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Original article

The results of intradermal skin tests (IDST) in dogs with atopic dermatitis from the Lublin voivodeship

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Abstract

The purpose of this study was to assess the positive immediate reactions received from intradermal skin tests (IDST) which confirmed the presence of IgE-dependent hypersensitivity in dogs with atopic dermatitis, which were patients of the Dermatology Consulting Section at the University of Life Sciences in Lublin between 2007 and 2009. Intradermal skin tests were performed on 142 dogs (72 females and 70 males) from the Lublin voivodeship of different breeds ranging in age from 1 to 6 years (average 2.8 years). The allergen set used in this study was the Artuvetrin Test (ARTU Biologicals Europe B.V, Holland). The owners of 84 dogs observed the presence of skin lesions all year round regardless of season, while 58 dog owners noted them only in spring and summer. Most immediate positive reactions were ascertained from mite allergens (70.61%), fewer from pollen allergens (19.55%), and the fewest from animal (4.15%) and mould allergens (1.66%). Immediate positive reactions for a flea allergen (4.03% of all positive reactions) were also ascertained. In 98.6% of dogs polysensitization was found.

Key words: intradermal skin tests, IDST, canine atopic dermatitis, aeroallergens, polysensitization

Introduction

Canine atopic dermatitis (CAD, AD) is a pruritic and inflammatory allergic skin disease, inherent from genetic predisposition to development of hypersensitivity (mainly IgE-dependent) for environmental allergens (Reedy et al. 1997, Scott et al. 2001, Hillier 2002). The disease occurs in 10 to 15% of the canine population (Hillier and Griffin 2001, Scott et al. 2001). The course of the disease is chronic and recurrent, meaning that the dogs suffer lifelong from the disease and clinical signs can be present seasonally or all year round. Canine atopic dermatitis can be coincident with other diseases with hypersensitivity with a similar clinical course – especially with adverse food reactions in 13-30% of cases (Hillier and Griffin 2001) and with flea allergy dermatitis in 31% of cases (Sousa and Halliwell 2001). The diagnostic procedure with a patient affected by AD is based on detailed anamnesis, dermatological examination of specific skin lesions localized in predilective sites, and on allergy tests contained in Willemse's criteria (Willemse 1986, Favrot et al. 2010). From among the allergy tests recommended in diagnostic and therapy of AD,

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skin tests are used in dogs – especially intradermal skin tests (IDST) with aeroallergens called airborne allergens (Silny and Czarnecka-Operacz 2001), as well as laboratory tests based on serological analyses confirming the presence of specific IgE and/or IgGd in blood serum (DeBoer and Hillier 2001). Positive tests results, correlating with the patient's clinical state, confirm the diagnosis and they are the basis of specific allergen immunotherapy (SIT). IDST in dogs are performed on animals in remission of clinical lesions' in the case of seasonal lesions, tests should be performed 2 months before allergen exposure (Hillier and DeBoer 2001). The IDST technique relies on intradermal injection of standardized (i.e. preclusive irritating and/or toxic effect) water solution of allergens. Intradermal administration of allergen is intended to cause a hypersensitivity reaction in the injection site (Reedy et al. 1997, Hillier and DeBoer 2001, Scott et al. 2001). In CAD, administration of aeroallergen causes degranulation of previously sensitized skin mast cells (type I immunological response according to the Gell and Coombs classification) which results in the release of inflammatory mediators, such as histamine, serotonin, tryptase, calicreine and proteases. A positive test result is visible 10-20 minutes after injection in the form of a wheal and skin erythema. This demonstrates the presence of an immediate immunological response of a humoral type caused by the presence of specific IgE in the skin, called an early allergic reaction (EAR) (Lasek 1995, Reedy et al. 1997, Scott et al. 2001, Kowalski 2006).

For diagnosis and treatment of AD, positive test results obtained 10-20 minutes after injection (usually after 15 mins) are important; they show the presence of early allergic reaction (Reedy et al. 1997, Hillier and DeBoer 2001, Scott et al. 2001, Kowalski 2006). Test reading (Reedy et al. 1997, Kowalski 2006), should be performed within 30 minutes of allergen administration. Positive results after this time are not accepted for consideration in AD diagnosis, because their clinical significance is unknown (Hillier and DeBoer 2001, Kowalski 2006).

Assessment of a positive test result can be subjective or objective – both methods are used in veterinary dermatology (Reedy et al. 1997, Hillier and DeBoer 2001, Scott et al. 2001). In subjective assessment, the presence of a wheal with concurrent erythema comparable to positive control, being a histamine solution (0.1mg/ml i.e. 0.001%), graded +4, is considered as a positive test result, while negative control is usually buffered saline, graded 0. Every wheal diameter larger than 3 mm with erythema present is considered as a positive reaction. All positive reactions less than positive control are visually graded +1, +2 or +3. In objective assessment, the wheal diameter is measured in millimetres with a transparent ruler. A wheal diameter equal to or larger than the mean value of a positive and negative control is considered as a positive test result (Reedy et al. 1997). In the case of an irregular shape of wheal, two diameters should be measured – the largest and smallest – and the mean value should be calculated.

For excluding false positive or false negative results, positive and negative control is performed. Positive control allows the detection of suppressed skin reaction, which can be caused by drugs, other diseases or stress during the test. Negative control allows the exclusion of skin reaction caused by trauma from inappropriate technique and an unspecific effect of preservative agents, such as 0.47% phenol. False negative results are noticed in 10-30% of investigated dog population (Hillier and DeBoer 2001), and false positive in 10-36% of dogs (Reedy et al. 1997, Hillier and DeBoer 2001).

The reliability of results obtained in an intradermal skin test depends on the standardization of allergen extracts, correct patient preparation for testing and the experience of the investigator.

The purpose of this study was to assess the positive immediate reactions obtained from intradermal skin tests confirming the presence of IgE-dependent hypersensitivity in dogs from the Lublin voivodeship affected by atopic dermatitis admitted to the Dermatology Consulting Section of Veterinary Clinics at the University of Life Sciences in Lublin (Poland) through the years 2007-2009. The study was carried out under research project No. 30801632/1409 'Assessment of selected parameters of immunological response in the course of canine atopic dermatitis' (in Polish).

Materials and Methods

Animals. Dogs were qualified for intradermal skin test according to approved requirements (Reedy et al. 1997, Griffin and DeBoer 2001, Scott et al. 2001) after fulfilling clinical criteria (Willemse 1986, Prelaud et al. 1998, Favrot et al. 2010) allowing the diagnosis of AD to be confirmed. Tests were performed on 142 dogs with AD admitted to the Dermatology Consulting Section for treatment. In 17 dogs (12% of investigated population), AD symptoms occurred for the first time, while the remaining dogs had been treated earlier. Owners of 84 dogs observed the occurrence of skin lesions all year round independently of the season, while owners of 58 dogs noted them only in spring and summer.

Experimental design. The Artuvetrin Test set (ARTU Biologicals Europe B.V, Holland) designed for dogs was used in this study. In this set, allergen extracts are suspended in buffered 0.9% NaCl solution with the addition of 0.47% phenol as preservative agent. The set contains 12 allergens including flea allergen:

a) Mite group allergens: *Dermatophagoides farinae, Dermatophagoides pteronyssinus, Lepidoglyphus destructor, Acarus siro, Tyrophagus putrescentiae,*

b) Pollen group: Grass pollen mixture *(Bermuda Grass, Orchard Grass, Sweet Vernal Grass, Timothy and Velvet Grass*), Tree pollen mixture I (*Birch, Alder, Haze),* Tree pollen mixture II (*English oak, European beech and American Elm*) and Weed pollen mixture (*Common mugwort, Stinging nettle, Common dandelion, English plantain*),

c) Mould group as Fungi mixture I: *Alternaria alternata, Aspergillus fumigatus, Cladosporium herbarum, Penicillium notatum,*

d) Animal allergens: Cat epithelium.

The set contains a positive control (0.1 mg/ml of histamine) and negative control (buffered 0.9% NaCl with 0.47% phenol).

Tests were performed according to the procedure recommended by the manufacturer. Result reading was performed 10-20 minutes after intradermal administration of allergens using objective and subjective assessment (the diameter of the test wheal with erythema):

++++ diameter equal to or greater than positive control i.e. >14 mm,

+++ diameter smaller than positive control i.e. 12-14 mm,

++ diameter close to half of the positive control diameter i.e. 8-11 mm,

+ diameter greater than negative control by 3 mm and less than half of the positive control i.e. 5-7 mm,

– diameter equal to or less than negative control.

Statistical analysis. The results were analyzed statistically using Statistica 9.3 (StatSoft) and Excel (Microsoft 2007) computer software. Arithmetical means (x), standard deviations (±SD) and medians (Nm) were determined. The Spearman test ($P<0.05$) was applied to evaluate the association between allergens used in intradermal skin tests. To reveal the statistical significance between IDST results obtained from the group of dogs suffering from AD, according to the owners, seasonally or all year round, Pearson's $\chi 2$-test was performed. In this test, pollen, mould and flea allergens were conceded as seasonal, while mite and animal allergens were conceded as occurring all year round.

Results

Tests were performed on 72 females and 70 males. The age of dogs qualified for intradermal skin tests ranged from 1 year to 6 years, the mean age (x) was 2.8 years (±S D = 1.37) and the median Nm = 2.5. 41 dogs were older than 3 years (28.9% of total population). Most IDST were performed on German Shepherds (26 dogs), mongrels (20), American Staffordshire Terriers (16), Boxers (14) and Dogue de Bordeaux (10). Tests were also performed on Labradors (5), French Bulldogs (5), Dachshunds (4), Dobermans (4), English Bulldogs (3), and Fox Terriers (3). The other 32 dogs belonged to other breeds. The mean value (x) of positive control (K+) was x±SD = 14.85±1.42 mm, median Nm = 15mm, while the mean value of negative control (K-) was x±SD = 0.22±2 mm, median Nm = 0 mm.

The number and percentage of dogs with positive immediate reactions from IDST is presented in Table 1. In 142 of the examined dogs, immediate positive reactions in IDST were ascertained in 844 cases, which shows that the majority of examined dogs demonstrated immediate positive reactions to two or more allergens. This demonstrates the polyvalence of allergy. Polyvalence of allergies is presented in Fig.1. Only 2 of the 142 examined dogs revealed monovalency of allergy. In 98.6% of the dogs polysensitization was noted. In 35 (24.65%) dogs, the polyvalence was ascertained for 5 allergens, in 26 (18.31%) for 6 allergens and in 22 (15.49%) for 7 allergens.

Positive immediate reactions to mite group allergens

In all examined dogs, immediate positive reactions were revealed to at least one allergen of this group. In 844 positive immediate reactions, 596 were noted to mite allergens (Table 1), representing 70.61% of all positive reactions. Most positive reactions were ascertained in allergens of *Dermatophagoides farinae* – reactions appeared in 93.66% of dogs. Slightly fewer positive reactions were confirmed to allergens of *Tyrophagus putrescentiae* (85.21%), *Lepidoglyphus destruktor* (83.8%) and *Acarus siro* (82.39%) and fewest to allergens of *Dermatophagoides pteronyssinus* (74.65%).

In 39 of 142 dogs a positive immediate reaction occurred exclusively to mite allergens. In the remaining 103 dogs, positive reactions to mite allergens were concurrent with positive immediate reactions to other allergens. Of the 103 dogs, 83 positive immediate reactions to pollen allergens occurred, 14 to mould allergens and 35 to animal allergens.

Positive immediate reactions to pollen allergens

Positive immediate reactions to pollen allergens (Table 1) were noted in 165 cases of 83 dogs i.e. 58.45% of dogs, and constituted 19.55% of all positive reactions obtained from intradermal skin tests. In all these dogs positive immediate reactions to mite allergens were also found, which demonstrates polysensitization. Grass pollen mixture revealed 47 positive

Table 1. Number of dogs and percentage participation of positive immediate reactions obtained from IDST in 142 dogs with AD.

Allergen	Concentration of allergen	Number of dogs (%) with immediate positive reactions in IDST
Grass pollen mixture I	1000 NU/ml	47/142 (33.09%)
Tree pollen mixture I	1000 NU/ml	41/142 (28.87%)
Tree pollen mixture II	1000 NU/ml	31/142 (21.83%)
Weed pollen mixture	1000 NU/ml	46/142 (32.39%)
Tyrophagus putrescentiae	100 NU/ml	121/142 (85.21%)
Dermatophagoides farinae	100 NU/ml	133/142 (93.66%)
Lepidoglyphus destructor	100 NU/ml	119/142 (83.8%)
Dermatophagoides pteronyssinus	100 NU/ml	106/142 (74.65%)
Acarus siro	100 NU/ml	117/142 (82.39%)
Cat epithelium	100 µg/ml	35/142 (24.65%)
Fungi mixture I	100 µg/ml	14/142 (9.86%)
Flea	1000 NU/ml	34/142 (23.94%)
All positive reactions to mite group allergens		596/844 (70.61%)
All positive reactions to pollen group allergens		165/844 (19.55%)
All positive reactions to mould group allergens		14/844 (1.66%)
All positive reactions to animal group allergens		35/844 (4.15%)
Flea allergen		34/844 (4.03%)
All positive reactions		844 (100%)

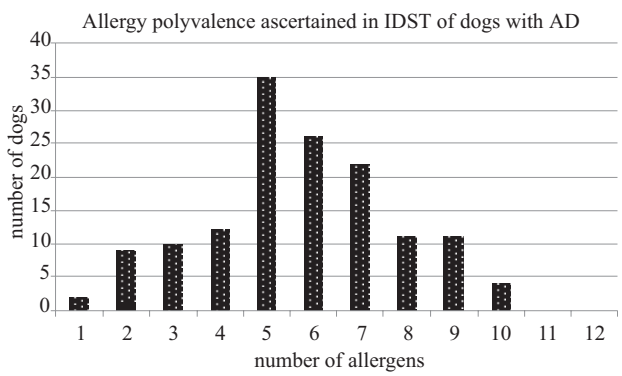

Fig. 1. Allergy polyvalence in 142 dogs examined by intradermal skin tests.

reactions (33.09% of examined dogs), tree pollen mixture I and II – 72 (50.7%) and weed pollen mixture – 46 positive reactions (32.39% of dogs).

Positive immediate reactions to mould allergens

In this group of allergens (Table 1) 14 positive immediate reactions were noted in 142 examined dogs, which is 9.89% of the examined dogs.

Positive immediate reactions to animal allergens

35 positive immediate reactions were noted to cat epithelium allergen (Table 1), while in all these dogs allergy was polyvalent – in 58.49% with mite group allergens, in 30.19% with pollen group allergens and in 11.32% with mould group allergens.

Positive immediate reactions to flea allergen

In the group of examined dogs, 34 positive immediate reactions to flea allergen were found i.e. 23.94% of dogs, but only in 8 dogs older than 3 years.

The intensity of positive immediate reactions obtained from intradermal skin tests is presented in Fig. 2. Most ++++ and +++ reactions were found in mite group allergens. Mean values (x), standard deviations (±SD) and medians (Nm) obtained from particular tests are presented in Table 2.

According to correlation analysis using Spearman's test at significance level $P<0.05$, a high correlation ($0.5 = <r, xy < 0.7$) was observed between allergens: *Tree mixture I*/*Tree mixture II* (R = 0.615), *Tyrophagus putrescentiae*/*Acarus siro* (R = 0.667), *Thyrophagus putrecentiae*/*Lepidoglyphus destructor* (R = 0.528), *Lepidoglyphus destructor*/*Acarus siro* (R = 0.572).

An average correlation ($0.3 = < r, xy < 0.5$) was revealed between allergens: *Tyrophagus putrescentiae*/*Dermatophagoides farinae* (R = 0.497), *Dermatophagoides farinae*/*Acarus siro* (R = 0.356) and *Dermatophagoides pteronyssinus*/Flea (R = 0.352).

Table 2. Intensity of positive immediate reactions obtained from intradermal skin tests to particular allergens. Mean values (x), standard deviations (±SD) and medians (Nm) of positive immediate reactions were given.

Allergen	Mean of diameter (x) measurement in mm	Standard deviation (±SD) in mm	Median (Nm) in mm
K+	14.85	1.42	15
Grass pollen mixture I	11	2.57	12
Tree pollen mixture I	10.85	2.55	12
Tree pollen mixture II	10.09	2.56	8
Weed pollen mixture	11.65	2.75	12
Tyrophagus putrescentiae	13.63	2.07	15
Dermatophagoides farinae	13.44	2.23	15
Lepidoglyphus destructor	12.5	2.32	12
Dermatophagoides pteronyssinus	10.99	2.21	12
Acarus siro	12.7	2.15	12
Cat epithelium	10.94	2.3	12
Fungi mixture I	11.07	2.75	12
Flea	11.35	1.81	12

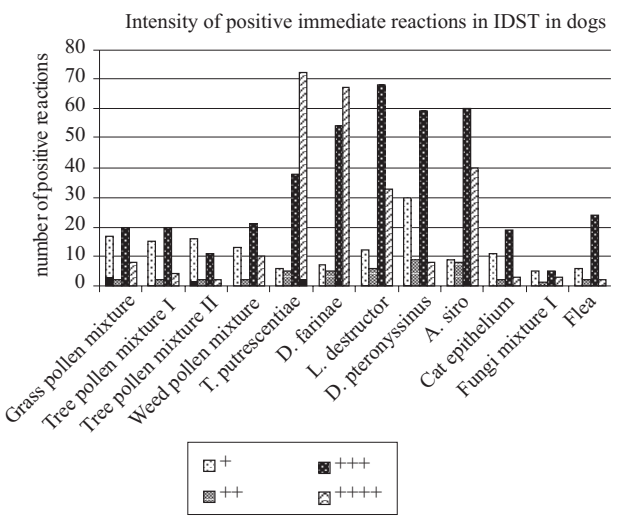

Fig 2. Intensity of reactions obtained from intradermal skin tests in dogs with AD

A correlation classified as weak (0.1 = < r, xy < 0.3) was found between allergens: Grass/ Tree mixture I, Grass/Weed, Grass/*Dermatophagoides pteronyssinus,* Grass/Fungi mixture I, Tree mixture I/Weed, Tree mixture II/Weed*, Dermatophagoides farinae*/*Lepidoglyphus destruktor, Dermatophagoides pteronyssinus*/*Acarus siro, Lepidoglyphus destructor*/Cat epithelium, Cat epithelium/Fungi mixture I and Cat epithelium/Flea.

The dependence between the positive IDST results and the owners' declaration about the seasonality of the presence of skin lesions was confirmed by results from Pearson's $\chi 2$ analysis test ($\chi 2 = 4.16$, $p = 0.0414$).

Discussion

Published studies (Pomorski 1990, Reedy et al. 1997, Hill and DeBoer 2001, Szczepanik et al. 2005) reveal that positive intradermal skin test responses to mite group allergens in dogs with AD in Europe are the most frequent. According to these studies, positive reactions to *Dermatophagoides farinae* allergens are present in 37% (Norway) to 54.5% (France) of disease-affected dogs, to *Dermatophagoides pteronyssinus* in 1.6% (Norway) to 21% (France), and to warehouse mites *(Tyrophagus putrescentiae, Acarus siro)* in about 23% (Germany) to 77% (Netherlands) of atopic dogs. According to Saridomichelakis et al. (1999), 84.6% of intradermally tested Greek dogs were positive to mite allergens. Sture et al. (1995), while conducting studies in Great Britain, found polyvalence of allergy in examined dogs with AD, and noticed the crucial role of mite group allergens – 49% of obtained positive test results for *D. farinae* allergens and 35% for *D. putrescentiae.* In 88.5% of dogs examined in Edinburgh and in 74.2% in London a positive reaction to one or both of these allergens was found. A published study from Australia (Mueller et al. 2000) performed on 1000 dogs shows that in 34% of dogs a positive reaction to *D. farinae* allergens and in 14% to *D. pteronyssinus* allergens was found. Hayasaki et al. (1998) in a study of dogs with AD found positive immediate reactions to mite group allergens in 32% of investigated dogs. However, Tzu-Yang Sung et al. (2009) in a study performed in Taiwan found positive IDST results both to *D. farinae* as well as *to D. pteronyssinus* allergens. Domestic and foreign literature data, similar to the author's results, show

that mite group allergens are diagnosed most frequently as responsible for the development of AD in dogs.

Positive immediate reactions to pollen group allergens i.e. grass, weeds or trees, are found (Reedy et al. 1997, Hill and DeBoer 2001) in 1.9% of dogs (France) to almost 30% (England). According to Sture et al. (1995), grass pollen allergens were responsible for 30% of positive reactions, weeds 26% and trees 26%; however, a difference between dogs living in London and in Edinburgh was noticed. Data from Greece (Saridomichelakis et al. 1999) show, that pollens comprise 36.3% of positive reactions in atopic dogs, animal allergens 42.9%, and mould group allergens 23.1%. Animal allergens (epithelium) were identified as causing a positive reaction (Reedy et al., 1997, Hill and DeBoer 2001) in dogs with AD in 0.8% (Norway) to 59.9% (Sweden) of patients, and mould group allergens in 3.3% (Norway) to 13.8% (Sweden) of atopic dogs. According to Sture et al. (1995), in England animal allergens can be responsible for 80% of positive IDST reactions, and mould allergens about 13%.

Polish literature data (Pomorski 1990, Adamska 2002, Szczepanik et al. 2005) emphasize the frequent occurrence of polyvalent allergies in dogs with AD, which are found in 62.8-97.53% of cases. These authors found positive immediate reactions in IDST of atopic dogs to *Dermatophagoides farinae* allergen in 93.8% and to *D. pteronyssinus* allergens in 40.5-66.7% at the same time. The results of IDST in dogs with AD from Greece (Saridomichelakis et al. 2008) indicated that allergy polyvalence to mite group allergens could reach 45% to all five allergens i.e. *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, *Tyrophagus putrescentiae, Acarus siro* and *Lepidoglyphus destructor*. The author obtained, in 63.38% of dogs, positive immediate reactions to these 5 mite group allergens.

The studies of Pomorski (1990), Adamska (2002), Szczepanik et al. (2005) indicate that in Poland positive reactions to pollen allergens are found in 60.5% of dogs with AD, including to grass allergens in 29%, trees in 7.9% and weeds in 8.8-43% of animals. The author obtained similar results – positive reactions with pollen allergens were found in 58.45% of examined dogs: with grass allergens in 33.09% dogs, tree allergens more than 21% of dogs and weed allergens 32.39%. The allergen set used contained birch, alder, hazel, oak and mugwort allergens, regarded (Rapiejko 2004) in Poland as having very large (+++++) or large (++++) significance for allergy in humans.

The author noted 9.89% of dogs with positive reactions to mould group allergens. Pomorski (1990) discovered such reactions in 30.7% of examined dogs, Adamska (2002) in 49.1% and Szczepanik et al. (2005) in 71.6%. This was probably conditioned by the different composition of allergen mixture used in tests. An Artuvetrin Set Test contains a mould allergen mixture of *Alternaria alternata, Aspergillus fumigatus, Cladosporium herbarum, Penicillium notatum,* the first two of which, according to Lipiec et al. (2000) and Jahnz-Różyk (2008), are identified most frequently as the cause of inhaled allergy in humans in Poland. In addition (Jahnz-Różyk 2008) among mould allergens, many differently built allergens are distinguished – 13 *Alternaria alternate* allergens, 12 *Cladosporium herbarium* allergens and 22 *Aspergillus fumigatus* allergens are known, which creates difficulty in extracting the main allergens used in allergy testing.

Positive immediate skin reactions for cat epithelium allergens are found in IDST of dogs with AD. Masuda et al. (2000) report their presence in 26.1% of dogs, Saridomichelakis et al. (1999) 1%, Pomorski (1990) 11.7%, Adamska (2002) 28% and Szczepanik et al. (2005) affirm them in 50.6% of examined dogs.

Some authors (Carlotti and Costargent 1994, Hayasaki et al. 1998, Szczepanik et al. 2005) point to the concurrence of atopy and flea allergy. Studies performed in Poland by Szymanek (2003) indicate that in young dogs (< 3 years) affected by AD, positive immediate reactions in IDST can occur in 20% of cases, and in older dogs in 92.9% of cases. Foreign authors obtained different results – Carlotti and Costargent (1994) found it in 57% of atopic dogs, Hayasaki et al. (1998) in 65.4% of dogs. The author noted 23.94% of dogs with positive immediate reactions to flea allergen.

The results obtained in intradermal skin tests show that the most common allergens causing allergy in dogs with atopic dermatitis are mite group allergens. Polyvalence found in intradermal skin tests in dogs with atopic dermatitis is characteristic, and comprises allergens of pollens, moulds, cat epithelium and flea.

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