



A Retrospective Study of Hyposensitization in Canine Atopy Based on a Polyclonal ELISA test

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ABSTRACT

Compliance with the treatment protocol and the most significant reasons encountered in general practice for the discontinuation of treatment in hyposensitized dogs are examined. The data are based on (1) a review of order forms for the hyposensitization mixture and information sheets for an ELISA test and (2) telephone interviews with dog owners. Most of the owners (81%) gave their dogs allergen injections at home. Non-compliance was defined as discontinuation of treatment in the induction period; 33.9% of the owners became non-compliant. A large proportion of non-compliant owners (51.2%) claimed to be unaware of the length of the induction period. Furthermore, 70.2% of the owners were not aware that treatment would most likely need to be lifelong if it was to remain effective. Although 67.5% of the owners perceived that their dogs had beneficial effects from hyposensitization, only 36.3% of the dogs were receiving maintenance injections at the time of the telephone interview, considerably reducing the long-term benefit from treatment. Canine atopy is a chronic disease characterized by remission and relapses. Since no control group was available in this study, the beneficial outcome of treatment reported by the owners could be partly due to the natural course of the disease. Nevertheless, the results indicated that the long-term effect of hyposensitization in canine atopy will be reduced by premature discontinuation of treatment in the maintenance period. The discontinuation of treatment could be a reflection of the treatment becoming less effective, owing to the development of new hypersensitivities or to a reduction in the placebo effect that may occur in 'new' treatments. However, poor client education and follow-up seem to be important reasons for both non-compliance and discontinuation of the treatment in the maintenance period.

Keywords: allergen, atopy, atopic, dermatitis, compliance, dog, hyposensitization, immunoglobulin E, serodiagnosis

Abbreviations: ELISA, enzyme-linked immunosorbent assay; IDT, intradermal test; IgE, immunoglobulin E; PNU, protein nitrogen unit (1 PNU $\equiv 10^{-5}$ mg of nitrogen precipitated with phosphotungstic acid)

INTRODUCTION

Atopic dermatitis is considered to be the second most common allergic skin disease in dogs after flea allergy dermatitis (Scott *et al.*, 2001). Management options available in canine atopy are avoidance or elimination of the allergen, symptomatic therapy, or

allergen immunotherapy (hyposensitization). Hyposensitization is the process of decreasing the hypersensitive response of an individual to exposure to an allergen (Scott *et al.*, 1995). It is a commonly accepted treatment for atopy in dogs (Scott *et al.*, 2001), although only one double-blind placebo-controlled trial of its efficacy in dogs has been reported (Willemse *et al.*, 1984).

No standardized hyposensitization regimen exists in veterinary medicine. Treatment regimens vary in the type of allergens used, the amount of allergens injected and the frequency of injections. Traditionally, intradermal testing (IDT) has been used to select allergens for inclusion in hyposensitization mixtures in canine atopy. Currently, *in vitro* allergy tests are widely used, although several studies have indicated a high occurrence of false positive test results in both atopic and non-atopic dogs (Miller *et al.*, 1992; Codner and Lessard, 1993; Bond *et al.*, 1994). However, *in vitro* allergy testing is easy to perform, and treatment recommendations and the allergens for hyposensitization are usually provided by a commercial laboratory (Codner and Lessard, 1992), making hyposensitization of the atopic dog readily available for general practitioners. Most studies evaluating the efficacy of hyposensitization in dogs and factors that might influence the outcome of treatment are nevertheless performed in a referral clinic.

For several years, a polyclonal ELISA test for the detection of allergen-specific IgE antibodies in dogs has been commercially available in Norway. The ELISA test results have frequently been used to identify allergens for hyposensitization. However, this test and the treatment regimen have so far not been independently evaluated. In a preliminary analysis of hyposensitization order forms, we found that most of the hyposensitization mixtures requested were composed of pollens or moulds, or a combination of these allergen groups. However, house dust mites and house dust have, in several studies, been found to be major allergens in canine atopy in Europe (Willemse and Van den Brom, 1983; Vollset, 1985; Sture *et al.*, 1995; Saridomichelakis *et al.*, 1999). This noticeable discordance from the established pattern of important allergens in canine atopy based on IDT could indicate a lack of diagnostic sensitivity and/or specificity in the ELISA test. Moreover, we found that hyposensitization was discontinued in many dogs in the induction period, as well as in the maintenance period. Since immunotherapy is assumed to be allergen-specific (Reedy *et al.*, 1997a), hyposensitization based on such a test would probably be less effective and lack of improvement could hence be one obvious reason for the discontinuation of treatment in many patients.

Drug compliance in veterinary medicine may be defined as the extent to which owners adhere to instructions when giving prescribed drugs to their animals (Grave and Tanem, 1999). In veterinary medicine, most dogs are given the hyposensitization injections at home, and compliance with the treatment protocol may strongly influence the outcome of the treatment. Additionally, studies in humans have revealed a much higher non-compliance rate in patients who receive their hyposensitization injections in facilities outside the allergist's office (Tinkelman *et al.*, 1995).

Therefore, a retrospective study was performed of hyposensitization based on the polyclonal ELISA test in dogs diagnosed and managed in general practice. The aims of the study were to determine the compliance with the immunotherapy regimen and to record the most significant reasons for discontinuing the treatment.

MATERIALS AND METHODS

Hyposensitization protocol

Hyposensitization solutions were composed on the basis of positive reactions in an ELISA test. The serum concentrations of allergen-specific IgE antibodies were measured at a commercial laboratory (Nycomed Allergenlab, Oslo, Norway), employing a polyclonal ELISA test (Alerchek Inc., Portland, ME, USA). The test used polyclonal rabbit anti-canine IgE and goat anti-rabbit IgG horseradish peroxidase conjugate to detect binding of antibody to allergen-coated microtitre wells, and measured the IgE content of the serum against 23 different allergens (Table I). The results were measured spectrophotometrically at 450 nm and, in accordance with the laboratory guidelines, an optical density value greater than 0.250 was considered positive for a specific allergen.

TABLE I
Allergens ($n = 23$) used in the ELISA test

Moulds:	<i>Penicillium</i> spp., <i>Aspergillus</i> spp., <i>Alternaria</i> spp.
Grasses:	<i>Dactylis glomerata</i> (orchard), <i>Festuca elatior</i> (meadow fescue), <i>Lolium perenne</i> (perennial rye), <i>Phleum pratense</i> (timothy), <i>Poa pratensis</i> (kentucky blue)
Trees:	<i>Betula verrucosa</i> (birch), <i>Salix caprea</i> (willow), <i>Populus</i> spp. (poplar)
Weeds:	<i>Artemisia vulgaris</i> (common mugwort), <i>Taraxacum vulgare</i> (dandelion), <i>Plantago lanceolata</i> (english plantain), <i>Chenopodium album</i> (lambs quarter), <i>Rumex acetosella</i> (red sheep sorrel), <i>Urtica dioica</i> (nettle)
Others:	House dust, house dust mites (<i>Dermatophagoides farinae</i> and <i>D. pteronyssinus</i>), flea, human dander, cat epithelium, goose feather

Although the ELISA test measured IgE antibodies against individual allergens, except for house dust mites, the hyposensitization solutions were prepared from mixtures of allergens as well as from individual allergens. Preparations of house dust mites, moulds, grasses, trees and weeds were mixtures of allergens, while those of house dust, cat epithelium, flea and goose feather were individual allergens. All extracts were aqueous, except for the house dust mites extract, which was glycerinated (Nelco Laboratories Inc., New York, NY, USA).

During the induction period, which consisted of three phases each lasting 3 months, an allergen injection was given subcutaneously every seventh day. After 9 months, the effect of the treatment was evaluated and treatment was discontinued in dogs with a

poor response. Dogs that responded positively to treatment then received maintenance injections at a frequency that maintained an optimum response (Table II). The hyposensitization mixture used for maintenance contained a total protein concentration of 20 000 protein nitrogen units (PNU) per ml. The injection volume recommended in the maintenance period was 0.25 ml, giving a total protein concentration of 5000 PNU per injection.

TABLE II

Hyposensitization schedule. During the three phases of the induction period, each lasting 3 months, an allergen injection was given subcutaneously every seventh day. After 9 months, a maintenance injection (0.25 ml) was given every 14–30 days, based on the observed frequency of injections that maintained the optimum response in each case

Phase no.	Volume (ml) injected at day no.											
	7	14	21	28	35	42	49	56	63	70	77	84
One ^a	0.05	0.10	0.15	0.20	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Two ^b	0.05	0.10	0.15	0.20	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Three ^b	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25

^aTotal protein concentration of 2000 protein nitrogen units (PNU)/ml

^bTotal protein concentration of 20 000 protein nitrogen units (PNU)/ml

Animals

One hundred and thirty Norwegian dogs, hyposensitized between June 1997 and June 1999, were included in the study. The dogs were of various breeds and mixed breeds, sexes and ages. All the dogs had been diagnosed as atopic by general practitioners and allergy-tested using the ELISA test. The referring clinician prescribed immunotherapy based on a positive test. The information sheets for dogs with positive ELISA tests provided information concerning the breed, sex, age, history, clinical signs and age at onset of clinical signs and the results of a physical examination. Seasonal variation in clinical signs, previous treatment and response to treatment were also recorded. Only dogs likely to have atopy based on a compatible history and clinical signs (Willemse, 1986), after exclusion of ectoparasite infestations, as assessed by physical examination, skin-scrapings, serological evaluation for *Sarcoptes scabiei* antibodies and response to appropriate treatment, were included. In Norway, cat and dog fleas are rarely a problem owing to the climate, and therefore tend not to interfere with the diagnosis and treatment of skin diseases. Additionally, to rule out adverse food reactions, 70 dogs (53.8%) were fed a hypoallergenic diet.

The dogs were divided into two groups. Group 1 consisted of dogs treated with immunotherapy for less than 9 months and group 2 consisted of dogs treated with immunotherapy for at least 9 months. The dogs in the second group were treated for a minimum of 9 months up to a maximum period of 7 years.

Collection of data

The results of the study were obtained by (1) a review of the order forms for hyposensitization mixtures and of the information sheets for the ELISA test and (2) telephone interviews with the owners. The first author performed all the telephone interviews.

To exclude the possibility that the owners discontinued the treatment in the maintenance period owing solely to a lack of improvement, information was collected on the owners' perception of the effect of hyposensitization. A good response was defined as a reduction of more than 50% in clinical signs, and a poor response was defined as less than a 50% reduction in clinical signs. If any additional treatment was required, the type of treatment and the frequency of administration were recorded. Information was collected on the injection routines, regularity of veterinary consultations, reasons for discontinuing treatment, and side-effects. Owners were also asked whether they were aware that treatment should continue for 9 months before final evaluation of the effect, and that treatment would most likely need to be lifelong if it was to remain effective. Owners who had discontinued treatment in the induction period were asked whether this was done in accordance with advice from the prescribing veterinarian, and whether they had started alternative treatment of their dog. Discontinuation of treatment during the induction period was defined as non-compliance.

RESULTS

Information concerning 121 dogs (93.1%) was successfully obtained by telephone interviews. Eighty dogs (66.1%) were treated for at least 9 months. The numbers of owners discontinuing hyposensitization at different stages within the first 21 months of treatment are shown in Table III. Allergens were ordered for maintenance injections for only 54 dogs (44.6%) and treatment was discontinued within one year in 18 (33.3%) of these 54 dogs.

The average number of allergens included in a hyposensitization solution was 2.4, ranging from 1 to 9 allergens. In total, 87 solutions (71.9%) were composed of pollens and/or moulds. Thirty-four hyposensitization solutions (28.1%) were composed of pollens only and 31 (25.6%) were composed of moulds only. Twenty-nine dogs (24.0%) had allergens from house dust mites included in the hyposensitization solution. Only two dogs were treated with allergens from house dust extract and house dust mites extract only.

TABLE III

Number and percentage of dogs ($n = 121$) in which treatment was discontinued at different stages

Discontinued treatment after	No. of dogs	%
3 months (phase one) ^a	27	22.3
6 months (phase two) ^a	14	11.6
9 months (phase three) ^a	26	21.5
12–21 months ^b	18	14.9
Total	85	70.2

^aInduction period

^bMaintenance period (first year)

Fifty-four owners (67.5%) perceived that their dogs had beneficial effects from the treatment. Twenty of these dogs (37.0%) received concurrent systemic anti-inflammatory treatment during all or part of the year (glucocorticoids, antihistamines or essential fatty acids). Nine of the 26 dogs (34.6%) reported to respond poorly to treatment received concurrent systemic anti-inflammatory treatment. At the time of the telephone interview, only 29 of the 54 dogs (53.7%) reported to benefit from the treatment were receiving maintenance injections.

The majority of the owners (81.0%) gave their dogs allergen injections at home. The average time between maintenance injections was 22.7 days, ranging from 7 to 70 days. Instructions to owners given by the prescribing veterinarian, however, seemed to be rather inadequate. Many owners were not aware of the length of the induction period, in particular, the owners of dogs in group 1. The same phenomenon was observed with respect to awareness that treatment would most likely be lifelong (Table IV).

The reasons for discontinuing treatment in the introductory nine months are given in Table V. Twenty-one owners (51.2%) claimed to be unaware of the length of the induction period, and hence became non-compliant. With three dogs (2.5%), pruritus increased one to two days after allergen injections and so treatment of these dogs was discontinued. None of the hyposensitized dogs displayed severe side-effects, such as urticaria, angioedema or anaphylaxis. Thirteen of the owners (31.7%) discontinued the treatment without approval from their veterinarian. Only one owner (0.8%) was instructed to modify the treatment protocol owing to lack of improvement with the standard protocol. None of the dogs that became non-compliant with the treatment protocol was treated with a modified hyposensitization protocol.

Six (23.1%) of the 26 dogs with a poor response to hyposensitization were euthanized, and 12 dogs (46.2%) received long-term treatment with glucocorticoids when hyposensitization failed. The corresponding numbers in group 1 were 9 dogs (22.0%) and 10 dogs (24.4%), respectively.

TABLE IV

Compliance with hyposensitization related to the information the owners had received from their veterinarian. Group 1 consists of dogs treated for less than 9 months (non-compliant group, $n = 41$) and group 2 consists of dogs treated for 9 months or more (compliant group, $n = 80$)

	Number and percentage of owners aware of treatment length			
	Group 1		Group 2	
	Yes	No	Yes	No
Information received				
Treatment should continue for 9 months before final evaluation of efficacy	20 (48.8%)	21 (51.2%)	66 (82.5%)	14 (17.5%)
Treatment would most likely need to be lifelong to be effective	12 (29.3%)	29 (70.7%)	24 (30.0%)	56 (70.0%)

TABLE V

Reasons for discontinuing hyposensitization injections in the introductory 9 months. While treatment was discontinued for 41 dogs, more than one reason for discontinuing was given by several owners, resulting in a total of 47 reasons

Reasons for discontinuing	Number of dogs
No noticeable improvement	21 ^a
Good response	12 ^a
Worsening of problem	3
Allergens too expensive	3
Unable to give the dog injections	2
Difficulty in obtaining renewal orders	2
Unrelated illness/death	2
Too time-consuming	1
Change of owner	1
Sum	47

^aTwenty-one of these 33 owners claimed to be unaware of the length of the induction period, and hence became non-compliant

DISCUSSION

In the present study, the hyposensitization solutions used were mainly composed of moulds or pollens, or a combination of these allergens. Moulds play a controversial role in canine atopy (Reedy *et al.*, 1997b), and pollens appear to be less important allergens than house dust mites. In a study by Vollset (1985), *Dermatophagoides farinae* was by far the most common allergen in atopic Norwegian dogs, as assessed by IDT. Most polyclonal ELISA allergy tests evaluated have been characterized by low specificity rather than low sensitivity (Miller *et al.*, 1992; Codner and Lessard, 1993; Bond *et al.*, 1994). However, in the referred ELISA test, house dust mites were supplied as a mixture consisting of *D. farinae* and *D. pteronyssinus*. *D. farinae* appears to be a more important allergen in canine atopy than *D. pteronyssinus* (Noli *et al.*, 1996), and results from a recent study using IDT have suggested a maximum possible cross-reactivity between *D. farinae* and *D. pteronyssinus* of 52% (Hillier *et al.*, 2000). Hence, the low number of positive ELISA test results against house dust mites could be due to the use of a mixed extract, resulting in lowered diagnostic sensitivity, with the consequent lack of the inclusion of important allergens in the hyposensitization mixture. Nevertheless, because of the 'threshold phenomenon', immunotherapy can still be successful, even though the hyposensitization solution does not include all the relevant allergens (Scott, 1981). If the ELISA test can detect enough of the implicated allergens, the dog should respond favourably to immunotherapy (Miller *et al.*, 1993). Moreover, since the mode of action of hyposensitization in dogs is unknown, other mechanisms might also explain a favourable response to hyposensitization when only some of the relevant allergens are included. It is also noteworthy that Nesbitt (1978) and Mueller and Bettenay (1996) observed better results following hyposensitization of dogs with strong reactions against pollens compared to those in dogs with strong reactions against moulds and house dust mites. This observation could imply that pollens play a more important role in canine atopy than that revealed by IDT. However, both these investigations were also open, uncontrolled studies.

In the present study, several owners (33.9%) discontinued treatment during the induction period. Other surveys have reported non-compliance rates ranging from 10% to 45% (Baker, 1969; Nuttall *et al.*, 1998; Rosser, 1998). A large percentage of the non-compliant owners (51.2%) in the present study claimed to be unaware of the length of the induction period. This could be due to poor client education and poor follow-up. Other possible explanations are owners' forgetfulness or disappointment with the effect of treatment and preference for alternative procedures (Thrusfield, 1995). Although the hyposensitization protocol used in this study is fairly time-consuming, only one owner (2.4%) discontinued treatment for this reason. Other practical problems, including the cost of treatment, were also reported to be of minor importance. A considerably larger number of owners in the second group compared with the first group was aware of the treatment regimen. However, recall bias could have more seriously affected the results for the non-compliant group, owing to the longer average time since these owners discontinued treatment.

The high percentage of dogs reported to benefit from treatment (67.5%) and the relatively small number of dogs receiving long-term treatment (only 29.8% received

treatment for more than 21 months) in the present study are somewhat conflicting, since a large majority of atopic dogs responding to hyposensitization is reported to require regular maintenance injections on a lifetime basis (Reedy *et al.*, 1997a; Nuttall *et al.*, 1998). If this is valid, hyposensitization should have been of long-term benefit in less than 29.8% of the dogs. A surprisingly low number of owners in both groups were aware that, to be fully effective, treatment would probably need to be lifelong. This finding could explain the relatively low number of owners continuing treatment on a long-term basis. However, it could also be a reflection that treatment became less effective, owing either to the natural course of the disease or to the perceived placebo effect that may occur in 'new' treatments. Willemse and colleagues (1984) reported a 21% placebo effect using aluminium as an adjuvant. In humans receiving hyposensitization therapy, the placebo effect observed has been reported to be as high as 40% (Kaufman and Roth, 1974).

The hyposensitization regimen utilized in the present study has some inherent shortcomings. First, although a positive ELISA test result against a single allergen was obtained, mixtures of grouped allergens were included in the hyposensitization solution. Second, the composition of allergen solutions was based only on the results of an ELISA test and did not take clinical relevance into consideration. Both these shortcomings could result in the inclusion of irrelevant allergens and hence in the dilution of important allergens. Finally, although more frequent injections were used, the recommended maximum dose of 5000 PNU per injection is low compared with previously published regimens for aqueous allergens (Scott *et al.*, 1993; Reedy *et al.*, 1997a; Rosser, 1998). Although the minimum effective dose of an allergen in immunotherapy is at present unknown (Reedy *et al.*, 1997a), results from one study indicate that hyposensitization is more effective with high doses than with low doses (Garfield, 1992). Conversely, Rosser (1998) reported a good response with a low-dose regimen. Consequently, the schedule supplied with commercially available extracts should be considered as a guide, and individual modification is often necessary. Hence, background knowledge in the field of hyposensitization is required to formulate a proper hyposensitization mixture and to design an optimal treatment regimen for each individual patient.

When hyposensitization fails, no alternative treatment other than lifelong symptomatic therapy is currently available. In the present study, many of the dogs that did not finish the induction period or responded poorly to hyposensitization were treated with glucocorticoids or were euthanized after cessation of hyposensitization. These figures emphasize the need for effective and specific long-term treatment and proper follow-up in canine atopy. On the other hand, unnecessary and inappropriate hyposensitization could delay more effective treatment. There is also a risk of fatal anaphylactic reactions and a possibility of inducing long-lasting hypersensitivity in non-sensitized individuals (Marsh *et al.*, 1972; Turkeltaub *et al.*, 1978). Adverse reactions to hyposensitization in dogs are reported to be uncommon (Angarano and MacDonald, 1992) and, in the present study, only three of the owners reported intensification of clinical signs after allergen injections. Furthermore, in a study by Codner and Lessard (1992), hyposensitization of normal dogs with irrelevant aqueous allergens did not induce clinical hypersensitivity. However, further studies are required to determine whether hypersen-

sitivities, or other immune-mediated disease, can be induced in dogs following injection of non-aqueous allergens or higher concentrations of aqueous allergens.

In interpreting the results of any study, it is important to recognize all of its potential limitations. Although an effort was made to include only atopic dogs in the present study, some pruritic, non-atopic dogs were probably included. Willemse's (1986) diagnostic criteria for canine atopy are commonly accepted, but the list of differential diagnoses is lengthy and a positive allergy test does not confirm a diagnosis of atopic dermatitis. Furthermore, an elimination diet was performed in only a proportion of the dogs and the compliance of owners in adhering to the elimination diet was difficult to evaluate. Hence, misclassification bias could have influenced the outcome of the study. On the other hand, by not being involved in the diagnosis and management of the included dogs, we could more objectively evaluate the owner compliance, and most likely also reduce interviewer bias.

Hyposensitization continues to be a valuable treatment option in canine atopy because of its potential advantages. However, there is an urgent need for controlled long-term trials evaluating the true efficacy of hyposensitization in canine atopic dermatitis. Another, yet unanswered, question is the required length of treatment. The results from this study indicate that poor client education and follow-up may lead to high non-compliance and a large proportion of owners discontinuing treatment within 21 months, despite the fact that they consider that their dogs had beneficial effects from the treatment. Owners should not be encouraged to begin hyposensitization unless factors such as the lag time and the necessary length of treatment are thoroughly discussed. Assuming that hyposensitization should be a lifelong treatment, a long-term benefit depends on owners continuing treatment. According to Nuttall and colleagues (1998), atopic dogs managed in a referral clinic had a significantly better success rate than externally managed cases. These authors suggested that this could be due to better recognition and management of flare factors in the dogs managed by dermatologists. The results found herein further imply that, if possible, allergy testing and subsequent hyposensitization should be within the remit of dermatologists.

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