

Paper

Characterisation of dog sensitisation to grass pollen in western France from 1999 to 2010

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Very few studies have investigated the seasonal aspect of grass pollen sensitisation or its evolution in the allergic canine population. The aim of this study was to evaluate both these aspects. A study of canine grass pollen sensitisation was performed throughout the statistical analysis of 261 intradermal skin testings (IDT=25 allergens tested on average) performed from January 1999 to December 2010. IDTs were performed on dogs with pruritic allergic skin disease. χ^2 Tests were used for statistical analysis. Two hundred and thirteen dogs (81.6 per cent) were sensitised to at least one allergen, and 56 (21.5 per cent) to at least one grass pollen. No increase in the rate of positive IDT was recorded over three periods: 1999–2002, 2003–2006 and 2007–2010. No statistical correlation was detected between sex, age or birth month and grass sensitisation. Moreover, no link was found between the season in which the IDT was performed and sensitisation, indicating that there is no seasonality to the sensitisation. However, considering the grasses tested over these three periods (grass mix and rye grass), a clear and significant increase in the percentage of dogs sensitised to grass pollen was observed between 1999–2002 and 2007–2010 (14.4 per cent and 27.7 per cent, respectively). The possible reasons for this sensitisation increase of dogs to grass pollen are discussed.

Introduction

In human beings, 'grass pollen allergy' is a common cause of polinosis that triggers a variety of seasonal clinical signs, especially rhinoconjunctivitis and/or bronchial asthma (Ring 2005a). Grasses represent a very common and abundant source of pollens due to their broad environmental distribution and their significant pollen producing capability. In dogs, very few studies indicate the real incidence of atopic patients sensitised to grass (White and Bourdeau 1995, Mueller and others 2000, Hill and Deboer 2001). Intradermal testing (IDT) remains the most common method used both in clinical practice and in research studies to investigate sensitisation aspects in allergic dogs. An analysis of the percentage of dogs reacting to grass pollen revealed disparities, with reported prevalences varying from 0 per cent to 89 per cent (Nesbitt 1978, Scott 1981, Willemse and Van Den Brom 1983, Nesbitt and others 1984, Schick and Fadok 1986, DeBoer 1989, Prélud 1990, Carlotti and Costargent 1994, Koch and Peters 1994, Beco and Fontaine 1995, Sture and others 1995, Zunic 1998, Saridomichelakis and others 1999, Mueller and others 2000, Zur and others 2002). These discrepancies could be explained by different geographical locations, and also by the lack of standardisation for allergen extracts used in canine IDT. Very little information is available in terms of the parameters that could characterise or influence grass pollen sensitisation in canine atopic dermatitis (CAD).

The purpose of this study was to determine the frequency of grass pollen sensitisation in allergic dogs in the Nantes area (western France) to characterise the population of these dogs, and to investigate the role of seasonality in grass sensitisation.

Material and methods

Animals

Intradermal tests performed on pruritic dogs with skin diseases consistent with atopic dermatitis (after the exclusion of other causes of pruritus, such as ectoparasites and infections) from 1999 to 2010 were analysed retrospectively. The animals were presented at the dermatology specialised referral consultation of the Dermatology-Parasitology-Mycology (DPM) Unit of the Nantes National Veterinary School.

Allergens tested

Each test utilised between 20 and 38 allergenic extracts, depending on the year and clinical context. In terms of the plants, 4 grass pollens (including 1 mix), 8 weed pollens and 17 tree pollens (including 2 mixes) were commonly used.

The grass allergens used included the following:

- ▶ Timothy grass (*Phleum pratense*),
- ▶ Perennial ryegrass (*Lolium perenne*),
- ▶ Common meadow grass (*Poa pratensis*),
- ▶ Grass mix: cocks foot (*Dactylis glomerata*), Timothy grass (*P. pratense*), sweet vernal grass (*Anthoxanthum odoratum*), velvet grass (*Holcus lanatus*), and Bermuda grass (*Cynodon dactylon*).

All these allergenic extracts were 1000 w/v aqueous preparations. A diluent solution (0.9 per cent phosphate-buffered saline) and histamine (0.1 mg/ml) were used as negative and positive controls, respectively.

From 1999 to 2010, the allergens originated from the same supplier (Laboratoire DESTAING, Grasse, France).

Veterinary Record (2013)

doi: 10.1136/vr.100710

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Provenance: Not commissioned;
 externally peer reviewed

Accepted May 17, 2013

Timothy grass was not available from our supplier from 2003 to 2010, and common meadow grass was not available between 1999 and 2006.

IDT technique

IDT was performed according to standard methods (Hillier and Deboer 2001, Scott and others 2001), and conducted in the same manner throughout the study period. The vast majority of IDTs were performed without sedation or anaesthesia. A volume of 0.05 ml of each control solution and allergenic extracts was injected intradermally on the clipped lateral aspect of the thorax.

Interpretation

Reactions were read 15–20 minutes after injection. Reactions were interpreted using the protocol developed at the DPM Unit. Three criteria were used: diameter of the wheal (in millimetres), erythema (presence or absence) and the thickness of the wheal as evaluated by digital palpation (0=negative to 3+).

A reaction was considered positive if distinctly different from the negative control, or if the wheal diameter was larger than the mean value of the negative and positive controls (histamine), or 3 mm larger than the negative control. Digital palpation and erythema were used as secondary indicators in cases of a doubtful reaction.

Data and statistical analysis

For each dog, the following data were extracted from the informatics data bank of the DPM Unit and/or the dog's files: age, sex, breed, birth month, IDT date, seasonality of the clinical signs and the IDT results.

The relationship between grass sensitisation and the parameters stated above were analysed using χ^2 tests. The results were considered statistically significant with a $P < 0.05$. These analyses were performed for each grass allergen (grass mix, Timothy grass, rye grass, or common meadow grass) and for the grass mix plus rye grass. When a dog was positive for both the grass mix and rye grass, it was counted only once.

Age at the time of IDT

Animals were classified into four age groups: puppies (0–1 year, $n=24$), young dogs (>1–3 years, $n=78$), adults (>3–8 years, $n=134$) and older dogs (>8–12 years, $n=24$).

Breed

The breeds chosen for comparison were selected if they added up to at least 3.0 per cent (minimum of three to eight dogs, depending on the allergen) of the tested population.

To assess the existence of a breed predisposition to grass sensitisation, we used a reference dog population. This population was composed of dogs presented to the preventive medicine clinics between 2002 and 2010 ($n=9746$) (information not available before 2002). For each grass tested, χ^2 tests were used to assess the relationship between the breeds and sensitisation ($P < 0.05$). ORs with a 95% CI were then used for precise breed predilections.

Birth month

To evaluate the possible relationship between birth month and the development of grass sensitisation and for statistical reasons, we grouped the period of birth into five periods (season 1 (S1): November to January, season 2 (S2): February to March, season 3 (S3): April to May, season 4 (S4): June to July, and season 5 (S5): August to October). S1 has a poor production of grass pollen; S2 and S5 have a moderate production of grass pollen and S3 and S4 include spring and summer, respectively, which supposedly have higher production rates of grass pollen.

Season in which the IDT was performed

Three periods were analysed: S1 (September–November), S2 (December–February) and S3 (March–June) to obtain a comparative number of dogs per period.

The months of July and August were not included due to the very low number of cases performed during the summer holiday period.

Evolution of sensitivity to grass pollens (1999–2010)

The evolution of sensitivity to grass pollens was evaluated through three periods of time: 1999–2002, 2003–2006 and 2007–2010. The proportion of dogs positive towards the rye grass, grass mix and rye grass plus grass mix was calculated for each of the three time periods, and the differences in incidence were evaluated using χ^2 tests.

Evolution of the abundance of grass pollens (1999–2010)

To evaluate the evolution of grass sensitisation, we studied the temporal evolution of grass pollination over the last 10 years in the Nantes area (France), from where most of the dogs originate. We used data provided by The Aerobiological Network of Surveillance (Réseau National de Surveillance Aérobiologique (RNSA)) (Thibaudon and Caillier 2002, Thibaudon and others 2003). For each year, we investigated the daily evolution of pollinic counts and the mean count of grass pollen grains, both stated in number of grains/m³/day.

Results

Animals

A total of 261 dog IDTs were included. The dogs tested were of 62 different breeds, with labrador retriever (13.4 per cent), boxer (7.3 per cent), West Highland white terrier and French bulldog (6.3 per cent) being the most commonly represented. Males and females were 46.4 per cent and 53.6 per cent, respectively, and the mean age at which the dogs were tested was five years old (a range of 1–12.5).

Sensitisation

The IDT results are shown in Table 1, where the percentages of positive reactions observed for each extract are accounted. From the 261 dogs tested, 81.6 per cent ($n=213$) were positive towards at least one allergen tested. *Dermatophagoides farinae* was the most common allergen to yield positive reactions (48.3 per cent, $n=126$). However, a majority of the tested dogs (77.4 per cent, $n=202$) were sensitised to at least one pollen (including trees, weeds and grasses), and 21.5 per cent ($n=56$) had a positive reaction to at least one grass. The grass mix was 15.1 per cent (39/259) positive, the perennial rye grass was 14.6 per cent (28/192), the Timothy grass was 14.1 per cent (9/64), and meadow grass was 13.3 per cent (6/45).

All the 56 dogs sensitised to the grass pollens also reacted to at least one other allergen. On average, dogs sensitised to grass pollen ($n=56$) also reacted to 8.8 other allergens.

Birth month

The percentage of dogs reactive to grass pollens born during S1, S2, S3, S4 and S5 is presented in Table 2. For each grass pollen tested (grass mix, rye grass, or common meadow grass), the period (season) of birth did not result in a greater frequency risk for developing grass sensitisation in atopic and suspected allergic dogs (P values varying from 0.246 to 0.804).

Season in which the IDT was performed

The percentages of dogs sensitised to grass during S1, S2 and S3 are presented in Table 2. No significant difference in the number of grass-sensitised dogs between these three periods was detected (P values varying from 0.091 to 0.953).

Season of clinical signs

Two hundred and forty-nine (95.4 per cent) dog owners answered the question concerning the seasonality of the clinical manifestations. Only 16.5 per cent (41/249) of the dogs suffered from seasonal pruritus. Of those dogs, between 0.0 per cent and 20.0 per cent reacted to the grass pollens tested (Table 2). Two hundred and eight dogs did not display seasonal pruritus. Among those dogs, between 12.2 per cent and 31.1 per cent (Table 2) were sensitised to grass pollens. No link could be found between grass sensitisation and the seasonality of the clinical signs (P values varying from 0.232 to 0.998).

TABLE 1: Number and prevalence of positive reactions

Allergens	Number of dogs tested	Positive reactions (number)	Positive reactions (%)
<i>Dermatophagoides farinae</i>	126	261	48.3
Tree # 1	13	53	24.5
Tree # 2	11	50	22.0
Alder (<i>Alnus glutinosa</i>)	26	139	18.7
Lamb's quarter (<i>Chenopodium album</i>)	36	194	18.6
Pine (<i>Pinus sylvestris</i>)	34	191	17.8
Cypress (<i>Cupressus sempervirens</i>)	31	192	16.1
Locust (<i>Robinia pseudoacacia</i>)	40	257	15.6
Olive tree (<i>Olea europaea</i>)	7	46	15.2
Grass mix	39	259	15.1
Birch (<i>Betula pendula</i>)	30	201	14.9
Perennial ryegrass (<i>Lolium perenne</i>)	28	192	14.6
Timothy grass (<i>Pleum pratense</i>)	9	64	14.1
Poplar (<i>Populus tremula</i>)	15	107	14.0
Ragweed (<i>Ambrosia elator</i>)	35	257	13.6
Common meadow grass (<i>Poa pratensis</i>)	6	45	13.3
Plantain (<i>Plantago lanceolata</i>)	33	255	12.9
Wall pellitory (<i>Parietaria officinalis</i>)	24	193	12.4
Oak (<i>Quercus robur</i>)	26	217	12.0
Mugwort (<i>Artemisia vulgaris</i>)	30	253	11.9
Stinging nettle (<i>Urtica dioica</i>)	24	203	11.8
Ash (<i>Fraxinus</i> species)	16	138	11.6
Sycamores (<i>Platanus occidentalis</i>)	22	193	11.4
Sheep's sorrel (<i>Rumex acetosella</i>)	21	192	10.9
Common hazel (<i>Coryllus avellana</i>)	19	202	9.4
Willow (<i>Salix</i> species)	7	86	8.1
Dandelion (<i>Taraxacum officinalis</i>)	16	203	7.9
Wild privet (<i>Ligustrum vulgare</i>)	2	26	7.7
European beech (<i>Fagus sylvatica</i>)	1	27	3.7

TABLE 2: Number and incidence of positive and negative dogs to the grass mix, rye grass, common meadow grass and Timothy grass

Sex	Grass mix (P=0.709)			Rye grass (P=0.778)			Common meadow grass (P=0.636)			Timothy grass (P=0.281)		
	+	-	% +	+	-	% +	+	-	% +	+	-	% +
Female	22	117	15.8	16	89	15.2	4	22	15.4	3	29	9.4
Male	17	103	14.2	12	75	13.8	2	17	10.5	6	26	18.8
Total	39	220	15.1	28	164	14.6	6	39	13.3	9	55	14.1
Age	Grass mix (P=0.123)			Rye grass (P=0.268)			Common meadow grass (P=0.839)			Timothy grass (P=0.689)		
0-1 year	7	17	29.2	3	14	17.6	0	4	0.0	0	6	0.0
>1-3 years	14	64	17.9	10	45	18.2	1	7	12.5	4	18	18.2
>3-8	15	118	11.3	15	85	15.0	4	23	14.8	4	27	12.9
>8-12 years	3	20	13.0	0	19	0.0	1	4	20.0	1	4	20.0
Total	39	219	15.1	28	163	14.7	6	38	13.6	9	55	14.1
Birth month	Grass mix (P=0.555)			Rye grass (P=0.804)			Common meadow grass (P=0.559)			Timothy grass (P=0.246)		
S1	12	52	18.8	8	37	17.8	0	6	0.0	2	16	11.1
S2	5	20	20.0	3	16	15.8	1	2	33.3	2	4	33.3
S3	8	39	17.0	3	33	8.3	1	8	11.1	3	7	30.0
S4	6	39	13.3	6	30	16.7	1	11	8.3	0	9	0.0
S5	7	65	9.7	8	45	15.1	3	11	21.4	2	16	11.1
Total	38	215	15.0	28	161	14.8	6	38	13.6	9	52	14.8
IDT season	Grass mix (P=0.693)			Rye grass (P=0.091)			Common meadow grass (P=0.316)			Timothy grass (P=0.953)		
S1	12	83	12.6	6	68	8.1	4	13	23.5	3	16	15.8
S2	10	49	16.9	5	29	14.7	1	12	7.7	3	21	12.5
S3	17	87	16.3	17	66	20.5	1	13	7.1	3	18	14.3
Total	39	219	15.1	28	163	14.7	6	38	13.6	9	55	14.1
Season of clinical signs	Grass mix (P=0.535)			Rye grass (P=0.998)			Common meadow grass (P=0.529)			Timothy grass (P=0.232)		
Yes	5	36	12.2	5	28	15.2	2	8	20.0	0	7	0.0
No	33	73	31.1	23	129	15.1	4	29	12.1	9	43	17.3
Total	38	109	25.9	28	157	15.1	6	37	14.0	9	50	15.3

IDT, Intradermal testing.

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Sex, age

The number of dogs sensitised to grass pollens did not statistically differ by sex or age (Table 2).

Breeds

The distribution of sensitisation among breeds is presented in Table 3.

No breed predilection was highlighted for sensitisation to the grass mix ($P=0.113$), rye grass ($P=0.092$) or common meadow grass ($P=0.154$). However, Timothy grass sensitisation appeared to be breed related ($P<0.05$). In particular, the West Highland white terrier appeared significantly predisposed to develop a sensitisation to Timothy grass (OR=21.2, CI 3.08 to 145.95).

Sensitisation evolution

The evolution of the percentages of dogs sensitised to each grass pollen is detailed in Fig 1. The percentages of dogs sensitised to the grass mix and rye grass increased significantly ($P=0.042$) between 1999–2002 and 2007–2010, with 14.4, 20.0 and 27.7 per cent of the dogs affected, respectively (Fig 1). By contrast, there was no statistically significant difference in the percentage of dogs with positive IDT, with 75, 81 and 70 per cent, respectively, of the IDT positive (except for the grasses) during the same periods.

Evolution of the abundance of grass pollens (1999–2010)

Fig 2 illustrates the strong seasonality of grass pollination in the Nantes area, with the highest airborne grass pollen concentration from May to August with a peak in May. Fig 3 shows that, surprisingly, the daily airborne grass pollen concentration has, in fact, decreased since 2006.

Cosensitisation with other allergens

A study on associated sensitisations shows that approximately 71.8 per cent (28/39) of the dogs sensitised to the grass mix also had a positive reaction to *D farinae* (Fig 4). Dogs positive towards the grass mix were more often sensitised towards weeds than to trees (Fig 5). Fig 6 shows that the majority of the dogs (8/9) that had a positive reaction towards Timothy grass were also sensitised to the grass mix; however, the sensitisation to the grass mix did not influence the reaction to rye grass.

Discussion

In human medicine, the results of some studies have suggested that children born during the grass pollination season (May–October) are more prone to exhibit signs of allergic rhinoconjunctivitis (Dutau and Rancé 2009). In veterinary medicine, data are scarce on this subject. In this study, no relationship was detected between the period of birth and the development of grass sensitisation. These results are consistent with those from a previous study performed in 1998 that indicated the lack of a correlation in dogs between the season of birth and IDT positive reactions (Bourdeau 1998). Furthermore, a recent study on CAD in labradors and golden retrievers failed to demonstrate any difference in risk for the development of the disease between dogs born in the warm months (March–August) or in the cold months (Meury and others 2011).

In theory, it is classically recommended that IDT should be performed after the pollen season for dogs with seasonal allergies (Hillier and Deboer 2001). However, there is little information to substantiate this recommendation (Hillier and Deboer 2001, Nesbitt and others 1984). In this study, the season of the IDT did not influence the positivity of the test. No data were included for the period from July to August. However, because of the absence of sensitisation variation for 10 months, it is unlikely that such changes could appear precisely and only during these two months.

According to previous studies, the percentage of atopic dogs that exhibit seasonal clinical signs at the time of examination ranges from 27 per cent to 62 per cent (Favrot and others 2010, Scott 1981, Nesbitt 1978, Saridomichelakis and others 1999, Zur and others 2002). In the present study, only 16.5 per cent of the dogs had seasonal pruritus, and among these dogs, few dogs (between 0 per cent and 20 per cent, depending on the grass tested) were sensitised. This result suggests

Table 3: Evaluation of breed predisposition

Breed	Reference population (%) n=9746	Grass mix ($P=0.113$)			Rye grass ($P=0.09$)			Common Meadow grass ($P=0.154$)			Timothy grass ($P=0.0004$)		
		Positive (%) (n=39)	Ratio/refer-ence	OR (CI)	Positive (%) (n=39)	Ratio/refer-ence	OR (CI)	Positive (%) (n=39)	Ratio/refer-ence	OR (CI)	Positive (%) (n=39)	Ratio/refer-ence	OR (CI)
Poodle	4.7	7.7	1.6	2.49 (0.63 to 9.79)	3.6%	0.0	1.9 (0.20 to 19.84)	/	/	/	22.2	4.7	7.57 (0.92 to 62.6)
Boxer	2.5	15.4	6.2	2.9 (1.03 to 8.16)	10.7	4.3	2.34 (0.58 to 9.42)	/	/	/	22.2	8.9	2.33 (0.39 to 13.89)
West Highland white terrier	1.3	10.3	7.9	1.56 (0.49 to 4.98)	0	0.0	0	0	0	0	44.4	34.2	21.2 (3.08 to 145.95)
French bulldog	1.2	7.7	6.4	1.33 (0.36 to 4.9)	17.9	14.9	3.02 (0.96 to 9.48)	0	0	0	/	/	/
German shepherd dog	2.5	5.1	2.0	0.65 (0.14 to 2.93)	0	0.0	0	0	0	0	0	0.0	0
Labrador retriever	8.7	7.7	1.1	0.53 (0.15 to 1.83)	14.3	1.6	0	0	3.8	9.25 (1.01 to 84.74)	11.1	1.3	0.64 (0.07 to 5.77)
Mixed-breed dog	28.4	0	0.0	0	0	/	0	0	/	0	0	0.0	0

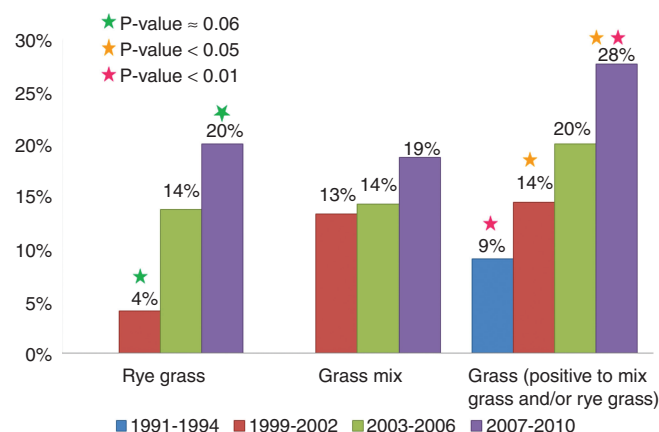


FIG 1: Evolution of grass sensitisation over time. Data from a study performed from 1991 to 1994 have been added

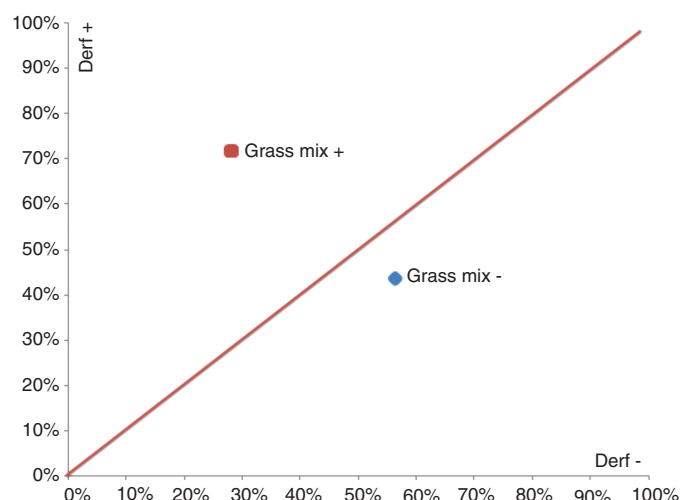


FIG 4: Relationship between *Dermatophagoides farinae* (Derf) and grass sensitisation. The abscissa represents the percentage of dogs that is negative towards Derf and the ordinate the percentage of dogs that is positive towards Derf

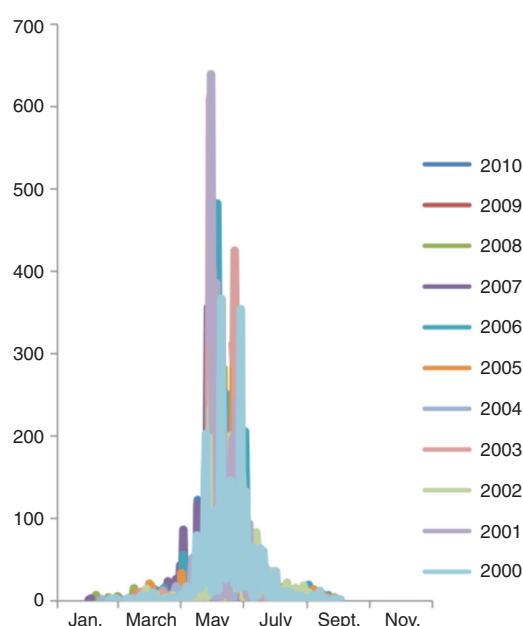


FIG 2: Daily airborne grass pollen concentration from 2000 to 2010 (number of grains/m³/day). Data provided by the RNSA

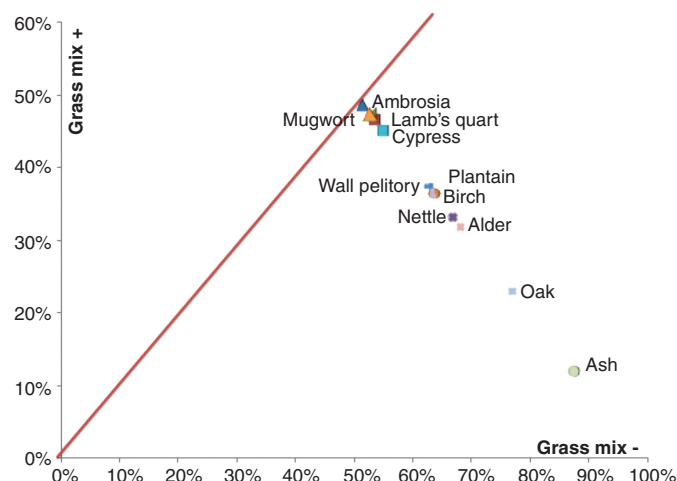


FIG 5: Relationship between the grass mix and other allergens. The abscissa represents the percentage of dogs that were negative towards the grass mix and the ordinate the percentage of dogs that were positive towards the grass mix

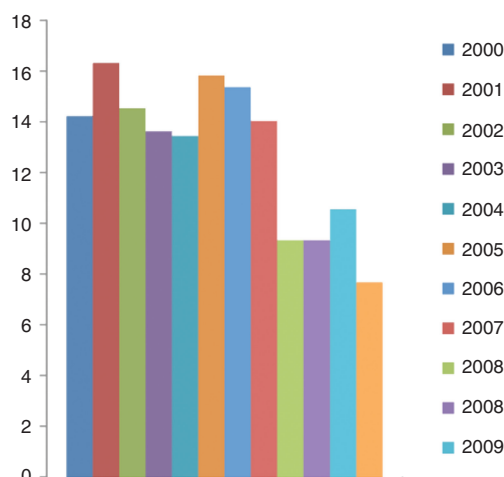


FIG 3: Mean number of grass pollen grains per day from 2000 to 2010 (number of grains/m³/day). Data provided by the RNSA

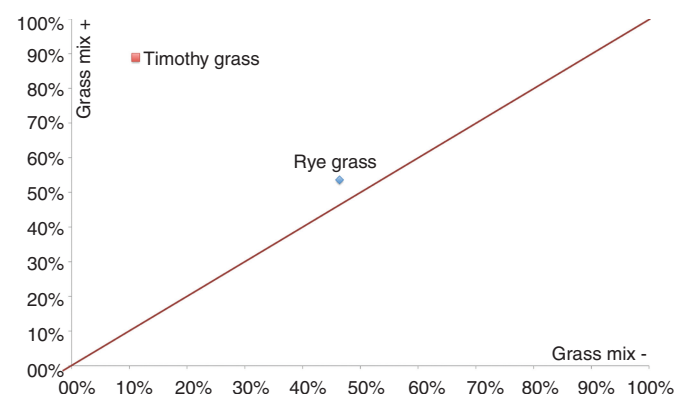


FIG 6: Relationship between rye grass, Timothy grass and grass mix. The abscissa represents the percentage of dogs that were negative towards the grass mix and the ordinate the percentage of dogs that were positive towards the grass mix

that sensitisation persists all year round in dogs, or that dogs could be sensitised even during periods of low pollen production. Fraser and others (2001) have shown that pollen grains are present in canine faecal samples throughout the months of February to July, suggesting that dogs can express signs of allergy outside of the peak season. Another explanation for the lack of a seasonality could be that clinical signs of CAD are often seasonal at their onset and then become perennial with chronicity (Favrot and others 2010, Griffin and Deboer 2001). Indeed, referral centres have to most often address the majority of difficult and chronic cases.

In a study on 266 dogs with CAD, Zur and others stated that among the 94 dogs sensitised to grass pollens, females were significantly more represented ($P=0.0514$) (Zur and others 2002). The results of this study do not support the hypothesis that female dogs are more likely to have positive reactions to grass. Similarly, the current study found no association between age and grass sensitisation.

In the current study, no breed predilection was highlighted for sensitisation to the grass mix, rye grass or common meadow grass. However, the West Highland white terrier appeared to be more susceptible to Timothy grass pollen sensitisation, or could have had a more significant exposure to factors triggering sensitisation. It has been proposed that an urban lifestyle is associated with an increased likelihood of atopic dermatitis (Favrot and others 2010, Meury and others 2011). Indeed, some breeds are likely over-represented in urban areas (ie, the West Highland white terrier, poodle, and French bulldog) and could be more often exposed to some particular environmental factors (eg, house dust and diesel exhaust particles) (Diaz-Sanchez and others 1997) or more frequent shampoos (Meury and others 2011), and these factors may explain their higher sensitisation rate to grass pollen. A recent study on the relationship between pollution and sensitisation in guinea pigs demonstrated that allergic reactions (IDT, IgE dosage) to polluted wheat pollens are higher compared with clean or unpolluted wheat pollens (Arbaban and others 2011). A variation in the breed predisposition to atopy may depend on geographical location (Jaeger and others 2010). Thus, such results must be interpreted cautiously, and further studies using a larger study sample will be required to confirm this result.

It is generally accepted that pollens are the most common allergens implicated in canine atopy in the USA (Scott 1981, Nesbitt 1978, Nesbitt and others 1984, DeBoer 1989, Zur and others 2002) but not in Europe (Willemse and Van Den Brom 1983, Carlotti and Costargent 1994, Koch and Peters 1994, Beco and Fontaine 1995, Sture and others 1995, Zunic 1998, Saridomichelakis and others 1999). Files 1 and 2 (see online supplementary data) summarise the sensitisation data to grass pollens from American, Australian and European studies. The results of the present study indicate a higher number of positive reactions to grasses than previously reported in France (Prélaud 1990, Carlotti and Costargent 1994). Several theories have been proposed to explain these discrepancies, such as geographical variation, lack of standardisation, and the wide disparity of allergenic extracts used (Hill and Deboer 2001). It is generally difficult to compare different studies. Interestingly, using the same evaluation techniques and the same allergens in two different places (Paris and Nantes) over the same periods of time, one group (PJB) demonstrated that the profile of dog sensitisation and the choice of allergens for desensitisation were different (unpublished data). Thus, sensitisation differences may exist within the same country.

A previous study on 373 IDT performed at the DPM Unit between 1991 and 1994 demonstrated that 8.8 per cent of the dogs were positive towards grasses (Bourdeau 1998). The present study suggests that the incidence of sensitivity to grass pollen has since increased (Fig 1).

This increase cannot be attributed to a general increase in the allergen sensitivity of the dogs tested nor to differences in the IDT techniques or their interpretation, or to major changes in the composition of the allergenic extracts. In this study, no significant increase in sensitivity to allergens was detected, and the IDT protocol remained the same during the study period. From 1999 to 2010, the allergens originated from the same supplier (Laboratoire DESTAING, Grasse, France), and the allergens were issued from three manufacturers: BIOPHADY and HAL (affiliate

of BIOPHADY; Lelystad, The Netherlands) from 1999 to 2003 and then ARTU since 2004.

The results of this study indicate a significant increase in sensitisation to grasses in allergic dogs that began before the change in supplier (since 1999, if we take into account the study performed from 1991 to 1994) and continued thereafter.

The lack of seasonality and increase in the number of dogs reacting to grass pollen are in contrast with the seasonal pattern of airborne pollens (Fig 2) and the downward trend in grass pollen counts between 2000 and 2010 (Fig 3).

This outcome raises a question about the value or significance of airborne pollen captors representing the real pollinic exposure or risk of sensitisation for dogs. Fraser and others speculate that dogs come into contact with pollen that has accumulated on the floor (Fraser and others 2001). Furthermore, another study shows a considerably high level of Phl p 5 (a major grass pollen allergen from *P. pratense*) in indoor dust even during periods when no grass pollen is present in the atmosphere (Fahlbusch and others 2000). Outside of the peak pollination season (May–August), indoor Phl p 5 levels appear to be independent of outdoor pollen counts. It is assumed that pollen grains from outdoor particles carried indoors via footwear and clothes may accumulate in house dust (Fahlbusch and others 2000). This idea could explain why the airborne pollen concentration does not reflect the increasing sensitisation of dogs to grass and may also help to explain the lack of a seasonality. This hypothesis could also be reinforced by the results of a large-scale study on the diagnostic criteria for CAD, which indicates that indoor life is one of the main criteria (Favrot and others 2010). Alternative methods must be developed to assess the pollens to which dogs are exposed.

Furthermore, it has been described in the human literature that pollen grains could be altered by air pollutants, leading (in vitro) to changes in surface structure and allergen liberation (Ring 2005b). In a humid milieu, the liberation of pollens occurs, and pollen grains release proinflammatory eicosanoid-like substances ('pollotrienes' or 'PALMs') (Behrendt and others 2001). Therefore, the pollen count does not always reflect the real allergen exposure, and the intensity of the allergic manifestation could vary based on the degree of pollution. This idea could contribute to the explanation for the lack of seasonality observed in this study, and the increasing rate of sensitisation to grass pollens (in cases of increased air pollution).

The majority of dogs reacting to the grass mix were sensitised to Timothy grass as well, which is expected, because the grass mix contains Timothy grass (Fig 6). On the other hand, sensitisation to rye grass appears to be independent of the sensitisation to the grass mix, which does not contain this pollen, indicating an inconsistency for cross-allergenicity between all the grasses, and for the usefulness of individual tests (Fig 6).

Knowledge of cross-sensitisation is essential for diagnoses and for choices of antigen-specific immunotherapies (Martinez-Cocera and others 2010). Cross-reactivity could be attributed to the existence of shared similar antigens in closely related plants. However, this phylogenetic approach does not explain why cross-reactivities may also exist between unrelated plants (Weber 2003). Such reactions have been attributed to the presence of panallergens, such as polcalcin and profilin (Asero and others 2010). In veterinary medicine, the traditionally aimed concept of a cross-reactivity between related plants is more controversial, and some authors recommend formulating allergen-specific immunotherapies on the basis of single allergen test results (Mueller and others 2002, Mueller and Chapman 2004). In human medicine, the detection of immunoglobulin E directed against panallergens allows us to distinguish between a cosensitisation to different protein allergens or to the corecognition of homologous allergens (Fontaine 2007, Asero and others 2010). In dogs, further studies will have to be performed to learn more about cross-sensitisation and panallergens.

Conclusion

This is the first study in France that reports such a high prevalence of dogs reacting to grass pollens (13.3–15.6 per cent, depending of the grass tested). These results are unlikely to be influenced by an interpretation bias, as shown by the relatively low percentage of positive reactions to *Dermatophagoides* species. In this study, positive reactions

to grass pollens were not influenced by the period of year in which the IDTs were performed, the period of birth of the dogs, nor by the seasonality of the clinical signs of the allergies. A hypothesis to explain the lack of seasonality could be the accumulation of aeroallergens in households. Finally, the most striking information extracted from this study is the progressive and regular increase of sensitisation of allergic dogs to grass pollens in western France over the 10-year period of time of this study. One can only wonder what the explanation for this increase in grass sensitisation in allergic dogs over time is.

► Additional material is published online only. To view please visit the journal online (<http://dx.doi.org/10.1136/vr-2012-100710>)

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Characterisation of dog sensitisation to grass pollen in western France from 1999 to 2010

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Veterinary Record 2013 172: 686 originally published online June 8, 2013

doi: 10.1136/vr.100710

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