

Some Allergens in Dogs and Their Evaluation via Hematologic, Immunologic and Intradermal Skin Test Methods

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Abstract: Allergic diseases with various symptoms affecting different systems and their evaluation formed this study. About thirty allergens choosed according to investigations and litterature have been applied to 100 dogs via IDST (Intradermal Skin Test) method. The allergens were mites, house dust, cat, dog, budgerigar and rabbit epithelia, duck feather, cotton, poplar, willow, grass, milk, chicken, cow, mutton, horse and turkey meat, tuna fish, wheat, tomato, potato, chocolate, orange, apple, pear, nut, walnut, egg white and yolk. The percentage of positive reactions as well as formul leukocyte and total IgE and IgG analyses between groups with and without positive reactions were evaluated. Neither haemogram and formul leukocyte nor total IgE and IgG values showed any statistical difference. It was noted that lesions differed significantly such as diet and living conditions having great influence on the allergic disease. We concluded that it was necessary to use multiple diagnostic methods and to evaluate them with clinical symptoms and the anamnesis since, the diagnosis of allergic diseases seems very complex.

Key words: Allergen, allergy, dog, Intradermal Skin Test (IDST), total IgE, total IgG

INTRODUCTION

Allergy is a disease state characterized by hypersensitivity responses to allergens and oftentimes mediated by IgE reaginic antibodies (Olivry *et al.*, 1996, 2001). Dogs' allergic diseases classified as atopy, hormonal, fungal, bacterial, parazitary and drug hpersensitivity, allergic contact dermatitis, respiratory and food allergy, urticaria and angioedema cause symptoms in integumentary, digestive and respiratory systems (Griffin *et al.*, 1993; Griffin and DeBoer, 2001; DeBoer, 2004). Although widely used, the term Allergy test is a misnomer and neither test has been demonstrated to be superior to the other and none of these tests is supposed to be used alone (Hillier and DeBoer, 2001; Hillier, 2002a, b).

IDST (Intradermal Skin Tests) is based on the evaluation of dermatologic reaction due to mast cell degranulation via mast cell originated IgE measurement (Kunkle, 1987; Kunkle and Horner, 1992; Hill *et al.*, 2001). The test is helpful for not only to diagnose the allergic disease but also to explain the sensibility (DeBoer and Hillier, 2001a, b). It allows to determinate the effective allergens for the allergic patient and to detect the necessary allergens for immunotherapeutic vaccine. The IDST battery can be individually organized and any factor

influencing the test such as drugs etc. may be investigated (Rees, 2001). When making allergen choice for IDST battery, cross reactivity among different allergens must be taken into consideration (Masuda *et al.*, 2000; Pauli *et al.*, 2006) while involving reported effective allergens in dogs (Halliwell and Schwartzman, 1971) within. It's of high importance to prepare the IDST battery according to patients' anamnesis (Mamikoglu, 2005). An optimal panel is made based on the regional location where live the patients, classic books and the anamnesis by veterinary dermatologists/allergists, veterinary faculties and allergy laboratories (Reedy and Miller, 1989) and cross-reactive allergens must not bein the battery (Masuda *et al.*, 1999; McCall *et al.*, 2001).

The standardization of allergen extracts being a complex mixture of antigenic components is essential to ensure the effectivity. In veteriary medicine, no such standardized extracts are available (Codner and Lessard, 1993; Codner and Tinker, 1995). Highly purified antigens permit the standardization of both allergy tests and immunotherapeutic vaccine (Hill and DeBoer, 2001) and since, no such standardized extracts are available for veterinary medicine, there is no evidence to suggest that allergens from any one company (human or veterinary) are superior to those of any other. Reports in dogs indicate differences in allergen extracts fom different

manufacturers or even between batches of the same allergen from the same manufacturer (Hillier and DeBoer, 2001). Although, there is a lack of optimal concentration knowledge for every allergen for use in IDST of dogs, typically recommended allergen dilutions are generally used (Kunkle and Horner, 1992; Hillier and DeBoer, 2001; Jeffers *et al.*, 2001).

IDST whose effectivity in definitive diagnosis is controversial (Park *et al.*, 2000) has some disadvantages due to sedation necessity, discomfort and increased risk of systemic reactions, one of which is anaphylaxis (Rees, 2001). Yet, the main problem is the false positive and negative reactions (Reedy and Miller, 1989; Park *et al.*, 2000). IDST is not recommended for flea allergy dermatitis frequently seen in dogs because it causes too much false positive reactions due to normal physiologic reaction against flea instead of doing so for allergic reaction itself (Loeffler, 2006).

MATERIALS AD METHODS

Dogs: About 100 dogs of either gender and various ages, breeds and body weight suffering from diverse but compatible allergy symptoms referred to Istanbul University, Veterinary Faculty, Internal Medicine Department Clinic were selected for the thesis. About 41 of the patients were female and 59 were male dogs. Their ages ranged from 3 months to 13 years old. About 12 dogs were <6 months old, 7 dogs' ages ranged from 6 months to 1 year old while 62 dogs' ages were between 1-5 years old and 12 of them were >5 years. About 7 adopted dogs whose age it was impossible to make a suggestion about were also in the group.

The breeds represented in the study included Terrier (20 cases), Golden Retriever (18), Mongrel dogs (17), German Shepherd dog (9), Rottweiler (5), Belgian Shepherd dog, Boxer, Husky and Setter (4 cases each), Kangal and Pooodle (3 cases each), Bulldog, Doberman Pinscher and Shar-pei (2 cases each) and Pug, Papillon and Great Dane (1 case each). After we observed detailed historical and clinical findings, we investigated the vaccine and antiparasitary applications, the diseases they suffered before and the therapy, they were receiving. Parasitic infestations and cutaneous infection were ruled out via dermatologic, microbiologic and parasitologic examinations and tests and restricted dietary trials were applied (Muller *et al.*, 1983; Leib and Monroe, 1997; Ettinger and Feldman, 2000; Willemsse, 2000; DeBoer and Hillier, 2001 a, Willemsse, 2006) at the same time withdrawal period of every drug which may adversely affect reactivity of the skin to allergens used during IDT (Barbet and Halliwell, 1989; Bond *et al.*, 1993; Leib and Monroe, 1997;

Kirkpinar, 1999; Ettinger and Feldman, 2000; Hillier and DeBoer, 2001; Rees, 2001; Bond *et al.*, 2002; Mamikoglu, 2005) was taken into consideration.

By this way, we had the opportunity to eliminate every disease showing same or similar findings to allergy and also we managed to include only the dogs detected to be suffering from just an allergic disease. We were also concerned with household characteristics of the dogs involved in the thesis. Detailed informations about their indoor conditions were obtained by investigating whether the environment, they were living was a house, a manufacturing plant or outdoor. For those living indoor at home, we took information about the frequency and the way of cleaning, the floor, the carpeting, the furniture and the heating system while we investigated the job definition and the presence of any chemical for those living in a manufacturing plant and we asked for both group whether, they were sharing their environment with another animals or not.

Between all dogs, 64 were living indoor at home, 25 in the garden and 11 were being housed at a manufacturing plant. About 48 dogs living at home was alone while 4 of 16 dogs sharing their environment with another animal were together with cats, 4 with another dogs and 8 with birds. About 14 dogs of all 25 living in the garden was alone at the same time 3 of 11 dogs were together with cats, 7 with other dogs and just one dog was sharing the environment with both cats and dogs. About 6 dogs of the manufacturer plants were alone. At the same time, 4 were sharing the environment with other dogs and cats were present where was living only one individual.

The houses where 30 dogs of 64 living indoor at home were carpeted and the number of climated houses were 20 for the same group. The diet of the dogs we involved in the study represented also aroused the interest. About 44 dogs were eating only home-cooked food, 22 dogs were eating commercial food and the number of the dogs eating both were 34. About 22 dogs of all eating home cooked food have been learned to be used to gobble. About 9 dogs from just eating commercial food group were eating dry food, one dog was eating wet dog food and 12 of them were eating both kind of dog food.

IDST: The specific selection and number of allergens included in the IDT battery was made on the basis of literature (Kunkle, 1987; Harvey, 1993; Codner and Lessard, 1993; Carlotti and Costargent, 1994; Codner and Tinker, 1995; Day, 1999; Kirkpinar, 1999; Saridomichelakis *et al.*, 1999; Ettinger and Feldman, 2000; DeBoer and Marsella, 2001; Bensignor and Carlotti, 2002;

Fujimura *et al.*, 2002; Foster *et al.*, 2003b; Biourge *et al.*, 2004; Bloom, 2006). Yet, we also added some trofoallergens detected to be oftenly given to the dogs when we conducted a poll before we started the study for detecting the performance of IDT for cutaneous adverse food reactions.

Positive and negative control solutions accompagned thirty allergen extracts to determine skin reactivity. The allergens used in this study were poplar, willow, grass, duck feather, dog, rabbit, cat and budgerigar epithelia, cow, mutton, chicken, horse and turkey meat, tuna fish, apple, orange, pear, walnut, nut, tomato, potato, wheat, milk, chocolate, egg white, yolk, cotton, house dust and house dust mites.

Allergens were supplied by allergopharma and brought by DHL kargo. Typically recommended allergen dilutions were performed (Kunkle, 1987, Hillier and DeBoer, 2001; Jeffers *et al.*, 2001; Ishida *et al.*, 2003) and diluted allergens were kept and stored with particular caution (Nelson, 1991; Hillier and DeBoer, 2001) and renewed according to expiration dates (Nelson, 1991).

We performed IDST on non-sedated dogs without the stress. However we used xylazine hydrochlorid (Rompun®; BAYER) (Kunkle, 1987; Moriello and Eicker, 1991; Frank and Kunkle, 1992) for agessive patients. We shaved gently lateral thorax without scrubbing, washing and using any chemicals (Kirkpinar, 1999; Saridomichelakis *et al.*, 1999; Masuda *et al.*, 2000). Despite of paying extreme care to choose an area free of inflammation and infection related to lesions in case of impossibility, we placed the injections at an appropriate distance from them. We marked every injection site with a permanent dark blue marker pen spacing at a distance of 3 cm. We administered the solutions and the extracts with the help of tuberculin syringues. Typically, an aliquot (0.05 mL) of each solution or allergen containing syringues prepared before the performance were put side by side in numerical order in order to be applied from left to right. All dogs were placed in lateral recumbency (Lian and Halliwell, 1998; Masuda *et al.*, 2000). The application started with positive and negative solutions (Reedy and Miller, 1989; Hillier and DeBoer, 2001) and continued with allergen extracts. About 15 and 30 min following, the last administration injection sites and reaction were judged either subjectively or objectively (Bellanti, 1978; Day *et al.*, 1996; Kunkle and Horner, 1992; Bond *et al.*, 1993; Leib and Monroe, 1997; Lian and Halliwell, 1998; Kirkpinar, 1999; Mueller *et al.*, 1999; Mueller and Bettenay, 2001; Rees, 2001; Bensignor and Carlotti, 2002; Bond *et al.*, 2002; Ishida *et al.*, 2003; Mueller *et al.*, 2002). Despite of following the criteria used by veterinary allergists in evaluating reactions

(Codner and Lessard, 1993; Codner and Tinker, 1995; Kirkpinar, 1999; Saridomichelakis *et al.*, 1999; Ettinger and Feldman, 2000; Fujimura *et al.*, 2002; Foster *et al.*, 2003b; Harvey and McKeever, 2003; Bloom, 2006) every reaction was noted in order to interprate different degrees of sensibility.

Blood analysis: Both blood with and without anticoagulant (5 cc. each) from V. jugularis of all dogs was prepared. Hemogram analysis from blood with anticoagulant (total leukocyte, erythrocyte, haematocryte, hemoglobin, thrombocyte) via otomatic MEDONIC CA 620 cell counter and formul leukosyete analysis via manual method were performed (Kirkpinar, 1999).

The results were compared between the group identified as allergic and the one involving the dogs having not reacted to an allergen. Total IgE and IgG from blood without anticoagulant were examined (Ucan *et al.*, 2003; Blount *et al.*, 2005; Schafer-Somi *et al.*, 2005) and absorbance values are determined according to the manufacturer's suggestions via ELISA method. BIOTEK L×50 washer and BIOTEK EL×800 reader were used for the analyses. Total IgE and IgG kits specific for dogs were purchased from Bethyl laboratories and brought by DHL kargo.

Statistics: Data were analysed using the statistical package SPSS 9.0 and Independent samples t-test.

RESULTS AND DISCUSSION

About 85 to all 100 dogs participated the study represented with positive IDST reactions. In dogs, we observed positive reactions, 52 were male and 33 female. The breeds diagnosed as having positive reactions included = Golden Retriever (17 cases 20%), Terrier (16 cases 18.8 %), Mongrel dogs (14 cases 16.4 %), German Shepherd dogs (9 cases 10.5%), Belgian Shepherd dogs and Husky (4 cases each 4.7%), Kangal, Boxer, Pooddle and Rottweiller (3 cases each 3.5%), Shar-pei, Bulldog and Setter (2 cases each 2.3%), Great Dane, Pinscher and Papillon (1 case each 1.2%).

According to these results, 17 Golden Retrievers from all 18 participated to the study (94.4%) and 16 terriers from all 20 from the same study group (80%) as well as 14 mongrel dogs of all 17 test group (82.3 %) represented with positive IDT results. About 12 dogs were younger than 6 months (14.1%) while the age of 4 ranged from 6 months old to 1 year old (4.7 %), 33 from 1-3 years old (38.8 %), 20 from 3-5 years old (23.5%) and 10 of those with positive reactions were >5 years old (11.7 %).

Meanwhile, the percentage of 6 dogs with positive reactions whose age was undetectable was 7%. With respect to the prevalence of positive IDST reactions, there were a large number of cases including 71 positive responses for mites, 23 for cat epithelia, 17 for house dust and milk, 16 for dog epithelia, 15 for chicken meat, 14 for cotton, 13 for cow meat, 12 for mutton and horse meat and tomato, 11 for poplar, 10 for tuna fish, 9 for wheat, egg white, duck feather and orange, 8 for willow and nut, 7 for budgerigar epithelia, pear, walnut, potato and yolk, 6 for grass, apple and chocolate, 5 for rabbit epithelia and 4 for turkey meat.

As for symptoms, the patients exhibited exematous lesions (41%), seborrhea and seborrheic plagues (26%), pruritus (75%), hyperpigmentation (15%), pyoderma (17%), otitis (13%), otomutilation (28%), erythematous and alopecic moist lesions (28%), lick dermatitis (32%), acne (14%), lichenification (11%) edematous pruritic lesions (22%), crusts (18%), alopecia (29%), erythema (11%), moist dermatitis (9%), diarrhea (6%), nasal (10%) and ocular (27 %) discharge, ear leak (14%), pododermatitis (35%), sneeze (13%), rhinitis (8%) and conjunctivitis (17%). About 55 dogs of all 85 we had positive reactions detected were living indoor while 20 were staying in the garden and 10 at a manufacturer plant; the percentages of whom were respectively 64.7, 23.5 and 11.7%.

The percentage of 41 individual with positive reactions living alone 48.3% and those of dogs living with another dogs, cats and birds were calculated, respectively as 2.3, 4.7 and 9.4%. About 13 dogs of all living in the garden (15.3%) were alone at the same time 2 of them were sharing their environment with cats (2%), 4 with another dogs (4.7%) and only 1 dog with both (1.2%).

About 10 dogs from different manufacturer plants formed a percentage of 11.7% within all patients with positive IDT results. The same percentage were respectively 7, 1.2 and 3% for 6 dogs staying alone, 1 dog living with cats and 3 sharing the same environment with another dogs. About 30 dogs with positive IDT reactions were learned to be living in carpeted and 5 in climated houses.

They formed a percentage of 35.2 and 5.9%, respectively within allergic patients. Home cooked diet was represented with a great percentage (47%) between all dogs with positive reactions while 20 dogs being used to gobbling formed 23.5% of them. About 14 dogs' diet were involving commercial food (16.4%) while 8 dogs were eating only dry dog food (9%), 1 dog only wet food (1.1%) and 5 were eating both (6%).

The percentage of the individuals eating both commercial and home cooked food was 36.4%. No

significant difference was observed between haemogram and formul leukocyte levels of dogs with positive IDST reactions and the other group (Table 1 and 2). No significant difference was present according to IgE and IgG values between the same 2 groups (Table 3).

Sex: Researchers' reports vary in regard to sex predisposition since, there is both publishings denying affinity (Harvey, 1993; Rosser, 1993; Saridomichelakis *et al.*, 1999; Masuda *et al.*, 2000; Willemsse, 2000, 2006; Jeffers *et al.*, 2001) and those describing male (Mueller *et al.*, 2000) and female predisposition (Halliwell and Schwartzman, 1971; Youn *et al.*, 2002; Zur *et al.*, 2002). In the study, females formed a percentae of 38.8% and males 61.2% of all dogs with positive IDST reactions which is similar to Mueller *et al.* (1999)'s study explaining sex predilection for males in spite of almost equal rates he observed for both sexes.

Age: The typical age of onset for allergies is reported to be two (Rosser, 1993) in spite of some researchers denying age predilection (Harvey, 1993; Griffin and DeBoer, 2001; Jeffers *et al.*, 2001). The age which seems to be a negative factor causes the symptoms to worsen and thus the older the dog gets, the stronger becomes the positive reactions to the allergens. It is very rare to observe allergy related findings before 6 months and after 8 years old. In the majority of dogs in the study, the

Table 1: Haemogram findings of allergic and non allergic dogs

Parameters	X±Sx	
	Non allergic dogs (n=15)	Allergic dogs (n=85)
RBC (×10 ⁶ μL)	5.86±0.200	6.08±0.100
WBC (×10 ³ μL)	10.46±0.650	11.36±0.480
PLT (×10 ³ μL)	353.40±42.55	301.73±13.75
HCT (%)	39.19±1.450	38.95±0.730
HGB (g dL ⁻¹)	12.86±0.670	13.19±0.250

Table 2: Formul leukocyte findings of allergic and non allergic dogs

Parameters (%)	X±Sx	
	Non allergic dogs (n=15)	Allergic dogs (n=85)
Neutrophil	70.80±0.58	70.09±0.370
Lymphocyte	21.27±0.80	22.15±0.330
Monocyte	3.13±0.35	3.39±0.160
Eosinophil	4.80±0.22	4.36±0.170
Basophil	0.20±0.11	0.14±0.100

Table 3: IgE and IgG analyses of allergic and non allergic dogs

Parameters	X±Sx		t-test
	Non allergic dogs (n=15)	Allergic dogs (n=85)	
IgE (ng mL ⁻¹)	147.52±40.28	154.0±15.53	0.172
IgG (ng mL ⁻¹)	281.39±19.80	263.22±11.83	0.618

Difference between 2 groups' averages is insignificant (p>0.05)

findings were similar to data. Yet, the evaluation of the historical information of the patients with symptoms' age of onset being >8 showed that the information was unreliable due to the number of owner the dog had previously.

Breed: Breed predisposition are common for terriers (Willemse, 2000, 2006). Terriers being represented with a percentage of 19% seemed to be similar to researchers' explanations. There is agreement with the findings for different breeds' positive reactions against allergens. We thought that the different result of other breeds is due to inadequate number of participants.

Researchers generally, agree about the dependence of breed predisposition on geographic and seasonal influence (Bollinger *et al.*, 1996; Maeda *et al.*, 2002). Lots of publishing do not take geography into consideration thus, breeds represented to have predisposition in one study may not be mentioned in another one (Harvey, 1993; Rosser, 1993; Kirkpinar, 1999).

The findings were similar to those published by Kirkpinar (1999) for the same geographic area. The differences obtained for some breeds are thought to be related to geographical, housing and dietary differences.

Housing characteristics: Willemse (2006) insists on the necessity of investigation of household characteristics in the anamnesis taken from allergic dogs' owners because studies (Randall *et al.*, 2003, 2005; DeBoer, 2004) related the dose response relationships between mite allergen exposure and sensitization.

Levels of indoor allergen are determined by house dust, house dust mites and pets (Warner, 2000; Olivry and Sousa, 2001). House dust mite allergens being the most important risk factor for allergic sensitivity having patients (Warner, 2000; Randall *et al.*, 2003), the amount of allergen plays role as a triggering factor (Warner, 2000; Simpson *et al.*, 2001).

The study representing, the mites as the most effective allergens with the percentages of 31.7% (*D. pterynossus*) and 51.7 % (*D. farinae*) is supported by data published. House dust mite allergen levels which seems to be significantly higher in individual homes tend to be influenced by geographical area, local climate and humidity (Warner, 2000; Simpson *et al.*, 2001). Dermatophagoi des *Farinae* dominates in drier climates, *D. pterynossus* in contrast is more common in humid climate (Simpson *et al.*, 2001).

High humidity and poor ventilation give rise to allergen levels (Randall *et al.*, 2003) which are strongly

associated with the presence of carpetted floors, soft furnishing, wool and polyester soft toys, clothes, blankets, bedding and covering mattresses (Warner, 2000; Simpson *et al.*, 2001; Mamoon *et al.*, 2002).

Cotton and polyester dog beds when unfrequently cleaned, provide the optimal environment for house dust which are the most effective allergens (Olivry and Sousa, 2001) by leaving their feces and extracts acting also as allergens (Codner and Tinker, 1995; Olivry and Sousa 2001).

We learned that no chemical was present in the living environment of the patients. However, the owners of those living indoor explained that the furnitures were old. Yet, they seemed to have further knowledge about house dust mite allergens and to avoid using chemicals in cleaning and to try to air the house frequently.

We found interesting, the higher percentage of the IDT reactions for house dust mites in the patients living in carpetted homes (35.2%) and the lower one in climated indoors (5.8%).

Diet: Dietary factors have a major role in the ethiology of allergic diseases (Watson, 1998). Both the efficiency of food allergies in allergic diseases (Harvey, 1993; Chesney, 2001, 2002; Harvey and McKeever, 2003) and of trofoallergens in atopy (Halliwell, 1992; Carlotti and Costargent, 1994; Paterson, 1995; Burks *et al.*, 1998; Hillier and Griffin, 2001a, b; Carlotti, 2003; Bloom, 2006) is well defined. Researchers (Paterson, 1995; Hillier and Griffin, 2001b; Ishida *et al.*, 2003; Foster *et al.*, 2003a) take into consideration the diet of dogs suffering from an allergic disease.

We organised the IDST battery and the trofoallergens included according to the detailed investigations, we made with the owners about their pet's diet before we started the thesis.

With respect to the high amount of positive IDT reactions for trofoallergens we observed, we have the opinion that both the gobbling and the one food diet style of dogs need to be investigated further.

Clinical manifestations: Clinical reports vary with regard to symptoms seen in dogs with allergic disease (Griffin *et al.*, 1993; DeBoer and Hillier, 2001a; Griffin and DeBoer, 2001). Definitive diagnosis is reported to be impossible to establish due to the lack of any pathognomic sign (DeBoer and Hillier, 2001a; Griffin and DeBoer, 2001; Marsella and Olivry, 2003). We observed in the represented study, the diversity of symptoms with regard to lesion, localisation and severity (Fig. 1-4).

Pruritus induced with a high percentage of 75% in all of the patients representing with positive skin reactions seemed to be definitively associated with allergy and supported by publishings (Kunkle, 1987; Kunkle and Horner, 1992) such as the otomutilation mentioned by some researchers (Kunkle, 1987; Kunkle and Horner, 1992; Zur *et al.*, 2002; Marsella and Olivry, 2003) from which 28% of positively reacted dogs were suffering. Hyperpigmentation (15%) and licenification (11%) were between unfrequently detected symptoms in spite of alopecia accompanying to diverse severity of pruritus and other complaints in almost all cases.

The high frequency of skin lesions were in concordance with the study published by Saridomichelakis *et al.* (1999) yet a wide variety of localisation of the lesions seemed us to be related to allergies' individual characteristics, species and dose of the effective allergen and the chronicity of the disease itself which makes the lesions to get worse as well as the housing conditions.

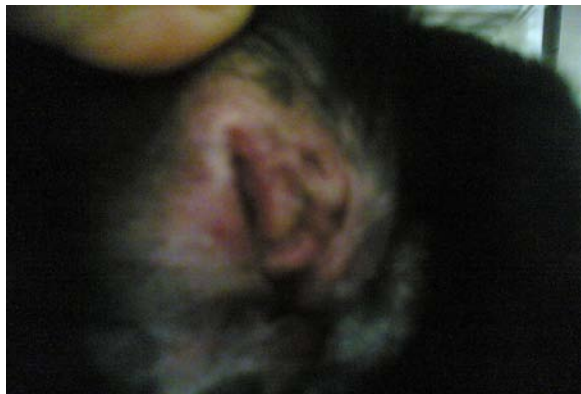


Fig. 1: Otitis and erythematous, pruritic lesions on the ear pinna



Fig. 2: Self-induced alopecia and erythematous pruritic lesions of the flexural site and interdigital area of the hindleg

The most common signs the dogs exhibited were exemateous lesions (41%), pododermatitis (35%) and lick dermatitis (32%). Yet, we had no occasion to explain any primary lesion. Inguinal, periorbital areas, dorsal, lumbar regions, ears and ear pinnae as well as flexural sides were also involved. About 15% of allergic dogs exhibited lesions of the face, neck and head while 23% represented with periorbital lesions and 15% with hyperpigmentation. The percentage of otitis externa we observed (13%) seemed to be contradictory to the data explaining higher ratios (Paterson, 1995; Leib and Monroe, 1997; DeBoer and Hillier, 2001a; Hillier and Griffin, 2001b; Zur *et al.*, 2002).

The reason was established to be related to ocular and ear symptoms which may be also due to an allergic disease are at 1st examined by surgery department which makes the percentages smaller than they really are. Food allergy associates atopy in dogs with a percentage of 7%. Dogs with food allergy complaint, develop both dermatologic and gastrointestinal symptoms (Harvey, 1993; Olson *et al.*, 2000; Hill and Olivry, 2001; Jeffers *et al.*, 2001; Chesney, 2002).



Fig. 3: Pruritic moist dermatitis between dorsal interdigital area

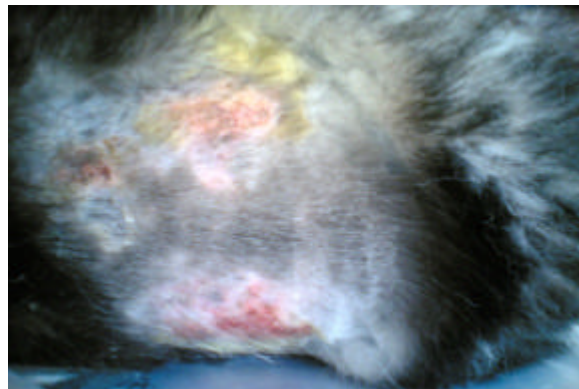


Fig. 4: Seborrhea ad pruritic exemateous lesions of the dorsal regio

In most cases, the skin exhibits a wide variety of symptoms making impossible to differentiate the food allergy from atopy (Halliwell and Schwartzman, 1971; Halliwell, 1992; Halliwell and DeBoer, 2001). Within the patients, only 6% of them had diarrhea. Even this low ratio is supported by data (Harvey, 1993), the cause is probably due to the rare estimation of food allergy when GI complaints exist compared to other GI diseases. Loss of body weight and abdominal pain did not represented. Signs appearing to effect both GI and respiratory symptoms while doing so with the integumentary system were supported by Marsella and Olivry (2003).

We concluded that the diversity and individual differences of the lesions, the patients exhibited were related to polymorphous characteristics of allergy, threshold effect, individual characteristics such as breed, age and sex as well as sensibility, the dose and the contact with the allergen.

Blood analysis: Eosinophilia which is generally related to allergy in classic books (Leib and Monroe, 1997) is not adequately researched due to similar mechanism explicating their role in allergic diseases (Hill and Olivry, 2001) since, cutaneous eosinophilic inflammation is rare in dogs.

Despite both dogs with inhalant or food allergy and those with allergic GI complaints exhibited eosinophilia, some researchers (Wilkie *et al.*, 1990; Collie *et al.*, 1997) reported that eosinophilia may not always be related to allergic diseases.

We did not detect any significant difference between 85 dogs represented with positive skin reactions and those not responding to the tests with regard to eosinophil levels (Table 2). This finding was supported by some reports (Wilkie *et al.*, 1990; Collie *et al.*, 1997) in spite of being different from classic books (Leib and Monroe, 1997; Day, 1999; Ettinger and Feldman, 2000; Harvey and McKeever, 2003) making us to conclude that their role in allergy needs to be investigated further.

Ig analysis: Studies yielded a wide variety of arguments (Hill *et al.*, 1995; Olivry *et al.*, 1996; Collie *et al.*, 1997; Hammerberg *et al.*, 1997; DeWeck *et al.*, 1998; DeBoer and Hillier, 2001b; Halliwell and DeBoer, 2001; Hill and DeBoer, 2001; Jackson *et al.*, 2002; Foster *et al.*, 2003a, b) that total IgE levels rise in allergic individuals (Hill *et al.*, 1995; Sicherer and Sampson, 1999; Halliwell and DeBoer, 2001; Halliwell *et al.*, 2006; Sicherer and Hugh, 2006) or any significant difference occurs (Collie *et al.*, 1997). Although, IgE value augmentation is explained to be due to the allergy (Hill *et al.*, 1995), reports also indicated

association with high rate of IgE excreting B cell presence (Jackson *et al.*, 2002) or with Th-2 immune response (Collie *et al.*, 1997).

Dogs may have IgE in their circulation without any allergic complaint or vice versa (Marsella and Olivry, 2003). IgE levels have been described to be higher in dogs allergic to inhalants (Collie *et al.*, 1997). However, total IgE levels of those with positive skin reactions to inhalants in the study did not reach higher levels than normal (Table 3).

As a high amount of IgE with diverse biologic characteristics exist (Foster *et al.*, 2003a, b; Provost *et al.*, 2003) and wide upper and lower levels is reported for dog IgE (Griot-Wenk *et al.*, 1999; Fraser *et al.*, 2003), serologic findings are already expected to be incompatible with IDTs reactions (Foster *et al.*, 2003a). There is no evidence of definitive IgG role in allergic disease in dogs (Hill *et al.*, 1995; Glickman *et al.*, 1998; Lian and Halliwell, 1998; Halliwell and DeBoer, 2001; Hou *et al.*, 2006). Studies suggest, there is established link between rise in IgE levels and age (Schreiber *et al.*, 1992; Ucan *et al.*, 2003), sex (Racine *et al.*, 1999) or allergy (Hill *et al.*, 1995; Hou *et al.*, 2005, 2006) at the same time in one study, it is commented that those are significantly independent (Glickman *et al.*, 1998). Hou *et al.*, (2005) revealing that the total IgG rise in dogs is associated with environmental allergens and house dust mites do not present any proven evidence for their role in pathogenesis.

We did not detect any significant difference between serum IgE and IgG levels of allergic and non allergic dogs (Table 3). These results being supported by some data (Collie *et al.*, 1997; Glickman *et al.*, 1998; Sicherer and Sampson, 1999; Sicherer and Hugh, 2006) or not (Schreiber *et al.*, 1992; Racine *et al.*, 1999; Zur *et al.*, 2002; Provost *et al.*, 2003; Ucan *et al.*, 2003) are probably related to dog immunoglobulins' different characteristics, their unidentified role in allergy as well as the explanation of Nuttall *et al.* (2001), explicating serologic tests are inappropriate in some individuals, since allergic diseases may occur without anticor response.

IDST

Trofoallergens: Trofoallergen sensitivity is rarely reported in dogs (Paterson, 1995; Martin *et al.*, 2004; Halliwell *et al.*, 2006). Food allergy whose pathogenesis has not been fully elucidated is manifested in a variety of dermatologic syndromes rather than associating with GI findings (Paterson, 1995; Hillier and Griffin, 2001b). Although, a variety of concerns have arisen regarding food allergy, a high amount of allergic dermatologic

signs is defined to be mediated by trofoallergens (Chesney, 2001) and food allergy is suggested to be taken into consideration in atopic dogs (Wills and Harvey, 1994; DeBoer and Marsella, 2001; Halliwell *et al.*, 2006).

To the researchers knowledge, documented allergy provoking foods in dogs include wheat, cow, lamb, mutton, chicken, turkey, fish, tuna and horse meat, milk, egg white, yolk and chocolate (Harvey, 1993; Ettinger and Feldman, 2000; Ishida *et al.*, 2003; Foster *et al.*, 2003a; Harvey and McKeever, 2003; Martin *et al.*, 2004).

The investigations we realised before we started the study revealed that potato and bread were the foods the dogs were fed most frequently as they were thought to be cheap and filling together which causes also the dogs to be fed only food diet. We concluded a percentage of 8.2% positive skin test response to potato. The finding seemed to be in concordance with documentations citing potato within allergy inducing foods (Halliwell, 1992; Teuber *et al.*, 2002; Foster *et al.*, 2003a; Halliwell *et al.*, 2006).

We realised chocolate being a reward for dogs for which we detected a percentage of 7% positive skin reactions with variable severity of sensibility was also considered to be allergic (Harvey, 1993).

Fujimura *et al.* (2002) established a connection between pollen and tomato allergy. We found the percentage of 14.1% positive reactions for tomato, significant especially with regard to the dogs being fed home cooked food.

Researchers (Masuda *et al.*, 2000; Maeda *et al.*, 2002) having included trofoallergens in their IDT battery with atopic dogs informed strong positive reactions to bread and egg and poor or moderate responses for chicken and cow meat while Ishida *et al.* (2003) explained strong responses for cow meat and bread and moderate reactions for egg and Olson *et al.* (2000) observed positive responses for chicken meat and milk.

The percentages of dogs having positive reactions in the study were 20, 14.1 and 15.2% for respectively milk, mutton and cow meat while the ratios being 10.5% for egg white and 8.2% for egg yolk.

The percentages, we detected for wheat and milk (10.6 and 20%) were significantly important since both were given together to the dogs living in a manufacturer plant and fed one food diet. This result agreed with what (Kunkle and Horner, 1992) have explained as positive reactions for bread (15%), milk (36%), fish (28%), chicken (21%) and cow meat (32%) and also with those of Jeffers *et al.* (2001) indicating positive test results for potato and bread.

Skin tested dogs in the represented study had the percentages of 10.6, 11.7, 17.7, 15.2, 14.1, 4.7 and 14.1%,

respectively for bread, tuna, chicken, cow, mutton, turkey and horse meat. The amounts were similar to recent reports (Kunkle and Horner, 1992; Olson *et al.*, 2000). Although, neither for turkey nor for horse meat was represented with quantitative results both were included within foods allergic in dogs. All were known to be foods given to dogs feeding with home cooked and commercial diet in addition to horse meat being included in commercial dog foods. Cow, mutton, chicken and horse meat and egg white, wheat, potato, chocolate, fish and tomato are explained as foods causing histamin release (WALTHAM, 1999).

Oral allergy syndrome identifying nut, walnut, apple, pear and potato as offending food allergens points the cross reactivity between the trofoallergens mentioned and the pollens (Pauli *et al.*, 2006).

In the study, 9 dogs showed positive skin reactions for orange, 8 for nut, 7 for pear and walnut and 6 for apple with the percentages being 10.5, 9.4, 8.2 and 7%. No quantitative comparison could be established due to the lack of amount information yet it seemed significant to observe the dogs detected to be sensitive to walnut and nut were also sensitive to pollens.

On the other hand, the positive results obtained for apple, potato and tomato seemed also significant with regard to cross reactivity published (Pauli *et al.*, 2006) between these three.

Although, food provocation test is considered to be a gold standard to identify food allergens (Kunkle, 1987; Kunkle and Horner, 1992; Watson, 1998; Ettinger and Feldman, 2000; Jeffers *et al.*, 2001), they do not indicate which trofoallergen to be included in diet (Hillier and Griffin, 2001b).

In spite of the confusion surrounding, the use of skin testing involving food allergens (Kunkle, 1987), their positive effects on clinical improvement being reported by Jackson *et al.* (2003). Burks *et al.* (1998) indicate the usefulness of any provocation diet used without IDTs results and report that in vitro test have no superiority compared to skin tests with regard to trofoallergen identification for restricted diet.

According to the results, the patients showed for trofoallergens we suggest to establish the diagnosis of an allergic disease with the help of multiple test results as well as the allergen identification or immunotherapeutic vaccination.

House dust and house dust mites: House dust invariably containing dust mites may also involve mould spores, animal allergens, insect debris and pollens. Therefore, it is so heterogenous that it can be regarded as a distinct allergen and testing for more specific allergens is strongly recommended (Mueller *et al.*, 2002). We related the

positive reactions percentage of 20% obtained only for house dust in the dogs to this explanation. Dermatophagoides allergens being the species of mite eliciting the highest number of positive results in dogs (Sture *et al.*, 1995; Park *et al.*, 2000; Yamashita *et al.*, 2002), a strong correlation between these two is determined (Foster *et al.*, 2003b).

Therefore, both are supposed to be involved in a IDT battery (Park *et al.*, 2000; Zur *et al.*, 2002) and since in most cases, positive reactions to only one mite is uncommon, a positive reaction to both *D. farinae* and *D. pteroyssus* is often associated (Sture *et al.*, 1995; Yamashita *et al.*, 2002). In the study, the percentage of dogs with positive results to only *D. farinae* was 28.2 and 8.2% for *D. pterynossus* alone which was similar with this explanation.

In the thesis of all dogs, testing positive 23.5% were sensitive to both allergen. Conversely, a percentage of 51.7% was represented with positive reactions to *D. farinae* and 31.7% was detected to be sensitive to *D. pteronyssus*. We explained, the higher amount of positive reactions to *D. farinae* compared to those obtained for *D. pteronyssus* by its higher indoor concentration and its stronger irritability as published in reports (Carlotti and Costargent, 1994; Saridomichelakis *et al.*, 1999).

Egli *et al.* (2002) established a relation between positive results for house dust mites and multiple IDST performances. Yet, no similar connection was present in the study because it was the 1st time the dogs were intradermally tested. Bensignor and Carlotti (2002) presented the evidence that associations exist between environmental house dust mite allergen levels, housing conditions and positive reaction amount. With regard to the number of the indoor patients represented with positive skin test reactions to house dust and dust mites, the results were supported by this explanation.

Kurata *et al.* (2004) having involved both house dust mites and trofoallergens in IDST battery, observed positive reactions for house dust mites while noticing negative responses for the others. The study, controversially resulted positive reactions for both group. Yet, the finding of a small amount of dogs with respiratory system complaints represented positive reactions to house dust and mites were learned to be living at home.

Not only does, there appear to be an association between allergic rhinitis and house dust mites but also between indoor conditions and house dust mite allergy. Since, we had asked specific questions to characterize type of housing, heating and cooling system, carpeting, number of indoor pets, dog bedding before we

started the study, the positive reaction findings seem both supported by data and significantly important.

Pollens: About <10% of pollens being able to act as aeroallergen are carried by air, dust and insects and they can influence dogs from other geographic regions. The wide diversity of IDT results in dogs has been published (Mueller *et al.*, 2000, 2002; Mueller and Bettenay, 2001)

The percentages of 12.9, 9.4 and 7% positive reactions for poplar, willow and grass, respectively, we detected were at the same time similar (Kirkpinar, 1999; Hill and DeBoer, 2001) and different (Saridomichelakis *et al.*, 1999) compared to data. We related the difference to geographic region, climate and humidity differences as well as individual housing conditions and contact rate of the dogs. In addition, the presence of similar epithopes possessed by these allergens does not require neither similar mast cell degranulation nor inflammatory mediation excretion.

Epithel: Epithel for whom different IDT reactions are published (Kirkpinar, 1999; Masuda *et al.*, 2000; Park *et al.*, 2000; Mueller *et al.*, 2002; Youn *et al.*, 2002; Herz *et al.*, 2004) is suggested to be involved in IDT battery.

Dog epithel is an important source of *D. farinae*. Sensitivity for cat allergen may be detected in dogs living cat free indoors (White and Ohman Jr., 1998; Maeda *et al.*, 2002). Attention must be paid when evaluating IDTs since, sensitivity in dogs for cat allergen does not prove clinic disease (Hill and DeBoer, 2001). The study revealed sensitisation for cat (27%), dog (18.8%), budgerigar (8.2 %) and rabbit (5.8 %) epithels and appeared similar to data.

Feather: Various IDT positive reactions percentages are published (Mueller *et al.*, 2002; Youn *et al.*, 2002; Foster *et al.*, 2003b) for feathers that allergic characteristics are controversial (Saridomichelakis *et al.*, 1999). Dog beddings whose filling material is feather cause allergic symptoms to begin or to worsen (Kirkpinar, 1999).

The percentage, we obtained for duck feather (10.5%) being supported by data (Kirkpinar, 1999; Saridomichelakis *et al.*, 1999; Mueller *et al.*, 2002) has an important role in allergy because it is used as filling material for indoor dogs' beds.

Cotton: Various percentages of positive reactions similar to those we detected (16.4%) are expressed in studies (Kirkpinar, 1999; Hill and DeBoer, 2001) for cotton which is a textil product gaining importance by being dog bed

filling material. Although, IDST is helpful for allergy diagnosis (Zur *et al.*, 2002; Loeffler, 2006), it is inadequate in proving the disease (Zur *et al.*, 2002). All dogs with clinical symptoms may not react to allergens at the same time young individuals may respond to only a few allergens even they are sensitive to a higher amount (Hilier and DeBoer, 2001; Hillier, 2002a). Yet, even dogs without any allergic disease and symptom are tested via IDST with high sensitivity, positive reaction to at least one allergen may be obtained (Egli *et al.*, 2002). Seasonal effects, age and IgE levels may also affect IDT results (Hillier, 2002a). We related the differences with data, we obtained and the individual differences to these characteristics of IDST.

CONCLUSION

In this study, we have the opinion that allergic diseases affecting multiple systems via polymorphous syndroms occur more frequently than they are thought to be in dogs and worsen their life quality.

Since, neither the allergic diseases nor the factors they are influenced by are not fully elucidated, we suggest they are both further investigated and evaluated via multiple test method and detailed anamnesis involving related factors such as age, sex, breed, genetic, diet and housing characteristics.

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