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Original article

# Comparison of IgE test results with intradermal skin tests for dust mites and storage mites in atopic dogs

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## Abstract

Atopic dermatitis is one of the most frequent allergic diseases in dogs. There are many methods of treating its symptoms but specific immunotherapy has recently gained high popularity. Before the application of specific immunotherapy, it is necessary to identify the allergens provoking the reaction of hypersensitivity in the selected animal. This raises a question about the method of allergen identification the medical practitioner decides to use in order to obtain the most credible result. The authors of the present study decided to compare the results of intradermal allergic tests and the results of an IgE screening test carried out using the Fc<sub>ε</sub>RIα receptor method. The aim of the study was to compare the results of both tests directed to dust and storage mites. The study proves that in case of the IgE screening tests (for a group of allergens), the sensitivity is quite high (85 to 90.69%) but the specificity of these tests is insufficient (25 to 50%). In case of antibodies for the selected mites the sensitivity and specificity was too low (65.1 to 89.4% for the sensitivity, with only 14.2 to 33.3% for the specificity). Only in case of *D. petronyssinus* the results were higher with the sensitivity calculated at 65.1% and the specificity at 80%. The IgE screening test carried out using the Fc<sub>ε</sub>RIα receptor method is reliable only in case of screening test for mites and the intradermal allergic test remains the gold standard for allergy testing.

**Key words:** intradermal skin tests, serum allergen-specific IgE, dogs, Fc<sub>ε</sub>RIα receptor

## Introduction

According to an international group of experts dealing with atopic dermatitis (International Task Force on Canine Atopic Dermatitis; ITFCAD), atopy is an inherited predisposition towards allergic reactions resulting from a contact with an antigen present in the environment. The allergy is related to the IgE antibo-

dies, but some dogs with the symptoms of atopic dermatitis do not have high levels of the IgE type antibodies (Halliwell 2006). Until recently, it was thought that high levels of the IgE type antibodies were present in atopic individuals as a manifestation of hypersensitivity of an immediate type and of a delayed type related to the presence of T lymphocytes. Hypersensitivity reaction of type I is characterized by a fast response

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(a dozen of minutes). The antibodies showing affinity to mast cells and basophilic granulocytes by means of  $Fc_\epsilon RI\alpha$  receptors in the target cell take part in this process. The criteria created by Favrot (Favrot et al. 2010) are presently considered as helpful in the diagnosis of atopic dermatitis in dogs. Intradermal skin tests and serological screening test IgE carried out by means of  $Fc_\epsilon RI\alpha$  receptor method enable identification of the allergens evoking the allergic reaction (DeBoer and Hillier 2001, Griffin and DeBoer 2001). The tests had proven that dust mites of the *Dermatophagoides* type are the most frequently identified allergens in dogs with atopic dermatitis (Hill and DeBoer 2001). Specific immunotherapy requires identification of allergens and aims at elimination or reduction of the intensity of clinical symptoms after contact with them (Bousquet et al. 1998, Olivry et al. 2001). Intradermal tests are described in the literature as more sensitive and specific than serological tests and are considered a gold standard (Hillier and DeBoer 2001). In case of serological tests, the period of carrying out the test itself is important as the IgE antibodies are present in blood for several weeks after the contact with the allergen. The season when the dog undergoes the test is relevant in case of seasonal allergens which, however, should be irrelevant in case of year-round allergens such as mites. Both kinds of tests have their advantages and disadvantages. The advantages of intradermal test are: the possibility of obtaining a credible result regardless of the season and fast results (15-20 min). Their disadvantages are the necessity of sedation and shaving of the hairs. Advantage of serological tests is that the material, in the form of blood serum, is easy to obtain. However, the period of retention of IgE antibodies in blood after the contact with the allergen is a drawback. The age of the animal is a limitation for both kinds of tests as they should not be carried out in animals younger than 1 year old. Moreover, despite there being studies proving the lack of influence of anti-histamine and anti-inflammatory medicaments on the level of the IgE type antibodies, Wassom and Grieve (1998) make a reference to the unpublished studies of de Weck who, however, expresses his doubts and firmly negates the lack of influence of glucocorticoid, which is confirmed by other studies (DeBoer and Hillier 2001, Olivry and Saridomichelakis 2013).

The aim of this study was to compare the results of intradermal allergic tests with the screening IgE test carried out using the  $Fc_\epsilon RI\alpha$  receptor method taking under consideration both general screening tests and the tests specifically directed to selected mites.

## Materials and Methods

Forty five dogs with clinical symptoms of atopic dermatitis were qualified to the experimental group.

The group consisted of dogs of various breeds and sexes and of age ranging from 1 to 8 years. There were 21 females and 24 males of the following breeds: 5 German Shepherds, 3 German pointers, 7 Labradors, 5 short haired dachshunds, 2 boxers, 4 beagles, 3 west highland white terriers, 1 poodle, 1 bulldog, 1 Belgian Shepherd, 1 briard, 1 jack russel terrier, 1 foxterrier, 2 Scottish terrier, 1 airedale terrier, 1 Tibetan mastiff, 1 central Asian Shepherd dog, 2 French bulldog, 2 shih-tzu and 1 cocker spaniel.

Diagnostic assessment using criteria of identification of atopic dermatitis according to Favrot (Favrot et al. 2010) was carried out in all the dogs. Detailed medical interview was conducted and general and dermatological test were performed. CADESI-03 (Olivry et al. 2001, Olivry et al. 2008) was used to assess the advancement of skin changes. Tests aiming at elimination of the possible presence of ectoparasites, yeast (*Malassezia* spp.) or bacterial infections (skin scrapings, cytological tests) were conducted in all animals qualified to the tests. Additionally, steroids were not administered for at least 4 weeks preceding the tests and antibiotics/chemotherapeutics were not applied for a minimum of 1 week before the test. No anti-histamine medicines were used in any of the dogs. None of the animals had earlier undergone specific immunotherapy.

Intradermal skin tests using Agroskin RTU 20, Central/North Europe panel, were conducted in the dogs qualified to the tests. Working solutions of allergens were prepared according to the producer's directions and then were kept in a fridge at the temperature not exceeding 0 to +4°C. The reactions for the selected allergens was tested in the following concentrations:

- *Dermatophagoides farinae* at the concentration of 1:1000 w/v;
- *Dermatophagoides petronysinus* at the concentration of 1:1000 w/v;
- *Tyrophagus putrescentiae* at the concentration of 1:1000 w/v;
- *Acarus siro* at the concentration of 1:1000 w/v;
- Histamine phosphate at the concentration of 0.0275 mg/ml.

Intradermal allergic tests results could be read and it was possible to qualify the reaction based on the size and quality of the positive skin reaction in all the animals.

Before running intradermal allergic tests, blood from cephalic vein was taken in order to obtain the serum for the IgE screening test which was carried out using recombinant chain of  $\alpha$  receptor of high affinity to IgE ( $Fc_\epsilon RI\alpha$ ). The serum was next sent to the IDEEX laboratory which carried out the test. To describe it in a simple way, the  $Fc_\epsilon RI\alpha$  receptor test consists of adding the serum with the specific IgE and IgG for the

allergen to the carrier so that the two combine. In the next stage, the Fc<sub>ε</sub>RIα receptor, which combines exclusively with IgE, is added. Next, a coloured marker is added and a spectrophotometric test is carried out. The count of over 150 units (including the clinical symptoms observed) was considered positive within the range of the test which was 0 to 4000 EA (ELISA absorbance) units.

The comparison of the intradermal allergic test (IDST) with the IgE screening test made with Fc<sub>ε</sub>RIα receptor method (ELISA) as well as calculations of sensitivity and specificity were carried out in the following groups:

- A screening test for mites including: *Dermatophagoides farinae*, *Dermatophagoides petronyssinus*, *Tyrophagus putrescentiae* and *Acarus siro*;
- A test involving only home dust mites: *D. farinae* and *D. petronyssinus*;
- A test involving only storage mites: *T. putrescentiae* and *A. siro*
- Tests involving all of the mites listed above individually.

Mites were chosen as the subject of this study as they are accounted to an example of year-round allergens which eliminates the seasonal factor of some allergens which in term could cause the disappearance of specific antibodies in blood serum. In the screening test for all groups of mites, that is both dust mites and storage mites, it was assumed that obtaining a positive result for at least one type of mite was considered a positive result for the entire group. When considering different types of mites separately, each positive result meant getting the antibodies titre >150 EA in a serological test for the selected mite.

The calculations of sensitivity and specificity of the test were carried out taking intradermal allergic tests as a baseline. Sensitivity (SE) was calculated based on the formula:  $SE = a / (a+c) \times 100\%$ , where "a" was the number of sick animals which were correctly diagnosed by the test and „a+c" the total number of sick animals. Specificity (SP) was calculated based on the following formula:  $SP = d / (b+d) \times 100\%$ , where „d" was the number of the healthy animals correctly diagnosed by the test and „b+d" the total number of the healthy animals.

## Results

Correspondence analysis between the results of the IgE screening tests carried out using the Fc<sub>ε</sub>RIα receptor method and the intradermal test referring to mites was performed. In case of blood test, a result of 150 EA or more units was regarded as a positive one and, in case of intradermal test, non-zero number of Agroskin RTU 20 test qualified the result as a positive one.

The test could yield a negative (-) and positive result (+) at various strengths (from 1+ to 4+). To make the statistical calculations easier, negative result was assumed to be 0 and the positive results were within a range from 1 to 4. By definition, specificity (SP) is related to the number of not sensitive dogs which will be correctly identified by the test as „negative" one. The sensitivity of the test (SE) showed how many of actually hypersensitive dogs were marked as „positive".

The percentage of dogs which had positive results in intradermal allergic tests and in IgE screening test carried out using the Fc<sub>ε</sub>RIα receptor method is presented in Table 2. Table 3 presents the tests results from all the dogs qualified to the experiment (n= 45) and obtained using intradermal allergic tests and the IgE screening test carried out using the Fc<sub>ε</sub>RIα receptor method. Table 4 presents the sensitivity and specificity of the the IgE screening test carried out using the Fc<sub>ε</sub>RIα receptor method directed exclusively to the selected allergens.

## Discussion

The introduction of serological tests in the diagnostics of allergy was a big step forward in the recognition of allergens provoking allergic reaction in people and animals. The initial tests, however, using polyclonal and monoclonal antibodies of IgE type turned out to be insufficient. So far, intradermal skin tests were considered to be more credible and burdened with a lower risk of a mistake which was to be changed by the introduction of serological test. This, however, did not happen and the applied antibodies reacted also with the antibodies of the IgG type and caused a high percent of mistakes. The IgE test carried out using the the Fc<sub>ε</sub>RIα receptor method (ELISA method) is the most modern serological method which is characterised by high specificity and eliminates cross reactions with the IgG immunoglobulin. The conducted experiment aimed at analysing results of the IgE tests carried out using the Fc<sub>ε</sub>RIα receptor method and of the intradermal skin test which, so far, has been considered a baseline (gold standard) for all other allergic tests. The comparison of the results and the verification of the sensitivity and specificity of the test in case of dogs with atopic dermatitis makes it possible to assess the advantages and disadvantages of the tests and to evaluate their usefulness in the identification of the allergens and specific immunotherapy. House dust mites are the most frequently identified allergens in atopic dogs both in Europe and in the United States which is why this study concentrated exclusively on this group of allergens (Sture et al. 1995, Scott et al. 2001, Tarpatki et al. 2006). Moreover, as year-round allergens, they are an excellent research model.

Table 1. The method of sensitivity and specificity calculations.

	Intradermal allergic tests (IDST) positive (+)	Intradermal allergic tests (IDST) negative (-)
Screening IgE test carried out using Fc <sub>ε</sub> RIα (ELISA) receptor method positive (+)	a	b
Screening IgE test carried out using Fc <sub>ε</sub> RIα (ELISA) receptor method negative (-)	c	d

Table 2. The percentages of dogs (%), which had positive results in intradermal allergic tests and IgE screening test carried out using Fc<sub>ε</sub>RIα receptor method.

Allergen	Positive result in intradermal allergic tests (%)	Positive result in IgE screening test carried out using Fc <sub>ε</sub> RIα receptor method (ELISA) (%)	Difference (%)
Screening test ( <i>D. farinae</i> , <i>D. petronyssinus</i> , <i>T. putrescentiae</i> , <i>A. siro</i> )	95.55	91.11	4.44
Home dust mites group: <i>D. farinae</i> , <i>D. petronyssinus</i>	88.88	82.22	6.66
Storage mites group: <i>T. putrescentiae</i> , <i>A. siro</i>	91.11	88.88	2.32
<i>D. farinae</i>	75.55	80	4.45
<i>D. petronyssinus</i>	77.77	55.55	22.22
<i>T. putrescentiae</i>	86.66	73.33	13.33
<i>A. siro</i>	84.44	88.88	4.44

Table 3. Test results in all the dogs qualified to the experiment (n= 45) using intradermal allergic test and IgE screening test carried out using Fc<sub>ε</sub>RIα receptor method.

	a	b	c	d	SE (%)	SP (%)
Screening test:						
<i>D. farinae</i>						
<i>D. petronyssinus</i>	39	1	4	1	90.69	50
<i>T. putrescentiae</i>						
<i>A. siro</i>						
Home dust mite group:						
<i>D. farinae</i>	34	3	6	2	85	40
<i>D. petronyssinus</i>						
Storage mite group:						
<i>T. putrescentiae</i>	37	3	1	4	90.24	25
<i>A. siro</i>						

a = positive ELISA and positive IDST, b = positive ELISA and negative IDST, c = negative ELISA and positive IDST, d = negative ELISA and negative IDST, SE = sensitivity, SP = specificity

Table 4. The sensitivity and specificity of IgE screening test carried out using Fc<sub>ε</sub>RIα receptor method directed only to selected allergens.

	a	b	c	d	SE (%)	SP (%)
<i>D. farinae</i>	28	8	6	3	82.3	27.2
<i>D. petronyssinus</i>	23	2	12	8	65.1	80
<i>T. putrescentiae</i>	29	4	10	2	74.3	33.3
<i>A. siro</i>	34	6	4	1	89.4	14.2

a = positive ELISA and positive IDST, b = positive ELISA and negative IDST, c = negative ELISA and positive IDST, d = negative ELISA and negative IDST

When comparing the percentage of positive test results of the intradermal skin test and the results of the IgE screening test carried out using the Fc $\epsilon$ RI $\alpha$  receptor method, in case the test is performed on all mites (screening tests on a group of mites), it can be observed that the two results are on a similar level. Notwithstanding, both in the screening test as described above, as in a separate one directed to a group of house dust and storage mites, better results were obtained with the use of the intradermal tests. The difference between the percentage of positive results in both tests oscillated from 2.31 to 6.66% (Table 2). The situation is less unambiguous when considering each type of mite separately. Both in the case of *D. farinae* as of *A. siro* the difference remains on a similar level (4.45 and 4.44%). However, in the case of *T. putrescentiae* it was 13.33% and as much as 22.22% for *D. petronyissinus*. Already at this initial stage of the assessment, it may be concluded that in the case of the screening test as in the case when mites are divided into groups, the results are similar. However, there may be significant differences when allergens are considered separately. The tests described in the literature show that a high percent of healthy dogs (about 20%) present detectable titres of antibodies against at least one species of mites, especially against *D. farine*, although they are lower than those found in atopic dogs (Koebrich et al. 2012). It must be pointed out, however, that the results of ELISA test should be assessed together with the presence of clinical symptoms of atopic dermatitis, the result itself being not sufficient. In the test comparing the results of atopic and healthy dogs, Roque et al. (2011) obtained even higher averages of specific antibodies of the IgE type in healthy dogs. Because of this, it was necessary to carry out more precise comparisons and assays of sensitivity and specificity of the IgE screening test performed with the Fc $\epsilon$ RI $\alpha$  receptor method. There are proofs, however, that not in all atopic dogs high titres of the IgE exist what can diminish the efficiency of the carried out test (Halliwell 2006). It is also assumed that the total IgE concentration in the serum of both healthy and atopic dogs is similar (Hill et al. 1995) and the presence of the IgE in the serum of puppies does not always correspond with them developing atopic dermatitis (DeBoer and Hill 1999). Additionally, the timing of the test is important as the antibodies are present only for a defined length of time after the contact with the allergen. The period of 4 weeks after the contact with the allergen is considered to be the moment of top values of the IgE antibodies in people. The data referring to dogs is limited. However, many authors agree upon their short period of presence in the blood serum; depending on the studies the period is up to 2 months long (Masuda et al. 2002). Also

Wassom and Grieve (1998) referring to the unpublished data of de Weck mentions the limited time of the presence of IgE antibodies in the serum. It should also be added that earlier specific immunotherapy probably influences the later lower level of specific IgE antibodies (Keppel and Campbell 2008). Thus, in the present experiment the dogs with the immunotherapy in their treatment history were not qualified to the test. What is more, the cross reaction between the mites has been proven. A dependence between *A. siro* and *T. putrescentiae* (Arlian et al. 2003) was observed. Saridomichelakis et al. (2008) also demonstrated that *D. farinae* strongly inhibited the reactivity of the IgE against *D. petronyissinus*, but it did not happen the other way round. They suggested that false positive results are more probable in the case of *D. petronyissinus* in dogs sensitive to *D. farinae* than the opposite way. It is significant, however, that cross reactions are not observed regularly which is also confirmed by the results of intradermal allergic tests. If the cross reaction was a rule, it would not be possible to obtain positive results in tests directed only on one type of the mentioned mites, which was also observed by Bensignor and Carlotti (2002).

The sensitivity and specificity of the IgE screening test carried out using the Fc $\epsilon$ RI $\alpha$  receptor test for the *D. farinae*, *D. petronyissinus*, *T. putrescentiae* group of mites and *A. siro* was respectively 90.69% and 50%. For the group of home dust mites the sensitivity was 85% and specificity only 40%; in case of the group of storage mites the sensitivity was on the level of as much as 90.24%, the specificity, however, only on the level of 25%. The results show that in case of the screening tests, the sensitivity is quite high. Unfortunately, the specificity of these tests is insufficient. The same is true when it comes to tests directed to selected allergens. The test demonstrated that the highest sensitivity among all tested allergens was obtained in the case of *A. siro* (89.4%), however with only 14.2% of the specificity. In case of *D. farinae* and *T. putrescentiae*, the sensitivity was also quite high (82.3 and 74.3% respectively) but the specificity was on the level of 27.2 and 33.3 % respectively, which is considered insufficient by the authors. Opposite results were obtained in the case of *D. petronyissinus* when the sensitivity was calculated on the level of 65.1% and the specificity was 80%. It is probable that the low level of specific antibodies of the IgE type was caused by the presence of atopic-like dermatitis in the patient where the clinical symptoms are identical with atopic dermatitis but without the proved participation of the antibodies of the IgE type (Halliwell 2006). It must be noted that the probability of qualification of a large number of animals with atopic like dermatitis to the carried out test is very low.

Summing up the results of the tests carried out, it can be concluded that intradermal skin tests are still burdened with a smaller mistake rate than the serological tests. It is worth noting, however, that serological tests have high sensitivity in a screening test directed to the whole group of the tested mites and also to the group of storage and dust mites. It suggests the possibility of using serological test in a screening test when a doctor diagnoses atopic dermatitis and initially wants to define the groups of allergens which the patient is allergic to. However, when analyzing the sensitivity and specificity of the IgE test carried out using the Fc<sub>ε</sub>RIα receptor method, a significant reduction in these values can be observed when detecting the antibodies for the selected mites. The experiments described in the literature still recommend the intradermal allergic test as a gold standard which has been proved by the present study. Additionally, the serological test is limited by the duration of the presence of antibodies in the serum; assuming that dust mites are year-round allergens, it should have no impact on the tests carried out in the present study. This fact, however, should be taken into account when assaying the antibody titre against seasonal allergens.

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