples. Can f 3 was only detected in a saliva sample from one of the dogs. The detection limit is higher for the Can f 3 assay than for the other assays, but, when present, the serum albumin Can f 3 is usually found at very high concentrations in fur and saliva samples.[6] All dogs were healthy with intact skin and thus at low risk for leakage of plasma proteins, possibly explaining why no albumin was found in fur samples.

For sampling of aerosol particles, each single dog spent two hours in a stainless-steel chamber of 22 m^3 together with its owner. The owners wore clean air suits (Mölnlycke Healthcare) to prevent human contribution to the aerosol samples. In order to release allergens to the air, the owner played with the dog and petted its fur. The chamber had a ventilation rate of 0.5 air exchanges per hour, similar to normal home ventilation. During the two hours period, released airborne particles were sampled using three sampling techniques. Allergens in the samples were recovered by extraction and quantified by ELISA.[6]

The distribution of dog allergens in airborne particle fractions was analysed using a multi-stage impactor with eight size fractions (Next Generation Impactor, Copley Scientific) from 0.14 to >8.1 μm aerodynamic diameter operating at an airflow rate of 60 L/min during the entire 2 h period. To extract allergens, each stage was swabbed with a wetted nylon swab (Copan Scientific) that was placed in 1 ml phosphate-buffered saline (PBS), vortexed to release the material from the swab and centrifuged. Can f 1 was present in the three fractions corresponding to the largest particle sizes, >2.8 μm, and highest concentrations were found in the size fraction >8.1 μm (Figure 1A), in concordance with earlier reported data.[5] In contrast, Can f 4 and Can f 6 were detected in all size fractions from 0.14 to >8.1 μm (Figure 1B,C). This wide distribution, including a significant portion in sub-micrometre particles, could impact on the allergenicity for two main reasons: the longer exposure time for inhalation of small particles due to their likelihood to stay airborne for extended time periods and the ability of small particles to deposit to all parts of the respiratory tract. Particles below 2 μm can to larger extent reach into the alveolar region of the lungs, providing a greater potential to act systemically, trigger mast cells and elicit allergic reactions.[7] Previously, 20%–30% of investigated pet allergens have been associated to particles <5 μm and linked to higher airway responsiveness, systemic inflammation and asthma.[8] In particular, Can f 6 was evenly distributed over the particle sizes. This allergen, with a reported sensitization rate of 38%, cross-reacts to cat and horse allergens.[9] Despite being a minor allergen, its aerodynamic properties together with its cross-reactive nature imply that it may have a significant impact on allergy to pets. No Can f 2, nor Can f 3, was detected in the cascade impactor samples (not shown).

Unfractionated aerosol particles were collected by two additional methods. To provide a measure of the total concentration of Can f 1–4 and 6 in the air, collection onto two PTFE filters (pore size

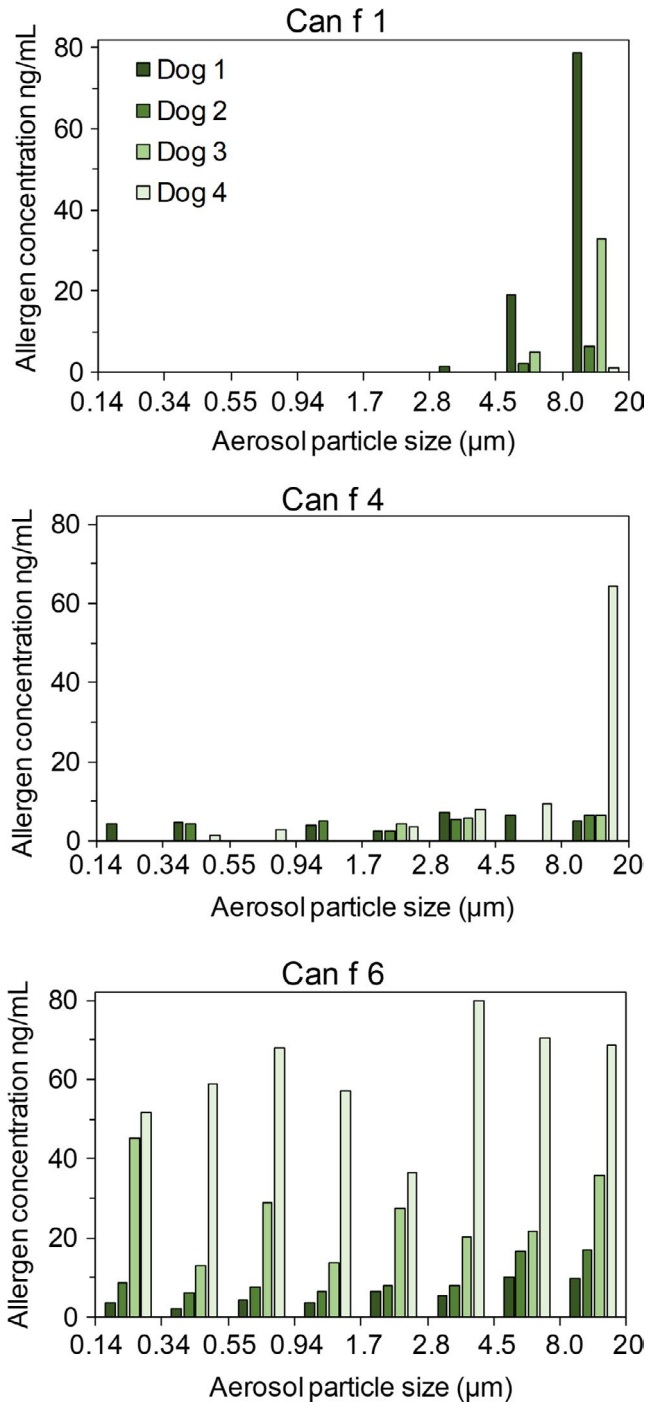

FIGURE 1 Dog allergen concentration in size fractionated air samples. Allergen concentrations measured in fractionated air samples collected with a multi-stage impactor with eight particle size fractions with aerodynamic diameters: 0.14–0.34, 0.34–0.55, 0.55–0.94, 0.94–1.7, 1.7–2.8, 2.8–4.5, 4.5–8.1 and >8.1 µm. (A) Can f 1, (B) Can f 4 and (C) Can f 6

0.3 µm, Whatman International Limited) was performed at an airflow rate of 20 and 40 L/min, respectively, over the 2 h sampling period. The collected material was extracted from the filters by overnight incubation in 1 ml PBS on a tube rotator and subsequent centrifugation at 4200 *g* for 10 min to retrieve the supernatant. Moreover, particles were collected using a liquid cyclone (Coriolis µ, Bertin Technologies) that sampled airborne particles into 20 ml PBS at a flowrate of 200 L/min for 10 min per sample. The samples were concentrated to 1 ml using Amicon Ultra centrifugal filters at 4000 *g* for 15 min (10 kDa, 15 ml, Merck Millipore) before analysis of Can f 1–4 and 6. The two sampling methods rendered somewhat divergent results. Can f 1, 2, 4 and 6, but not Can f 3 (not shown), were found in all filter samples, with highest concentrations detected for Can f 1 (Figure 2A). In contrast, only Can f 6 was found in liquid cyclone samples from all dogs. Can f 6 was also detected at higher concentrations than Can f 1 and 4, that were detected in samples from one and three dogs, respectively (Figure 2B). Can f 2 and 3 concentrations were both below the detection limit (not shown). The fact that Can f 4 and Can f 6 were detected on smaller particles than Can f 1 (Figure 1) implies that they might spread further or remain airborne longer, possibly explaining their higher abundance compared to Can f 1 in the cyclone air samples.

The strength of the present study is that five individual dog allergens were measured simultaneously in air samples collected at controlled conditions with a minimal impact of environmental contaminants. Limitations include the small study population and the short sampling time. The distribution pattern of allergens over different particle sizes is however consistent. Our results also indicate that the likelihood of aerosols from saliva of staying airborne is lower than for fur-derived particles. This would explain why Can f 2, exclusively present in saliva in all four dogs, was only detected in filter samples in negligible amounts (Figure 2A) and not at all using the other sampling methods. The prostatic allergen Can f 5 was not analysed in the study, since only one male dog participated and Can f 5 is only found in unaltered males. The dogs in the study were of three different breeds (Table S1). Previous data show that the individual variation in allergen levels from fur within a breed is similar to the variation between breeds.[6,10] The sample size in the present study was too small for drawing any conclusions on differences between breeds regarding the capacity to shed airborne allergens from the fur, although this is an interesting topic for future investigation.

In conclusion, we here present data on airborne properties of individual dog allergens beyond Can f 1. Despite the limited size of the study population, we show that dog allergens are present in a wide span of aerosol particle sizes, from 0.14 to >8.1 µm, each allergen with a distinct particle size distribution. Notably, Can f 4 and Can f 6 were in contrast to Can f 1 detected in sub-micrometre fractions. This may have implications on the allergenicity of the individual dog allergens.

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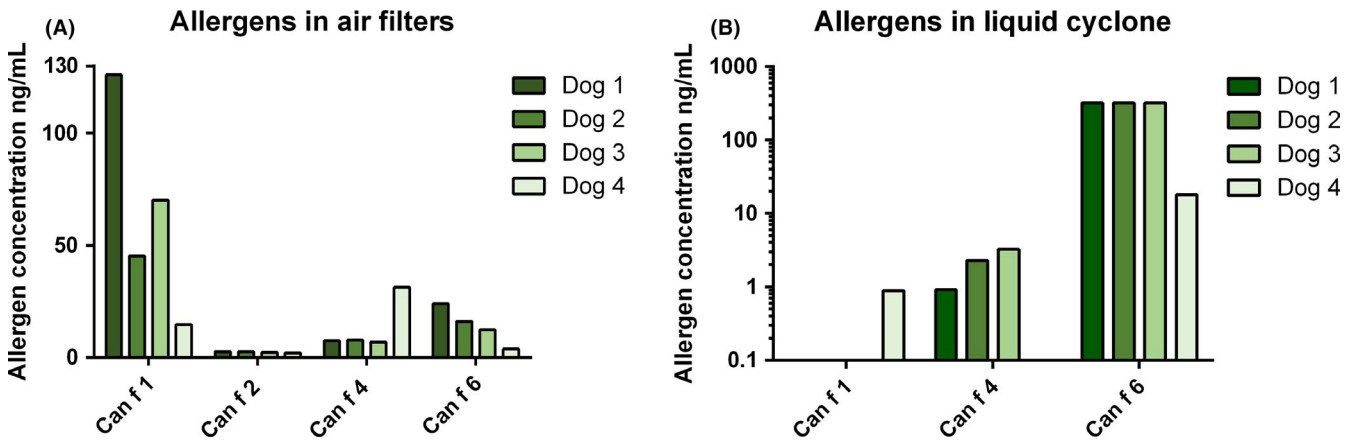

FIGURE 2 Allergens collected on filters and by a liquid cyclone. (A) Amount of allergen (ng/ml) collected on filters (average concentration from two filters operating at 20 and 40 L/min) from each dog. (B) Amount of allergen (ng/ml) collected by the liquid cyclone (average concentration of two samples per dog). *Y*-axis is logarithmic. Low levels (<1.8–3.6 ng/ml) of Can f 4 were detected from dog #1–3 and high levels of Can f 6 (>320 ng/ml)

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CONFLICT OF INTEREST

All authors declare no conflict of interest regarding this manuscript.

AUTHOR CONTRIBUTION

AW involved in data collection and analysis, experimental design and manuscript writing. MA involved in data collection and analysis and manuscript review. JJ involved in data collection and manuscript review. SS involved in hypothesis and manuscript review. HG involved in hypothesis, data analysis and manuscript review. JL involved in experimental design, data analysis and manuscript review. GG involved in hypothesis, experimental design, data analysis and manuscript writing.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available in the Supporting Information of this article.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.