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EFFECT OF DESENSITIZATION THERAPY IN DOGS WITH ATOPIC DERMATITIS

EFECTUL UNEI TERAPII DE DESENSIBILIZARE LA CÂINII CU DERMATICĂ ATOPICĂ

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ABSTRACT | REZUMAT

This study documents the results of a desensitization therapy used in allergic dogs. The Polycheck® test was used to identify the reactivity of 20 dogs to various environmental allergens. The dogs with allergic symptoms aged between 3 and 7 years were tested. This study demonstrated a statistically sustained significant improvement in the cutaneous lesions and a reduction of pruritus in 75% of the evaluated dogs. Withdrawal of oral glucocorticoid treatment was achieved in 12 (60%) cases. The dose of glucocorticoid treatment was decreased by more than 50% in 15% of the dogs who still needed corticotherapy. The pollen hypersensitivity responded very well to the allergen-specific immunotherapy, with positive results observed in approximately 50% of the tested dogs. In the 11th month, 45% of the dogs manifested mild pruritus (grade 1), 30% had moderate pruritus (grade 2) and 25% had severe pruritus (grade 3). The immunotherapy represents the future treatment for allergic animals and humans, even if the methods used today give variable results.

Keywords: desensitization therapy, dogs, atopic dermatitis

Acest studiu documentează rezultatele unei terapii de desensibilizare utilizate la câinii alergici. Testul Polycheck® a fost utilizat pentru a identifica reactivitatea a 20 de câini la diferiți alergeni din mediu. Au fost testați câinii cu simptome alergice cu vârste cuprinse între 3 și 7 ani. Acest studiu a demonstrat o îmbunătățire semnificativă a leziunilor cutanate și o reducere a pruritului la 75% din câinii examinați. La finalul studiului 12 (60%) cazuri nu am mai necesitat tratament cu glucocorticoizi. Doza de tratament cu glucocorticoizi a scăzut cu mai mult de 50% la 15% dintre câini care au necesitat în continuare corticoterapie. Hipersensibilitatea la polen a răspuns foarte bine la imunoterapia specifică, rezultatele pozitive fiind observate la aproximativ 50% din câinii testați. În luna a XI-a a studiului, 45% dintre câini au manifestat prurit ușor (gradul 1), 30% prurit moderat (gradul 2) și 25% au manifestat prurit sever (gradul 3). Chiar dacă metodele utilizate astăzi dau rezultate discordante, imunoterapia reprezintă viitorul tratamentului animalelor și oamenilor alergici.

Cuvinte cheie: terapie de desensibilizare, câini, dermatită atopică

Canine atopic dermatitis is considered to be a very common skin disease in dogs. The diagnosis of the allergic conditions is made by the correct interpretation of the anamnesis, associated with a differential diagnosis. Unfortunately, no single test is able to differentiate between the atopic and non-atopic dogs (12, 26). Allergen immunotherapy (AIT) is considered to be the only efficient treatment for atopic dermatitis. The immunotherapy is able to change the pathogenic mechanisms of the disease (1, 14).

Nowadays, the administration of the immunotherapy is performed subcutaneously. Other protocols like intralymphatic, sublingual and epicutaneous immunotherapy have also been described (21). The effectiveness of the allergen immunotherapy is considered to ranging between 50-100%, depending on the used protocol, study design and the severity of the disease (3, 4, 13, 20, 29, 32).

The disease represents a challenge for the practitioners and for the owners, and it involves long term compliance with the therapeutic protocol. Over the last decades, several studies conducted in different climatic conditions of different countries, have investigated the efficiency of different treatment protocols in the canine atopic dermatitis (6, 7, 11, 13, 17, 23).

In this regard, even if the obtained results leaded to the promising progresses, the remaining knowledge gaps raise great challenges in daily routine activities of small veterinary practitioners. Thus, additional studies using different diet trials based on the administration of different drug formulations are still re-

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quired. The therapies should also include drugs against the resistant bacteria that are involved in the cutaneous lesions and otitis externa which are very common in allergic dogs (8, 16).

The present study was undertaken to compare the effectiveness of an allergen immunotherapy, commercially available in Romania, using the conventional and the cluster protocols.

MATERIALS AND METHODS

The study was conducted during the period September 2017- August 2018, enrolling twenty dogs diagnosed with atopic dermatitis, according with standard methods and excluding other pruritic skin diseases. Before testing acaricidal treatment to exclude infestation with scabies and *Demodex canis* was performed. In order to exclude any adverse cutaneous food reaction an elimination diet was provided to the tested dogs during six weeks. If the clinical signs did not show any improvement during the diet trial, the food allergy was excluded. After the applied test diet, no dietary modifications were allowed.

During the study, the administration of corticosteroids (Medrol®; Pfizer, Italy ,1-2 mg/kg) was allowed up to the seventh month, and it was discontinued for the remaining four months.

Animals

The 20 animals studied belonged to the following breeds: Bichon (n = 4), French Bulldog (n = 3), Pekingese (n=3), West Highland White Terrier (n=2), Mops (n=1), Labrador (n=2), German Shepherd (n=2), and Mix breed (n = 3) (Table 1).

Depending on the sex, the distribution of the animals was as follows: eight females (40%) and 12 males (60%). Of these, 70% were neutered. The dogs that were included in the study were between 3 and 7 years old with a median of 4,5 ± 2 years, the average onset of the disease was 3.2 ± 1 years, and the average evolution of the disease was 1.4 ± 1.2 years.

Allergen testing

A quantitative serum allergen-specific IgE ELISA test was used in order to identify the reactivity of the dogs to various environmental allergens (Polycheck®, Biocheck GmbH, Münster Germany). Dogs with IgE directed against *D. farinae* and other allergens with a value of >2 kU/L were considered positive and eligible for this study. The working methodology followed the instructions provided by the manufacturer (https://polycheck.de/wp-content/uploads/2015/12/Polycheck_Catalogue_2020.pdf).

Treatment

Written consent was obtained from dog owners for the tested animals to be used in research, as consequence of positive result showed by the allergenic test.

For the desensitization protocol the Alutek® (Inmunotek, Madrid) extract was used, it consists of native, standardized allergen extracts, adsorbed on aluminium hydroxide gel and contains the following excipients: aluminium hydroxide, sodium chloride, phenol, and saline for inoculation. The Alutek® (Inmunotek, Madrid) was presented in a set of three vials containing increasing concentrations (100 UT/ml, 1.000 UT/ml and 10.000 UT/ml) of the active allergen ingredient that were identified using the Polycheck® test (Biocheck GmbH, Münster Germany). The product was administered by sub-cutaneous injection (s.c.) in two phases: an induction phase and a maintenance phase by two methods the conventional and the cluster method.

The induction phase of the conventional therapy was caried out in a period of 35 days in which the immunotherapy was administered in ascending order of concentration, starting with the 1.000 UT/ml extract with a dosage of 0.1 ml/s.c in day 1, 0.3 ml/s.c in day 7, and 0.5 ml/s.c in day 14 and continuing with the 10.000 UT/ml in day 21 (0.1 ml/s.c), day 28 (0.3 ml/s.c), and day 35 (0.5 ml/s.c). The maintenance phase was continued with the 10.000 UT/ml extract administered monthly.

For the cluster method the induction phase was caried out in a period of 21 days, with double dosages at intervals of 30 minutes, starting with the 1.000 UT/ml extract with a dosage of 0.1 ml/s.c and 0.2 ml/s.c in day 1 and continuing with 0.3ml/s.c and 0.5 ml/s.c in day 7. In day 14 the extract was changed with the 10.000UT/ml extract in a dosage of 0.1 ml/s.c and 0.2 ml/s.c and was repeated in day 21 with 0.3 ml/s.c and 0.5 ml/s.c dosage, respectively. For the maintenance phase the 10.000 UT/ml extract, administered monthly, was used.

Evaluation of clinical efficacy

The severity of the cutaneous lesions was evaluated using the Six Areas Six Sign Atopic Dermatitis severity index (SASSAD) by the same veterinarian before the initiation of allergen immunotherapy (pre-AIT) three, seven and eleven months, respectively after the induction phase of the therapy. A reduction of the SASSAD score under 25 points was desirable and considered an indicator for the efficacy of the immunotherapy. The owner evaluated the intensity of the pruritus using a Visual Analog Scale (scored 0–3) weekly.

The recorded data were statistically analysed using the STATISTICA 8.0 program (TIBCO Software).

RESULTS AND DISCUSSIONS

Evaluation of clinical efficacy

The hypoallergenic diet, tested for a period of six weeks, had no visible effects on the reducing of pruritus in the experimentally evaluated dogs, confirming the diagnosis of atopic dermatitis.

Table 1

Epidemiological data and the allergen reactivity of dogs assessed by allergen-specific immunotherapy

No	Breed	Age (years)	Allergen reactivity
1	Maltese Bichon	3.5	Df, Dp, As, Mix 2, Mix 3
2	Maltese Bichon	5	As, Ld, Mix 2, M
3	Maltese Bichon	4	Rg, Mix 3, Sp
4	Frisé Bichon	3.5	RP, Mix 3, Mw
5	French Bulldog	4	As, Mix 3, So
6	French Bulldog	6	As, Tp, Mix 1
7	French Bulldog	5	Df, Ld, Sp
8	Pekingese	3	Df, Mix 3, M
9	Pekingese	4	Df, Dp, RP, Mix 1
10	Pekingese	3	Df, Dp, Mix 3
11	Mops	5	Df, Dp, Sp
12	German Shepherd	6	Df, Dp, As, RP, M
13	German Shepherd	7	Df, Mix 1, Mw
14	Labrador	6	Df, Mix 3, M
15	Labrador	3.5	Df, Tp, RP
16	West Highland White Terrier	4	Df, Mix 3, M
17	West Highland White Terrier	4	Df, Dp, Mix 2
18	Mix breed	5	Df, RP, Mw
19	Mix breed	5	Df, Tp, Mix 2
20	Mix breed	4	Df, Mix 3, So

Legend: Df: *D. farinae*; Dp: *D. pteronyssinus*; As: *Acarus siro*, Tp: *Tyrophagus putrescentiae*; Ld: *Lepidoglyphus destructor*; Rg: ragweed; Mix 1: birch, alder, hazelnut; RP: rye pollen; Mix 2: plantain, willow, poplar; Mix 3: herb mixture; M: *Malassezia* spp., Sp: wild spinach, Pt: plantain, Mw: wormwood, So: sorrel.

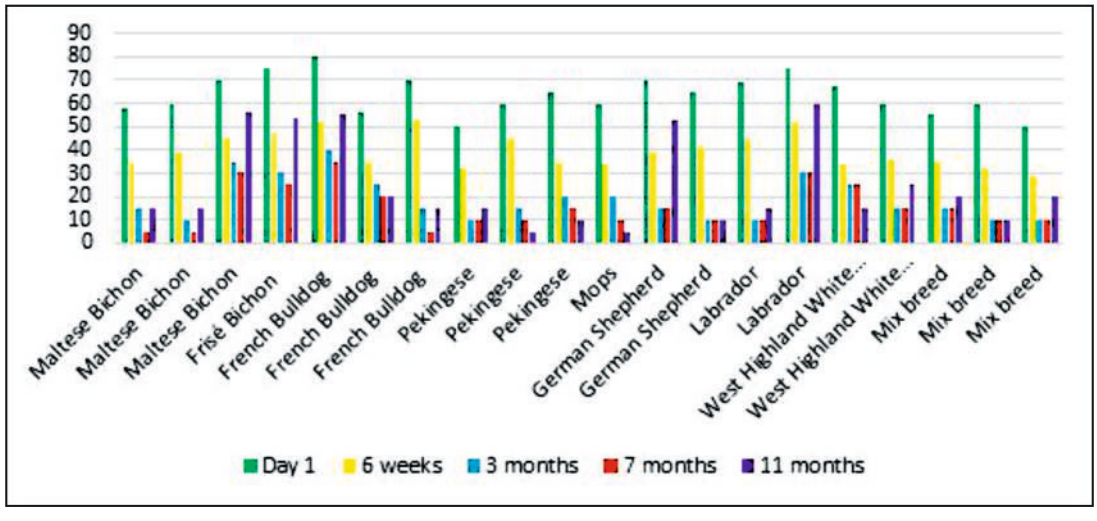

Fig. 1. The evolution of the clinical signs among the tested animals during the eleven months of the investigation period

The reactivity of the animals assessed using the Polycheck® test is summary presented in the Table 1.

The obtained results showed that 80% (16/20) of the investigated animal animals, which constituted the experimental group, expressed reactions towards mites. A positive reactivity to *Dermatophagoides farinae* was observed in 75% (15/20) of the dogs, while other 30% (6/20) of dogs positively reacted to *Dermatophagoides pteronyssinus*, 25% (5/20) to *Acarus siro*, 15% (3/20) to *Tyrophagus putrescentiae*, 10% (2/20) to *Lepidoglyphus destructo,* respectively.

Likewise, 45% (9/20) of the dogs reacted to the mixture of herbs, and other 20% (4/20) expressed reactions towards birch / alder / hazelnut, rye pollen and *Malassezia* spp., Also, 15% (3/20) of the animals reacted to plantain / willow / poplar, wild spinach and wormwood, and only 10% reacted to sorrel.

At the beginning of the study the clinical signs, assessed with the SASSAD index, recorded the following values: ten dogs recorded a score between 50-60

Table 2

The evolution of the clinical signs during the investigation period

No		Tested animals	Evaluation day				
			Day 1	6 weeks	3 months	7 months	11 months
Conventional method	1	Maltese Bichon	58	35↓	15↓	5↓	15↑
	2	Maltese Bichon	60	39↓	10↓	5↓	15↑
	3	Maltese Bichon	70	45↓	35↓	30↓	56↑
	4	Frisé Bichon	75	47↓	30↓	25↓	54↑
	5	French Bulldog	80	52↓	40↓	35↓	55↑
	6	French Bulldog	56	35↓	25↓	20↓	20
	7	French Bulldog	70	53↓	15↓	5↓	15↑
	8	Pekingese	50	32↓	10↓	10	15↑
	9	Pekingese	60	45↓	15↓	10↓	5↓
	10	Pekingese	65	35↓	20↓	15↓	10↓
	11	Mops	60	34↓	20↓	10↓	5↓
	12	German Shepherd	70	39↓	15↓	15	53↑
Cluster method	13	German Shepherd	65	42↓	10↓	10	10
	14	Labrador	69	45↓	10↓	10	15↑
	15	Labrador	75	52↓	30↓	30	60↑
	16	West Highland White Terrier	67	34↓	25↓	25	15↓
	17	West Highland White Terrier	60	36↓	15↓	15	25↑
	18	Mix breed	55	35↓	15↓	15	20↑
	19	Mix breed	60	32↓	10↓	10	10
	20	Mix breed	50	29↓	10↓	10	20↑
Total points			**1275**	**796**	**375**	**310**	**493**

points, seven dogs had a score between 61-70 points, and three dogs they had the score between 71-80 points. The total score of the experimental group was 1275 points. The evolution of the SASSAD score, in the 11 months of the study period, is summary presented in Table 2. Thus, comparing to the first day of the experiment, a decreasing of the SASSAD score value with 61.3% was observed, this meaning from 1275 to 493 points. Also, compared to the 7th month of the investigation period, an increase of the score from 310 to 493 points was observed (Fig. 1).

In the last month of the investigation period, 67% (8/12, 95% CI: 11-64) of the desensitized dogs by the conventional method expressed a reduction or a stagnation of the SASSAD score with values below 25 points (Table 2). The cluster desensitization method showed a reduction or stagnation of the SASSAD score in the 11th month of the study period in 88% (7/8 95% CI 13-69) of the tested dogs.

The evolution of pruritus, evaluated by animal owners, was influenced by the drug treatment received by the animals during the first seven months. Therefore, all dogs showed a significant reduction ($p<0.05$) in the intensity of pruritus, reaching the stage of mild pruritus (grade 1) in the first month. Furthermore, in the seventh month, only 25% (5/20 95% CI 11-46) of the dogs manifested an intensification of the pruritus, reaching the stage of moderate pruritus (grade 2).

In the 11th month, 45% (9/20 95% CI 25-65) of the dogs manifested mild pruritus (grade 1), 25% (5/20 95% CI 11-46) had moderate pruritus (grade 2) and 25% (4/20 95% CI 11-46) had severe pruritus (grade 3) (Fig. 2). After the investigation period, 75% (15/20, 95% CI 58-91) of the evaluated dogs continued the desensitization therapy, maintaining the SA SSAD score in the desiderated interval (under 25 SA SSAD point).

Adverse effects

Temporary side effects were reported in 2% (4/20; 95% CI 1-4%) of the dogs such as vomiting and diarrhoea. Also, in two (1%; 95% CI 1-3%) patients, as consequence of the inoculation of the therapeutic solution aiming the desensitization, pain at the inoculation site was recorded.

Statistical analyses revealed that the recurrence of lesions in dogs that had an SASSAD score of less than 25 points in the 7th month was significantly lower ($p <0.05$) compared to that of dogs that in the 7th month had a SASSAD score higher than 25 points. Significant differences ($p <0.05$) were obtained in terms of the evolution of the cutaneous lesions, from day 1, compared to all four control periods (six weeks, three, seven and 11 months, respectively), but also between the four control periods. Significant differences ($p < 0.05$) were obtained using the Fisher's test between the efficacity of the applied two protocols, the cluster protocol having a higher efficacy than the conventional protocol. Overall, the study results demonstrated a significant improvement in the skin lesions and a reduction of pruritus using the Alutek® (Inmunotek, Madrid) allergen test in 75% (15/20 95% CI 53-88%) of the enrolled dogs.

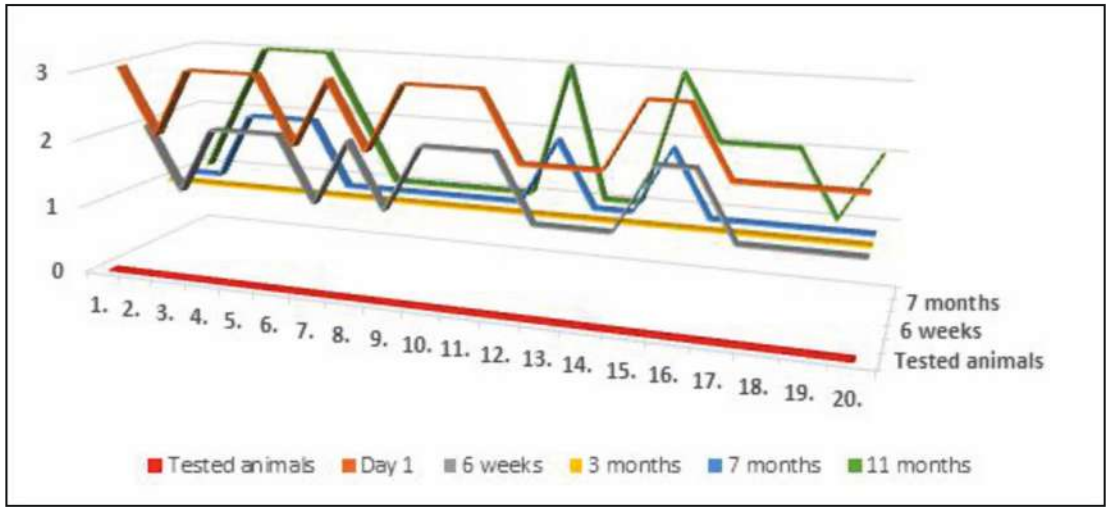

Fig. 2. The evolution of the pruritus intensity counted as mild, moderate and severe, during the eleven months of the study

Withdrawal of the oral glucocorticoid treatment, achieved in 60% (12/20 95% CI 38-78%) of cases, suggested higher response rates on ASIT (5.0–21.5 %) comparing to those reported in previously conducted studies (9, 19, 30). The dose of glucocorticoid treatment was decreased by more than 50% in 15% (3/20, 95% CI 5-36%) of the dogs.

Results of previously conducted investigations using anti-inflammatory drugs in combination with a specific desensitization treatment confirms that low-dose anti-inflammatory drug therapy is useful for positive results and does not interfere with the immunotherapy (2, 5, 36).

Mellerup et al. (2000) observed that, the use of cetirizine, cyclosporine, oclacitinib or glucocorticoids in order to reduce pruritus in the induction phase, did not interfere with immunotherapy (22), although in the case of using oclacitinib a delay of epicutaneous desensitization has been recorded (21).

In accordance with our results, several other published studies support the high reactivity of the dogs to mite allergens (4, 10, 18, 31, 35).

The pollen hypersensitivity responded very well to the allergen-specific immunotherapy, with positive results observed in approx. 50% of the tested dogs. Therefore, the using of immunotherapy often allows the control of atopic dermatitis, without the use of oral glucocorticoids or antihistamines treatments (27, 28).

Although, the flea bite allergy is considered to be one of the major causes of pruritus in dogs, in the present survey the dogs have not expressed any hyperreactivity to fleas. Results of several investigations conducted in Romania, suggest that the flea bite allergy has the highest incidence among allergic diseases in dogs (24, 25, 33).

As consequence of the analysis of the occurrence of adverse reactions in dogs in the present study, the desensitization protocol used during investigations, can be considered a safe protocol, due to the gradual increase of the inoculated dose, in the induction phase. Systemic reactions can range from mild to severe, even anaphylactic shock. These reactions depend by the type of the used allergen and the administration route. Moreover, the safety of allergen extracts adsorbed onto aluminium hydroxide gel, like the one tested in this study, is confirmed by numerous other studies (20, 34, 37), in contrast to aqueous extracts which have a higher probability of inducing a systemic reaction (15).

Even if there are multiple studies in this field with promising results, there is a category of patients that do not show an improvement in the cutaneous lesions after the desensitization protocol, that is why additional studies using different protocols with low and high dosages extracts will fill the present gasps. The sublingual and intranasal therapies represent new approaches in this field and could represent the missing link for the unresponsive patients.

CONCLUSIONS

The study highlighted that the desensitization protocol with the cluster method was more effective (88%) in reducing the cutaneous lesions compared with the conventional method (67%).

The desensitization protocol also demonstrated a reduction of the pruritus in 75% of the evaluated dogs, demonstrating that this approach can be considered a useful tool in the therapeutic management of clinical cases.

A key role in the protocol is the owner's compliance, any discontinuity without consulting the clinician could result in the loss of the effectiveness of the immunotherapy.

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