

Original article

Dog factor differences in Can f 1 allergen production

Background: The clinical importance of dog allergy is well known, but it is unknown if all types of dogs represent the same risk for allergic patients. The purpose of this work was to evaluate among 288 healthy dogs if the levels of Can f 1 on fur vary between breeds (German Shepherd, Pyrenean Shepherd, Poodle, Cocker spaniel, Spaniel, Griffon, Labrador retriever and Yorkshire terrier), gender, hormonal status, hair length, and according to the presence of seborrhea.

Methods: Each dog was shaved in a limited area and Can f 1 concentrations were measured in µg/g fur by ELISA. The results (geometric mean values and 95% confidence intervals) were analyzed using analysis of variance and with nonparametric tests.

Results: A wide variability in Can f 1 levels was found between dog breeds, from Labradors [1.99 (0.03–129.91)] to Yorkshires [16.72 (3.67–76.16)] and Poodles [17.04 (2.79–103.94)] but only the Labrador levels were significantly different from each other breed. Males produced more Can f 1 than females, 11.75 (1.27–108.40) vs 8.89 (0.91–86.39). No difference was found according to hair length or hormonal status. The seborrheic status highly ($P = 0.0019$) influenced the presence of Can f 1 on hair: 16.66 (1.59–173.96) vs 9.40 (1.03–85.70).

Conclusion: Breeds (Labrador retriever), sex and seborrhea seem to influence the levels of Can f 1 on fur.

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Most respiratory allergies due to pets are caused by cats and dogs. The prevalence of pet allergy has been estimated by our group to be 2.5% in a general population (1). The prevalence of dog sensitization was found to reach 8% in a general population (2). For children, the prevalence found to be 5% at the age of 7–8 years, reaches 10% 4 years later in the same group (3). Both antigens Can f 1 for dogs and Fel d 1 for cats are ubiquitous and have been found in dogless homes, classrooms, public places and day nurseries (4, 5). Moreover, presence of a dog in a house, currently or previously, is not associated with the incidence of sensitization to dog allergens (6). Rates found in the air may differ widely in homes housing a dog, from 0.3 to 99 ng/m³ (7). These variations may be linked to environmental factors, to the presence of reservoirs in homes, especially for the main allergen, to the number of dogs housed, and to the kind of avoidance measures used. The rates found in the atmosphere could also vary according to the dog characteristics. Indeed the level of allergen produced can vary from one dog to another. This is the hypothesis tested in this work, by assessing the Can f 1 levels according to breed, sex, hormonal status (castrated, noncastrated, nonpubescent, under hormonal treatment) and hair length (short, semi-long, long). Moreover, we demonstrated a few years ago that the major cat allergen Fel d 1 was produced by sebaceous glands (8) and, if an

analogy between cat and dog allergens exists for some epitopes (9), we may suppose that presence of seborrhea may be linked to high level of Can f 1 allergen.

Material and methods

Dog selection and hair sampling

Dogs were selected, throughout the year, by a veterinarian and included in the study when entering the clinic for a benign operation (such as castration) or euthanasia. Verbal consent for inclusion in the study was obtained from the dog owner.

For each dog, sex, breed, presence of seborrhea, hormonal status, hair length and disease or traumatism (if diagnosed) were assessed by the veterinarian. Seborrhea was diagnosed by the veterinarian on the basis of 'excessive greasiness of the skin and hair coat' (10). Dogs selected for this study were all healthy to avoid a possible influence of pathology on the Can f 1 production. Dogs were shaved as close to the skin as possible in one (for 179 dogs) or many areas (90 had two samples, 19 had three samples) including neck, abdomen, thorax, flanks and lumbar zone. It was verified that all these zones were statistically similar for Can f 1 production and only one area was retained to represent one dog. Can f 1 concentrations found for each area were: abdomen [$n = 74$; 11.92 (0.74–192.48)], neck [$n = 50$; 10.31 (0.93–114.78)], flanks [$n = 16$; 13.75 (2.33–81.21)], lumbar zone [$n = 26$; 9.46 (1.34–66.75)] and thorax [$n = 122$; 9.19 (1.32–63.94)] with $P = \text{NS}$. Thorax area was preferentially kept for statistical analysis because it was the most often represented. If thorax sample was not available for a dog, we chose abdomen area.

A total of 288 dogs were included in the study, 150 males and 138 females. For the breed study, only 157 dogs were retained because mongrels and breeds with less than eight representative dogs were excluded from this analysis.

For the hormonal status study, we excluded two dogs under hormonal treatment and nine nonpubescent dogs.

Can f 1 assay

The major allergen Can f 1 was assayed by ELISA (11). Extraction was carried out by incubation of 100 mg of hair for 24 h at 4°C with 2 ml of BBS (borate buffered saline pH 8.2) and spun at 1200 *g* for 20 min. Supernatants were recovered and diluted at 1 : 5 and 1 : 50 for assay.

Microplates were coated by the monoclonal antibody 6E9F6, anti Can f 1 (Indoor Biotechnologies, Cardiff, UK).

Nonspecific sites were saturated by PBST-BSA (Bovine Serum Albumine; Sigma, Saint Quentin Fallavier, France). Can f 1 standard ranged from 250 ng/ml to 1.95 ng/ml. Detection antibody was a polyclonal rabbit serum raised against dog extract (Indoor Biotechnologies). Samples were then incubated with a peroxidase-conjugated goat anti-rabbit IgG 6154 (Sigma), an *O*-phenylenediamine (OPD) substrate was added (Abbot, Rungis, France) to reveal the reaction and optical densities were read with a 405 nm filter after 10 min.

Statistical analysis

Statistical analysis was achieved by using arithmetical values, converted into logarithmic values in order to lower the influence of extreme values. Data distributed normally were treated by analysis of variance. Nonparametric Kruskal–Wallis and Mann–Whitney tests were used for small samples, particularly as soon as the notion of breed was introduced.

For breeds, comparisons in pairs by circular permutation were done, taking breeds two by two, and results were calculated by an analysis of variance and verified with the nonparametric Mann–Whitney test. Results were given in µg/g of hair.

Results

Hair Can f 1 levels according to breeds

The analysis was limited to the most represented breeds, i.e. with more than eight dogs in each group: German

Shepherd (*n* = 40), Pyrenean Shepherd (*n* = 9), Poodles (*n* = 43), Cocker Spaniel (*n* = 8), Spaniel (*n* = 12), Griffon (*n* = 14), Labrador Retriever (*n* = 9) and Yorkshire Terrier (*n* = 22). A great variability in Can f 1 levels was found between the different dog breeds. The observed difference between breeds is highly significant ($P < 0.0001$), as confirmed by the nonparametric Kruskal–Wallis test ($P = 0.0017$). The breed with the lowest level of Can f 1 on hair is the Labrador retriever (1.99 µg/g of hair) and the highest levels were found for Poodles (17.04 µg/g of hair) and Yorkshire Terrier (16.72 µg/g of hair) (Table 1). The comparisons in pairs by circular permutations showed that only the Labrador retriever had a Can f 1 concentration significantly lower than all other dog breeds. The other breeds studied did not differ from each other. On the other hand, in the same breed, very high variability was observed from one dog to another. When individual values for each dog were compared, breed by breed, the breed effect was obvious (Fig. 1), whichever the dogs studied, although some dogs might be 'high' or 'low' Can f 1 producers among the same breed.

Hair Can f 1 levels according to gender

Males displayed significantly higher concentrations of Can f 1 on hair than females: 11.75 µg/g of hair for males vs 8.89 µg/g of hair for females ($P = 0.0365$) (Table 2). Within breeds, there was no statistically significant difference between levels of Can f 1 in hair from male and female dogs, but for all breed there was a trend for the levels to be higher in males, reflecting the small numbers in each group.

Hair Can f 1 levels according to hormonal status and hair length

No significant difference was found according to hormonal status: 48 castrated dogs for a concentration of 8.97 µg/g of hair vs 222 noncastrated dogs for a level of 10.79 µg/g of hair. The comparison of castrated dogs and

Table 1. Concentrations of Can f 1 (µg/g of hair) according to breeds

Most represented breeds	Number (<i>n</i>)	Geometric mean	95% Confidence intervals	Minimum	Maximum	Samples (157)		
						Quartiles (%)		
						25	50	75
German Sh.	40	8.69	0.83–90.54	0.74	129.70	2.96	9.96	15.50
Pyrenean Sh.	9	13.13	1.49–115.91	4.01	136.60	5.27	15.01	19.63
Poodle	43	17.04	2.79–103.94	2.21	122.60	9.19	15.46	31.40
Cocker Sp.	8	8.45	1.13–63.04	1.21	28.87	5.12	10.27	17.25
Spaniel	12	11.07	1.20–101.68	2.58	84.10	4.28	11.87	25.23
Griffon	14	11.84	2.01–69.95	3.22	44.40	4.43	15.65	24.40
Labrador	9	1.99	0.03–129.91	0.01	16.28	2.10	2.60	4.91
Yorkshire T.	22	16.72	3.67–76.16	2.98	118.80	10.83	16.28	22.75

Analysis of variance $P < 0.0001$.

Checking by nonparametric analysis of Kruskal–Wallis $P = 0.0017$.

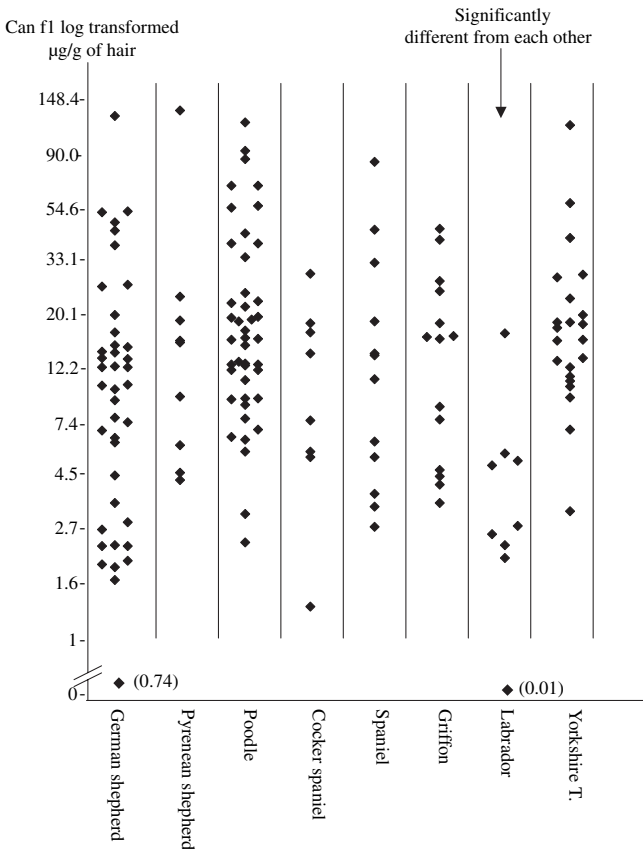


Figure 1. Repartition of Can f 1 concentrations. Can f 1 was assayed by ELISA in various breeds of dogs. Individual data are given per breed in function of dog populations.

noncastrated dogs was also made in males and in females separately. No statistically significant difference was found between castrated and noncastrated dogs, respec-

tively, 9.51 (1.62–55.80) µg/g of hair vs 8.70 (0.73–104.36) µg/g of hair for females and 8.07 (0.91–71.80) µg/g of hair vs 12.65 (1.44–110.81) µg/g of hair for males.

Similarly, no difference was found according to hair length (Table 2).

Hair Can f 1 levels according to seborrhea

As shown in Table 2, dogs with seborrheic skin displayed significantly ($P = 0.0019$) more Can f 1 allergen on hair than dogs without seborrhea: 16.66 µg/g of hair vs 9.40 µg/g of hair. This difference between seborrheic and nonseborrheic dogs was found independently of the studied variables: breed (except Spaniel), gender, noncastrated dogs, and hair length. However, in some particular cases where the number of dogs in a subgroup was low (for example only seven dogs were castrated and seborrheic), the difference was not significant.

Discussion

The two major dog allergens are Can f 1 and Can f 2 (10). 75% of serums taken from people allergic to dogs contain specific IgE to Can f 1. About 20% of Can f 1 allergens are to be found on small particles of less than 5 µm across. cDNA corresponding to both major dog allergens was found in salivary glands (12) but a great concentration of protein is to be found on dog hair. So far, no study has been conducted on Can f 1 concentration on hair according to various parameters such as dog breeds, sex, hormonal status, hair length or presence of seborrhea. In 1980, Lutsky et al. (13) published a study of cutaneous reactions in patients sensitized to dog, with epithelium, serum, and urine extracts from different dog breeds. This

Table 2. Concentrations of Can f 1: univariuous analyses according to sex, hormonal status and hair length (µg/g of hair)

Samples (288)									
	Number (n)	Geometric mean	P-value	95% Confidence intervals	Minimum	Maximum	Quartiles (%)		
							25	50	75
Sex									
Females	138	8.89	0.0365	0.91–86.39	0.01	135.74	5.26	9.49	16.28
Males	150	11.75		1.27–108.40	0.58	278.00	5.24	12.83	23.92
Hormonal status									
Castrated	48	8.97	NS	1.33–60.69	0.58	47.10	5.71	9.15	15.58
Noncastrated	222	10.79		1.05–111.14	0.01	278.00	5.38	11.98	19.51
Nonpubescent	9	9.10		0.76–108.40	1.69	88.40	3.54	8.62	20.57
Hair length									
Short	68	8.41	NS	0.60–117.90	0.01	93.00	4.53	8.72	19.00
Semi-long	141	10.52		1.36–81.52	0.54	278.00	5.32	11.54	18.08
Long	77	11.83		1.22–115.22	0.51	136.60	6.11	11.94	21.00
Seborrhea									
No seborrhea	241	9.40	0.0019	1.03–85.70	0.01	278.00	5.16	10.13	17.86
Seborrheic	44	16.66		1.59–173.96	1.54	129.70	7.67	16.58	38.15

study suggested that qualitative variations existed among the different dog allergens though this study did not demonstrate that real differences exist between different dog breeds. In the same way, the study by Lindgren et al (14) aimed at characterizing the allergenicity of eight dog dander extracts taken from eight different breeds. Results showed that the studied extracts contained both allergens common to the eight breeds and components specific to some breeds only. Again, in this work, it was not possible to say whether quantitative variations of major allergens existed as a function of breed or other parameters. Finally, the study by De Groot et al. (15) showed great variations in allergen concentrations of 27 hair extracts of nine different dog breeds. This study also showed that saliva contained important quantities of Can f 1 compared with urine and faeces which are other sources of allergens. However, this study also did not allow the authors to analyse whether quantitative differences existed in the presence of Can f 1 on hair according to dog breeds.

In our study, Table 1 shows a great variation in the quantity of Can f 1 on hair according to dog breeds. The two breeds that produce by far the most are Yorkshire terriers and poodles. The Labrador retrievers produce the least and this very low level of Can f 1 could explain the global variation. These results were still significant after exclusion of the Labrador retrievers (not shown). The differences between breeds could be related to different dog behaviour according to their breed. For instance, Labradors may display lower levels of Can f 1 because they frequently jump into water. Such an effect cannot be excluded. As has been shown for cats (16), it appears that male dogs display a higher quantity of allergens on hair than female dogs. Finally and overall, it appears that if hair length and hormonal status have no significant effect, presence of seborrhea is a highly important and significant factor associated with Can f 1 production. An effect of dog washing in our results is unlikely. Indeed, as the primary treatment of seborrhea is shampoo, seborrheic

dogs are probably the most frequently washed and these dogs still display higher levels of Can f 1. Finally, in this study, it is impossible to affirm that the production of Can f 1 by a seborrheic skin is higher than that of a nonseborrheic one. Actually, it could simply be because of the fact that the presence of seborrhea allows a greater accumulation of Can f 1 on hair even though the skin production is not greater. With our experience of major allergen production by cat Fel d 1, which is mainly linked to the presence of sebaceous glands (8, 17), we can assume that the activity of sebaceous glands is higher in the skin of seborrheic dogs than in nonseborrheic ones; this activity may be linked with the production of Can f 1 by the skin. Further work will be required to confirm this assumption or not.

In any case, this work shows for the first time that factors exist which can modulate the concentration of Can f 1 on the hair of different dogs. This study also shows that Can f 1 quantities on the hair itself may vary significantly within the same breed. Independently of the qualitative nature of allergens, these results may explain why some allergic patients say they fear certain dogs more than others. The clinical relevance of these results is unknown so far, encouraging further study in order to better understand the mechanisms of Can f 1 production in dogs. In conclusion, there are few practical consequences. We can advise patients to choose Labrador dogs over another breed but the variability from one dog to another in the same breed shows that a 'hypoallergenic dog breed' does not exist. As dogs with seborrhea present more Can f 1 levels on hair than nonseborrheic ones, we can only advise patients to treat their dogs when they suffer from seborrhea.

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