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Allergens in dog extracts: Implication for diagnosis and treatment

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Abstract

Background: Five to ten percent of the population in affluent countries are allergic to dog. Diagnosis and treatment is based on allergen extracts from natural sources where composition and concentration are poorly defined.

Objective: We aimed to quantify six dog allergens (Can f 1-6) in commercial skin prick test (SPT) solutions and to determine individual allergen profiles in dogs.

Method: The allergen content of SPT solutions from five vendors and allergen source material from three anatomical sites were analyzed. Fur and saliva samples were collected from a mixed population of 120 dogs. Can f 1-6 were quantified by inhibition ELISA using purified recombinant or natural allergens and polyclonal or monoclonal antibodies. Allergenicity was analyzed by basophil activation test.

Results: Extensive variation in allergen composition was observed in commercial SPT vials resulting in a patient-dependent ability to activate basophils. Extract heterogeneity depended on collection site and allergen composition in individual dogs and source materials. Can f 2 and Can f 6 exhibited low levels in fur and SPT solutions, whereas Can f 4, which was the dominating allergen in fur samples, did not display similar high proportions in SPT solutions. Can f 3 varied most among SPT solutions.

Conclusion: There is a great variation of dog allergens in natural extracts raising questions of source, sampling, processing and ultimately of standardization and minimum allergen levels for accurate diagnosis and treatment.

KEYWORDS

allergy, dog allergen extract, dog allergens, skin prick test

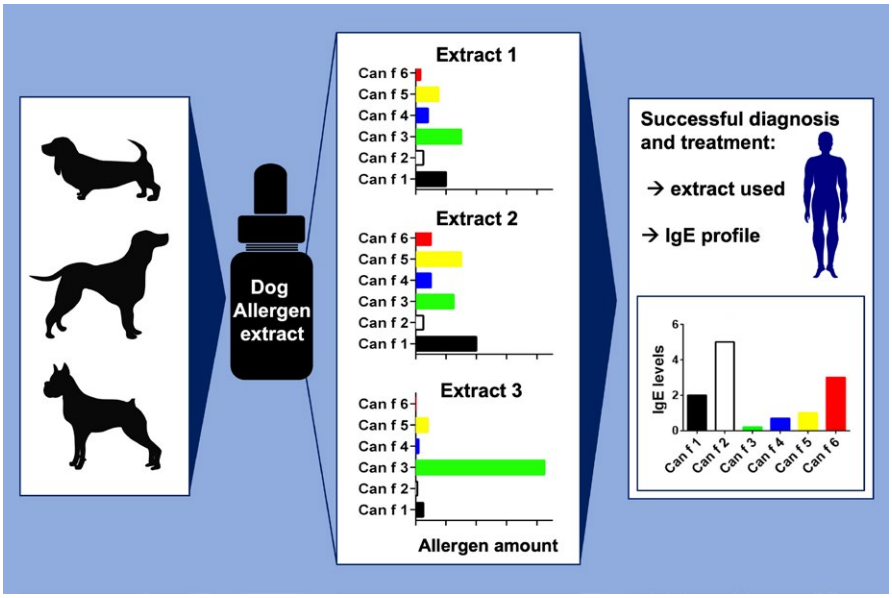
1 | INTRODUCTION

Dogs are common in society. Only in the UK, the dog population is estimated to 11.6 million[1] and in Australia the number equals 4.2 million.[2] Dogs are often kept as pets, but may also serve in assistance by, for example, the police, health care, and customs, effectively placing them in many public places. Dogs produce allergens which easily become airborne and can cause sensitization and subsequently allergy.[3] This is a steadily increasing affliction worldwide, being responsible for respiratory symptoms such as rhinitis and asthma.[4] This in turn leads to reduced professional performance and quality of life for affected individuals.[5]

Dog allergen source material is excreted in individually unique compositions and concentrations at distinctly different anatomical sites, the skin (dander/hair), mouth cavity (saliva), and

Abbreviations: AIT, allergen-specific immunotherapy; HSA, human serum albumin; SPT, skin prick test.

Gafvelin and Grönlund shared last authorship.



GRAPHICAL ABSTRACT

Raw material for dog allergen extracts comes from dogs with various allergen profiles. Commercial dog allergen extracts vary in allergen content. Successful diagnosis and treatment of a dog allergic patient depends on the extract used for diagnosis/treatment and on the IgE profile of the patient.

prostate/bladder (urine).[6] The composition complexity increases by cross-contamination of these sources within the dog. To date, seven dog allergens have been identified. Can f 1, Can f 2, Can f 4, and Can f 6 are members of the lipocalin protein family. Can f 1 is considered a major dog allergen to which 50%-70% are sensitized.[7,8] High IgE levels to Can f 1 early in life have been proposed as the most important predictor for developing dog allergy later in childhood.[9] Can f 2 causes sensitization in around 25%.[10] Both these proteins are abundant in saliva,[6] but Can f 1 is also found in fur.[11] Can f 3, dog serum albumin, displays a high homology to other mammalian albumins. It is present in dog dander, saliva, and urine in addition to serum.[12] Can f 4 appears in at least two isoforms.[13] The prevalence of sensitization varies between geographic regions from 46% to 81%, indicating that there might be a difference in reactions to the isoforms.[13,14] Can f 5 is a prostatic kallikrein homologous to human prostate-specific antigen (PSA). It is found in the urine of intact male dogs causing IgE responses in up to 70% of dog allergic patients, thus classified as a major allergen.[15] Can f 6 cross-reacts with the cat allergen Fel d 4 and horse allergen Equ c 1, indicating symptomatic reactions in additional species. The prevalence of sensitization is around 38% in patients with allergy to dog.[16] The latest discovered allergen, Can f 7, or NPC2 protein, belongs to the MD-2-related lipid recognition (ML) family. The prevalence of sensitization is 10%-20%.[17] The amount and composition of allergens has been shown to differ between dogs, and the difference is greater between individuals than breeds.[11] Likewise, each patient displays a unique IgE profile and will thus respond to different combinations of dog allergens. This is a likely reason to why some individuals state that they react to certain dogs but not to others.

Skin prick test[18] and allergen-specific IgE (sIgE) serologic measurements[19] are the most common methods to aid diagnosis of allergy.[20-22] Diagnosis, as well as treatment by allergen-specific immunotherapy (AIT), is almost exclusively performed with extracts from natural allergen sources such as dander or epithelia.[23] This poses an uncertainty since not all of the allergens are present in skin. Moreover, those that are present may vary in quantity and quality depending on the starting material and extraction procedure. Of note, manufacturers use in-house methods for quality assessment and do not provide information on individual allergen content or composition in their products.[24] This obstructs evaluation and comparison of extracts.[25] A previous study has indeed shown qualitative differences between Can f 1 and Can f 3 in extracts intended for SPT from different manufacturers,[26] and others have pointed out inconsistency in reproducibility of extracts.[27-29] Here, we have systematically characterized and compared the allergen content in dogs and different commercial dog allergen extracts in order to evaluate the implication of extract quality on diagnosis and treatment of dog allergic patients.

2 | MATERIAL AND METHODS

2.1 | Dog fur and saliva samples

Randomly included dog owners (n = 120) sampled their dogs. The sampling method is detailed in the supplement. In short, fur samples were collected from a defined area (35 × 30 mm) on the dog's neck and groin using a sampling kit according to instructions (Medi-Tec Research & Development AB, Stockholm, Sweden). A saliva swab (Oracol, Malvern

Medical Developments, Worcester, UK) was applied on the inside of the dog's cheeks until saturated (30-60 seconds). Fur and saliva samples were collected in parallel from each individual dog. Fur samples were prepared by extraction in 1 mL PBS for 1 hour. The saliva samples were centrifuged at 1000 g for 10 minutes.

2.2 | Dog allergen extracts

Diagnostic dog allergen extracts were obtained from ALK (ALK-Abelló Nordic, Hørsholm, Denmark), Bencard (München, Germany), Greer (Cambridge, MA, USA), Inmunotek (Madrid, Spain), and Stallergenes (Antony, France), randomly labeled no 1-5 (a and b for different batches from same manufacturer), Table S2. Evaluation was made on ready-to-use solutions for SPT, except for one that was for skin scratch testing. In this report, they are all categorized as "SPT solutions." From two manufacturers, Allergon (Ängelholm, Sweden) and Greer, lyophilized extracts not intended for clinical use were purchased. These were denoted as dog source material, that is of different anatomical origin, hair, epithelia, and dander, and were randomly labeled extract no 6-9. The source materials were reconstituted in phosphate buffered saline, pH 7.4 (PBS). Protein concentration was determined using Pierce™ BCA protein assay (Thermo Fisher Scientific, Waltham, MA, USA).

2.3 | Allergen production

Recombinant (r)Can f 1, Can f 2, Can f 4, and Can f 6 were produced with and without biotinylation tag and purified as previously described.[10,13,16] rCan f 5[15] was produced in HEK293 cells using FreeStyle™ 293 expression system (Thermo Fisher Scientific). Natural Can f 3 was purified from dog serum (a kind gift, Djurakuten, Stockholm, Sweden).[30] Can f 3 and Can f 5 were in vitro biotinylated (Sigma-Aldrich, Darmstadt, Germany).

2.4 | Antibody production

Can f 1, Can f 2, and Can f 3 monoclonal antibodies were kind gifts from Medi-Tec Research and Development Stockholm AB, Sweden. Polyclonal affinity purified mouse antibodies were obtained from mice immunized with rCan f 4 or rCan f 6, respectively (ethical permission from Stockholm Norra Animal Ethics Board). The antisera were passed over 1 mL HiTrap® NHS-activated columns (GE Healthcare, Uppsala, Sweden) with covalently linked rCan f 4 and 6, respectively, and washed with 20 column volumes of PBS, followed by elution with 0.1 M glycine-HCl, pH 2.5. The pooled antibodies were neutralized using 1 M carbonate buffer pH 9.6. Can f 5 monoclonal antibodies were a kind gift from Phadia, Uppsala, Sweden.

2.5 | ELISA

Allergen concentrations in extracts were measured by competitive inhibition ELISA, further described in the Data S1. In short, mouse antibodies directed to each dog allergen, Can f 1-6, were added to ninety-six–well MaxiSorp plates (Thermo Fisher Scientific) precoated with goat-anti mouse IgG (Jackson Immunoresearch, Ely, UK). The plates with allergen-specific antibodies were incubated with equal amounts of sample and competitor, corresponding to in vivo biotinylated recombinant Can f 1-2-4-6[31] for Can f 1, Can f 2, Can f 4, and Can f 6 measurements. In *vitro* biotinylated native Can f 3 and *in vitro* biotinylated recombinant Can f 5 was used for the Can f 3 and Can f 5 measurement, respectively. To generate sensitive assays, the biotinylated antigens were titrated to an optical density (OD) value around 1.0. For detection of bound biotin-labeled antigen, the plates were incubated with streptavidin-horseradish peroxidase (Jackson Immunoresearch) followed by incubation with substrate 3,3′,5,5′-Tetramethylbenzidine super slow (Sigma-Aldrich) and finally absorbance was measured at 450 nm. Concentration was determined against a reference curve obtained with eight concentrations of recombinant or purified allergens, typically in the range of 2.5-320 ng/mL.

Human serum albumin (HSA) was assessed using a HSA-specific ELISA kit (Abcam, Cambridge, UK) according to manufacturer's instructions. The specificity of the assay was confirmed by using our internal Can f 3 standard curve as control for cross-reactivity.

2.6 | IgE assay

Patients' allergen-specific IgE antibodies were analyzed using ELISA, described in detail in the Data S1. In short, 96-well microtiter plates (Thermo Fisher Scientific) were coated with respective allergen and for the reference curve with polyclonal rabbit anti-human IgE (kind gift from MIAB AB, Uppsala, Sweden). Dilutions of chimeric anti-Bet v 1 human IgE (kind gift from Professor Rudolf Valenta, University of Vienna, Austria) were used to form the standard curve.

2.7 | CD-sens

Basophil activation was measured using the CD-sens method.[32] Three dog allergic patients gave their informed written consent to donate blood (ethical permission from the Regional Ethical Review Board, Stockholm, Sweden). Whole blood was stimulated with 15 dilutions of the extracts (5000-0.0005 ng/mL), red blood cells lysed using ACK lysing buffer (Gibco, NY, USA), and the white blood cells stained using PE-conjugated anti-CD63 and APC-conjugated anti-CD203c antibodies (BD Biosciences). Activated degranulated basophils were analyzed by flow cytometry (FACS-Verse, BD Biosciences, Franklin Lakes, NJ, USA) and the percent of CD203c-positive basophils co-expressing CD63 out of the total number of CD203c-positive cells recorded.[33] Rabbit anti-human IgE (1000ng/ml, kind gift from MIAB, Uppsala, Sweden) was used as positive control and unstimulated cells as negative control. The CD-sens value was calculated as the allergen concentration giving 50% of maximum upregulation of CD63 inverted and multiplied with 100.[34] A result was considered positive ("+," without stating a value) when only the highest concentration of extract triggered a positive response, and thus, a value could not be calculated. The cutoff value for determining a

positive value was set to minimum 5% upregulated CD63 of total number of basophils.

2.8 | Statistical analysis

Statistical analyses were made with GraphPad Prism 6 (GraphPad Software, Inc); Mann-Whitney *U* test was used to compare gender differences, Sidak's multiple comparisons test to compare breeds, and Dunnett's multiple comparisons test to compare Can f 4 levels to other allergens. *P*-values <0.05 was considered significant.

3 | RESULTS

3.1 | Allergen content and composition of extracts

The total protein content in SPT solutions from five manufacturers ranged between 1.53 and 3.07 mg/mL (Table 1a). The six dog allergens, Can f 1 to 6, constituted between 0.26% and 10% of the total protein content. Can f 1 to 6 were present in all extracts, although the concentrations of each allergen varied greatly between SPT extracts from different manufacturers (Table 1 and Figure 1). The composition of extract number 3 singled out, having the highest total allergen content. Generally, Can f 2 and Can f 6 were detected at comparably low amounts, corresponding to minor proportions of the total allergen content (Table 1a, Figure 1A). Can f 3 showed the largest variation between manufacturers, ranging from 19% in extract 2 to 98% in extract 3. The detected Can f 3 did not correspond to HSA supplemented to the extracts. An assay specific for HSA confirmed that the extracts contained HSA, equivalent to 0%-0.6% of the total Can f 3 concentrations. Except for extract 3, the proportions of Can f 1, Can f 4, and Can f 5 each comprised at least 8% of the total allergen content (Figure 1A). Both the proportion and the amount of these three allergens still varied considerably between the extracts (Table 1a and Figure 1A).

Allergen composition in two different batches of SPT solutions from two of the manufacturers was investigated (Figure 1B). Comparison of the composition of two batches from each company showed more consistency than the composition of extracts from different manufacturers. Still, we observed some batch variation, especially for extracts from company 4, which could mainly be attributed to variation in Can f 3 content (Figure 1B).

In the source material obtained from epithelia, hair, and dander, that is extracts 6, 7, and 8, respectively, the total protein content varied between 0.8 and 2.7 mg/mL and the allergen content between 1.3% and 3.2% (Table 1b). The highest total proportion of allergens (28%) was found in extract no 9 (origin—dander). Comparison of the allergen composition of extracts from different origins exhibited distinct distributions of the six dog allergens (Figure 1C). The epithelial extract 6, similarly as SPT solution 3, was dominated by Can f 3 (99% of total allergen content), which also constituted the largest part of the dander extracts no 8 and 9 (60 and 54%, respectively). In contrast, the hair extract 7 mainly contained Can f 1 (67%), which was also a dominating allergen in extract 9 (43%).

3.2 | Basophil reactivity to dog allergen extracts

The allergenic activity of the different extracts was determined by CD-sens in a basophil activation assay. Three dog allergic subjects, displaying different profiles of IgE to Can f 1-6, were selected (Table 2b). The basophil activation capacity varied between extracts and between subjects (Table 2a). Patient 1, sensitized exclusively to Can f 6, displayed CD-sens values to extracts 7 and 8 and reacted to the highest allergen concentration of extracts 1, 2, and 5a. Patient 2, with low serum IgE levels to all of the tested allergens, reacted only to the highest concentration of two extracts. Finally, patient 3, highly sensitized to all the allergens, exhibited positive CD-sens values to all but one extract to which the subject responded to the highest concentration.

TABLE 1 Concentration (ng/mL) of six dog allergens and total protein concentration (mg/mL) in (a) ready-to-use skin prick test (SPT) solutions from five suppliers (1-5) and (b) source material extracts from dog, epithelia (6), hair (7), and dander (8 and 9)

	Can f 1	Can f 2	Can f 3	Can f 4	Can f 5	Can f 6	Total protein concentration	Allergens of total protein (%)
(a) SPT solution								
1	9050	597	10 000	2530	3720	874	1.55	1.7
2	1210	58.7	1530	1920	3190	157	3.07	0.26
3	14.6	14.4	177 000	2560	786	10.7	1.72	10
4a	6950	52.1	5280	6540	3290	191	1.53	1.5
5a	8980	167	38 500	12 900	5300	284	2.28	2.9
(b) Source material								
6	86.8	7.12	40 600	450	29.1	36.9	2.41	1.7
7	17 100	230	6660	461	151	895	0.80	3.2
8	2180	767	20 900	206	7640	3390	2.70	1.3
9	95 000	911	119 000	20.6	6020	49.7	0.80	28

The allergen content in extracts is calculated as percentage (%) of aggregated concentration of the six allergens out of total protein content

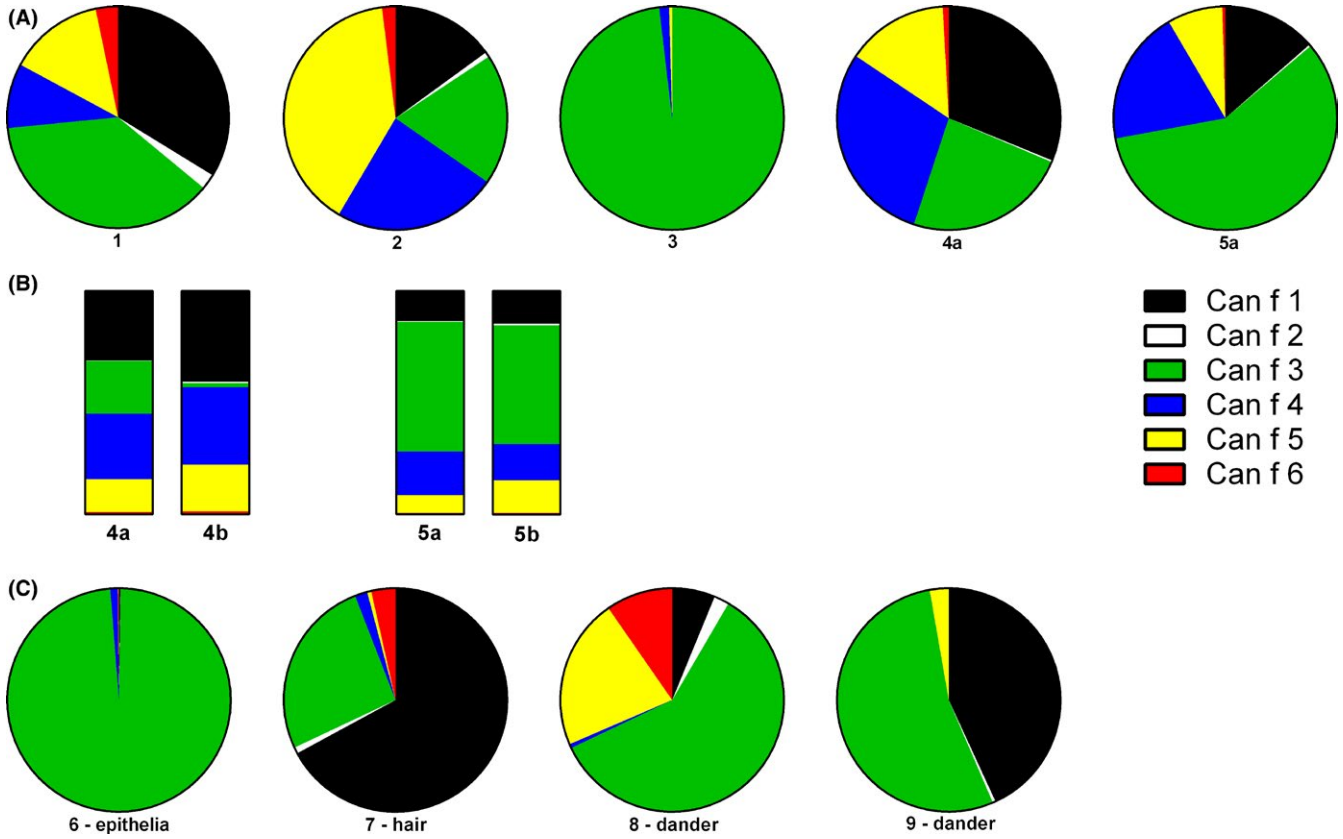


FIGURE 1 Distribution of six dog allergens, Can f 1-6, in ready-to-use SPT solutions (1-5) from five suppliers (A), two batches (a and b) of skin prick test (SPT) solutions from two manufacturers (B) and in allergen source material extracts (6-8) from one vendor and (9) from another supplier (C)

TABLE 2 (a) Basophil reactivity (CD-sens) to skin prick test (SPT) solutions (no. 1-5) and source material extracts (no. 6-8) of three dog allergic patients (Patients 1-3). Plus (+) indicates a positive result below the CD-sens limit criterion. (b) Serum IgE reactivity (kU/L) to six dog allergen allergens for Patients 1-3

	Patient 1	Patient 2	Patient 3
(a) Extract			
1	+	–	0.1
2	+	–	+
3	–	–	1.0
4a	–	–	0.1
5a	+	–	0.1
6	–	–	1.8
7	1.0	+	0.8
8	0.2	+	1.0
(b) Allergen			
Can f 1	0.0	0.6	21
Can f 2	0.0	0.3	8.8
Can f 3	0.0	0.0	75
Can f 4	0.0	0.1	19
Can f 5	0.0	0.0	1.8
Can f 6	7.4	0.2	8.1

3.3 | Allergen profiles of dogs

Can f 1 to 6 were quantitatively analyzed in fur and saliva samples from 120 dogs (Table S1). Higher allergen levels were detected in saliva than in fur, and the significantly highest concentrations were recorded for Can f 4 (Figure 2).

All allergens exhibited great individual variation. Can f 2 was exclusively found in saliva and Can f 5 in fur (Figure 2). Can f 5 was only present in trace amounts in samples of female dogs, while significantly higher Can f 5 levels ($P < 0.0005$) were found in fur samples from the groin of male dogs (Figure 3A). Such gender difference could not be detected for the other allergens (shown for Can f 1, Figure 3B). Allergen profiles of individual dogs varied both within and between breeds, exemplified by a short haired (Labrador retriever) and a long haired (Border collie) breed (Figure 4 and Figure S1).

4 | DISCUSSION

Exposure to dog allergens is a common worldwide cause of allergy. Allergen extracts are routinely used for diagnosing and treating allergy. However, the content of the extracts remains poorly defined, which may lead to a fragmentary diagnosis and insufficient treatment. In this study, we evaluated ready-to-use SPT ampules from five vendors and four nonclinical grade allergen source materials for

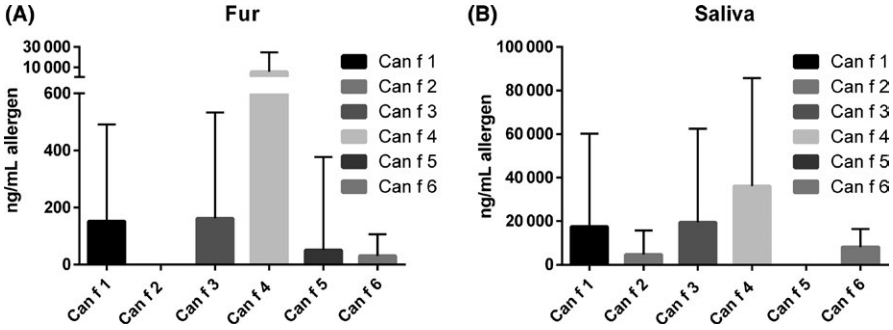


FIGURE 2 Concentration (ng/mL, *y*-axis) of six allergens (*x*-axis) analyzed in extracts of fur sampled from the neck (A) and of saliva (B) collected from 120 dogs. Mean with standard deviation shown. Can f 2 is not detectable in fur. Saliva concentration is approximately 100-fold higher compared to fur

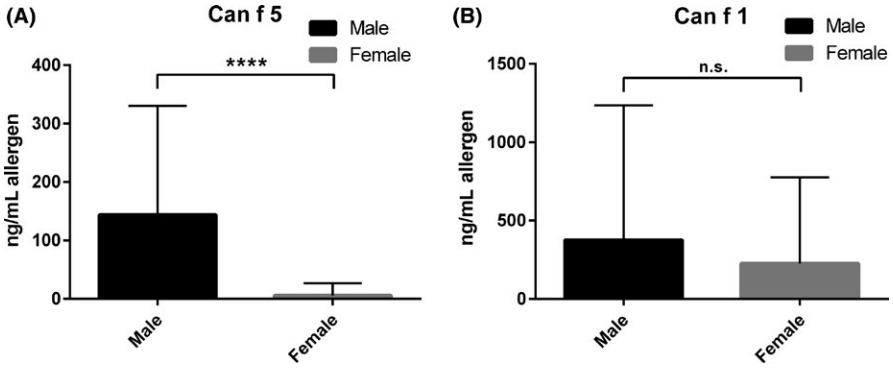


FIGURE 3 Concentration (ng/mL) of Can f 5 (A) and Can f 1 (B) in extract of fur sampled from the groin of male (n = 44) and female dogs (n = 76). Mean with standard deviation, ****$P < 0.0005$; ns, no significant difference

their levels of the six dog allergens Can f 1-6. The processed commercial dog allergen extracts were compared with allergens extracts of fur samples from a representative number of dogs (n = 120).

Here, we show a remarkable range of allergen component concentrations in the different SPT and allergen source solutions. In most cases, the allergen proportion out of the total protein content did not constitute more than a few percent.

One of the SPT solutions, no 3, contained a considerably higher allergen content than the other extracts. Notably, 98% corresponded to the minor allergen albumin, Can f 3, suggesting the use of an alternative allergen source material or supplementation of serum albumin for stabilization purpose. However, no HSA could be detected in this extract. Moreover, solution no. 3 contained a particularly low concentration, 0.01 µg/mL, of the major allergen Can f 1. Such low level may result in reduced diagnostic sensitivity. It should however be noted that solution no 3 is labeled for skin scratch test, a slightly more invasive test than SPT. The SPT solutions from three of the five manufacturers contained Can f 1 concentrations in the range 7-9 µg/mL, extracts that also showed consistency in the major dog allergen Can f 5 levels (range 3-5 µg/mL). Yet, the other four allergens varied greatly between these extracts.

In contrast to other allergens, Can f 2 and Can f 6 were found at low amounts in SPT solutions. Can f 2 is primarily present in dog saliva.[6] The low Can f 2 levels can thus be expected since the extracts derive primarily from hair and dander. Still, one fourth of the dog allergic population reacts to Can f 2.[10] These patients may not be reliably diagnosed with currently available SPT extracts. The concentrations of Can f 6 were detected at 20 to 100 times lower concentrations than Can f 1 and even lower in extract 3. Can f 6 is an important minor allergen due to its cross-reactivity to other furred animals.[16] Low amounts of Can f 6 in the extracts are therefore troublesome. The widely varying allergen composition in SPT solutions prompts the question; which allergen level should be present in an extract to be diagnostically reliable?

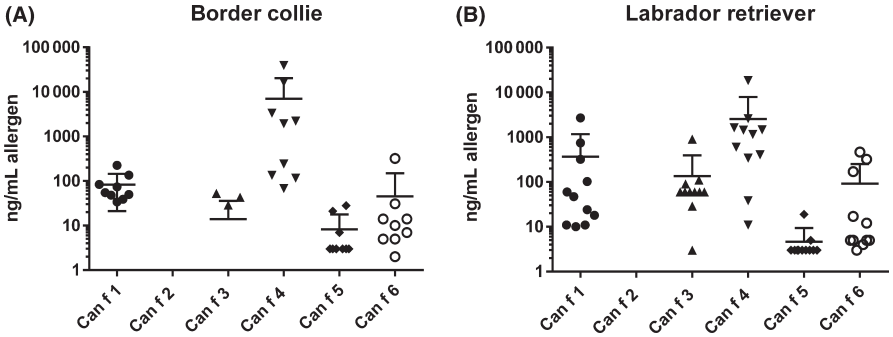


FIGURE 4 Concentration of allergens (ng/mL) in fur samples from different breeds, Border collie (n = 9) (A) and Labrador retriever (n = 11) (B). Mean values with standard deviation shown, *y*-axis is logarithmic

Inter batch variation was investigated for two manufacturers. Even batches from the same manufacturer varied in allergen content, especially for Can f 3, although to less extent than variation between manufacturers. This indicates that differences in source material and extraction methods impact the final allergen composition.

One reason for the allergen variation might be choice of extraction method. Different precipitation and extraction protocols may lead to varying yield and integrity, affecting each allergen independently. One convenient method is protein concentration by acetone precipitation (21), which might lead to partial denaturation and selective loss of antibody binding. Our results support the assumption that the extraction process may lead to partial loss of activity. Can f 4 was found to be the most abundantly detected allergen in fur of dogs. This observation is remarkable since Can f 4 did not exhibit a similar dominant feature in any of the SPT solutions or source materials. Thus, the activity of important allergens may be lost during manufacturing. An indirect proof of the importance of nCan f 4 is the reported prevalence of sensitization of more than 80%,[14] suggesting that sensitization is underestimated when diagnosed with commercially available SPT extracts.

Allergen source material from epithelia, hair, and dander was examined. The analysis revealed that anatomical origin has a major impact on allergen composition. For example, epithelia were mainly composed of Can f 3 while hair contained a large proportion of Can f 1.

Moreover, the heterogeneity in the source materials may also reflect differences in allergen profiles between dogs. To investigate this, we analyzed samples collected from fur and saliva of 120 randomly selected dogs. The sampling was performed according to a well-specified procedure. A weakness of the method is that allergen levels are not related to sampling recovery. As the allergens were collected from a defined area on the neck, no statement can be made about the total allergenicity of particular dogs. Nevertheless, the relative allergen levels in samples from each dog provide individual allergen profiles irrespective of protein recovery. Moreover, although data on absolute allergen levels should be interpreted with caution, the large individual heterogeneity in allergen levels observed in our dog cohort is in line with previously published data on Can f 1 in dog hair and coat samples.[11] Quantification of six dog allergens in fur samples from the mixed dog population revealed individual variations in the distribution of allergens regardless of breed. Our data support that there are no hypoallergenic breeds thus extending previous data for Can f 1.[11] Furthermore, we show that the length of the coat does not influence on allergenicity. Gender mattered only for Can f 5, which was expected, since Can f 5 is a prostatic protein. Can f 5 was also detected in low amounts in a few female dogs, speculatively due to male dog kallikrein cross-contamination. Hence, not only does the individual dogs used for allergen collection matter, but also the origin of material, anatomical site, and method of extraction affects the final allergen composition of the SPT solution.

Basophil activation analyses were performed to determine the allergenic potential of extracts in relation to patients' sensitization profiles. For this purpose, three patients with widely different sensitization profiles were chosen and basophil degranulation measured. The allergenic activity to the extracts varied greatly for each patient, essentially mirroring the content of allergens in the extract. For instance, the patient monosensitized to Can f 6 reacted to the extracts with highest content of this allergen, while the polysensitized patient, highly sensitized to Can f 3, also was particularly responsive to extracts with high content of this allergen. Basophil reactivity demonstrated that the content of single allergens may be crucial for extract allergenicity. Accordingly, patient 2, with low IgE levels to four of the six allergens, did not respond to any of the SPT solutions.

The same type of naturally derived extracts as used for SPT also forms the basis for vaccine formulation. Reported varying success to treat allergy to dog by AIT may speculatively be ascribed to poor or uneven quality of the vaccines.[35,36] Successful treatment is ultimately dependent on how the amount and composition of allergen components in the extracts matches treated patients' sensitization profile.[37,38]

In conclusion, here we show that natural dog allergen extracts intended for clinical use are highly unreliable. Factors such as individual dog allergen production within and at different anatomical sites and producer unique manufacturing all contribute to the demonstrated enormous variability and complexity in the mixture of allergens. Our data imply an urgent need for content declaration of dog allergen extracts, which would form the basis in a harmonized standardization and determination of minimum allergen levels for accurate diagnosis and effective treatment.

CONFLICT OF INTEREST

Hans Grönlund is co-founder of Medi-Tec Research & Development Stockholm AB; further, AW, KA, EH, and GG have acted as scientific advisors. The other authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

HG conceived the study and designed the experiments together with AW and GG. OBN and EH produced recombinant allergens. AW, KA, and JB performed immune analyses. AW, GG, and HG were the main responsible for data analysis and interpretation, with input from all co-authors. AW wrote the manuscript under supervision of HG and GG, and all co-authors reviewed and approved the final version.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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