



Evaluation of cyclosporine-sparing effects of polyunsaturated fatty acids in the treatment of canine atopic dermatitis

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ABSTRACT

A randomised, double-blinded, placebo-controlled multicentre trial was conducted in 36 dogs with atopic dermatitis to evaluate the cyclosporine-sparing effect of polyunsaturated fatty acids. Dogs were stable on their individual cyclosporine dosage and received either a mainly omega-3 fatty acid product with a minor omega-6 fatty acid fraction or placebo, orally for 12 weeks. Dogs were examined every 4 weeks and the Canine Atopic Dermatitis Extent and Severity Index (CADESI-03) was determined by a clinician. Pruritus, quality of life, global condition and coat quality were scored by the owner. If the dog's CADESI-03 and/or pruritus score improved by at least 25% compared with the previous visit, the cyclosporine dosage was decreased by approximately 25%. If the scores deteriorated by at least 25%, the cyclosporine dosage was increased by the same percentage.

The median daily cyclosporine dosage/kg bodyweight decreased in the active group from 4.1 mg to 2.6 mg and in the placebo group from 3.5 mg to 3.3 mg over the study period. The difference between the two groups was significant ($P = 0.009$). The improvement in median pruritus score from inclusion to completion was significantly greater in the active group than in the placebo group ($P = 0.04$). There was no significant difference in CADESI-03 changes between groups ($P = 0.38$). The results of this study indicate a cyclosporine-sparing effect of a mainly omega-3 fatty acid supplement in dogs with atopic dermatitis.

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Introduction

Canine atopic dermatitis (CAD) is a common skin disorder in small-animal practice (Scott and Paradis, 1990; Hill et al., 2006) and is defined as a hereditary predisposition to develop pruritic inflammatory skin disease associated with IgE antibodies, which typically target environmental allergens (Halliwell, 2006). The most consistent clinical feature is pruritus of the face, ears, paws, limbs and ventrum where secondary lesions may also arise (Griffin and DeBoer, 2001).

The sole causal treatment for CAD is allergen-specific immunotherapy (Mueller and Bettenay, 1996; Loewenstein and Mueller, 2009). However, response to this treatment may take several months (Loewenstein and Mueller, 2009) and it is not effective in all dogs (Mueller and Bettenay, 1996). Consequently, atopic dogs are often treated symptomatically with glucocorticoids (Olivry and Sousa,

2001), cyclosporine (Steffan et al., 2006), antihistamines (DeBoer and Griffin, 2001), oclacitinib (Cosgrove et al., 2013) and polyunsaturated fatty acids (PUFA) (Mueller et al., 2004; Olivry et al., 2010). Cyclosporine has proved to be no less effective than glucocorticoids in reducing the clinical signs of CAD (Olivry et al., 2002; Steffan et al., 2003).

PUFA can be divided into omega-3 and omega-6 fatty acids and are considered a safe therapeutic option for the treatment of CAD (Mueller et al., 2004; Olivry et al., 2010). The omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) may modulate eicosanoid synthesis, as they compete with arachidonic acid (AA) for the enzymes cyclooxygenase and 5-lipoxygenase, leading to an increased production of anti-inflammatory eicosanoids instead of proinflammatory eicosanoids derived from AA (Vaughn et al., 1994). PUFA may also inhibit cellular activation (Stehle et al., 2010) and proinflammatory cytokine secretion (Caughey et al., 1996; Novak et al., 2003) as well as stabilise the epidermal lipid barrier (Hansen and Jensen, 1985).

PUFA appear to be insufficient as sole therapy for CAD (Olivry and Sousa, 2001; Olivry et al., 2001, 2010) although a steroid-sparing

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effect of PUFA has been shown (Saevik et al., 2004), making PUFA supplementation with glucocorticoid therapy reasonable. To the authors' knowledge, there are no published data on the cyclosporine-sparing effects of PUFA in the treatment of CAD. The purpose of this study was to evaluate whether orally administered PUFA (Omega-3 Support, WDT)¹ are effective in reducing cyclosporine (Atopica, Novartis) dosage in atopic dogs.

Materials and methods

Study population

Dogs with atopic dermatitis were eligible for inclusion and were diagnosed by history, clinical examination and ruling out differential diagnoses with tests and treatments such as flea control, an elimination diet of 6–8 weeks (home cooked or commercial, containing food proteins that were novel to each dog; Favrot et al., 2010) and skin scrapings or trial therapy against superficial mites as indicated (DeBoer and Hillier, 2001; Olivry, 2010).

All dogs were stable on their individual cyclosporine (Atopica, Novartis) dose for at least 8 weeks prior to inclusion in the study. Other medications were permitted unchanged in the 8 weeks prior to and during the study. Dogs with a proven food adverse reaction had to be on the same diet for at least 3 months prior to inclusion and during the study. Allergen-specific immunotherapy was permitted as long as it was initiated more than 12 months before inclusion and not changed during the study. Oral and topical fatty acids were withdrawn for at least 12 weeks and 8 weeks before commencement of the study, respectively.

Dogs with skin infections in need of antibiotic therapy at recruitment were excluded. Serious adverse reactions to the study-drugs as well as lack of owner compliance were further reasons for withdrawal from the study.

Study design

The study was carried out as a randomised, placebo-controlled, double-blinded multi-centre study. The study protocol was approved by the Ethics Committee of the Centre for Clinical Veterinary Medicine, LMU Munich (number 8-20-06-13, dated 3 July 2013). Dogs were assigned to one of two treatment groups using a computer-generated randomisation list. Study medications were labelled A, B, C, D with two letters assigned for each group and neither dog owners nor evaluating clinicians were aware of the coding.

Participating clinics

Participating clinics were referral dermatology clinics in Germany (Prof. Dr. Ralf Mueller, Medizinische Kleintierklinik LMU Munich; Dr. Monika Linek, Tierärztliche Spezialisten Hamburg; Dr. Christine Löwenstein, Tierärztliche Gemeinschaftspraxis Dr. David, Dr. Krützfeldt Frankenthal; Dr. Anja Röthig, Klinik für Kleintiere, Justus-Liebig-Universität Giessen; Drs. Kerstin and Brett Wildermuth, Tierdermatologie Dr. Wildermuth Wiesbaden; Drs. Sonya Bettenay and Nina Glos, Tierärztliche Fachklinik für Kleintiere Haas und Link Germering), France (Dr. Emmanuel Bensignor, Centre Hospitalier Vétérinaire Paris), Austria (Dr. Lucia Panakova, Vetmeduni Vienna) and Switzerland (Dr. Claudia Nett, Dermatologie und Allergologie für Tiere Hünenberg). Clinical evaluations were performed by participating dermatology specialists.

Clinical evaluation

Dogs were examined on the day of inclusion before receiving study medications (week 0) and after 4, 8 and 12 weeks of therapy. At each visit, a dermatologist examined the animal and determined a validated lesion score, the Canine Atopic Dermatitis Extent and Severity Index (CADESI-03) (Olivry et al., 2007, 2008). Briefly, 62 body sites were evaluated for marks of acute inflammation (erythema), chronic inflammation (lichenification), trauma (excoriation) and alopecia on a scale of 0 (non-existent) to 5 (severe) and the additive total score was calculated. Additionally, owners determined the intensity of their dog's pruritus using a validated pruritus severity visual scale ranging from 0 (pruritus not observed) to 10 (severe pruritus) (Hill et al., 2007; Rybníček et al., 2009). Owners also completed a validated quality of life score (Noli et al., 2011a, 2011b) at every visit, where 0 points indicated 'no disturbing influence' and 45 points indicated a 'great disturbing influence' on the quality of life. They also judged the dog's global condition compared to the previous visit from 'deteriorated' (total minimum of –2 points) to 'strongly improved' (total maximum of 5 points) and evaluated the coat quality on a scale from dull/greasy (–1 point) to shiny (2 points). All adverse events occurring during the study were reported in accordance with the current pharmacovigilance regulations.

Intervention

Dogs received either a preparation of plant oil and fish oil containing omega-3- and omega-6 polyunsaturated fatty acids in a ratio of 3.75:1 (Omega-3 Support, WDT) or placebo (paraffin oil for medical purposes) daily for 12 weeks at a dose of 3 mL/10 kg/day, given with food by the owner. Each 3 mL of the PUFA supplement contained approximately 360 mg alpha-linolenic acid (ALA), 410 mg eicosapentaenoic acid (EPA), 249 mg docosahexaenoic acid (DHA) and the omega-6 fatty acids linoleic acid (LA) and docosapentaenoic acid (DPA) in minor fractions. CADESI-03 and pruritus scores were determined every 4 weeks. If the dog showed an improvement of either CADESI-03 or pruritus or both of at least 25% compared with the previous visit, the dose of cyclosporine was decreased by approximately 25%. If lesions or pruritus deteriorated by 25% or more, the dose of cyclosporine was increased again by approximately 25%. Dosage changes were performed as shown in Fig. 1.

Dogs were evaluated for cutaneous infections by clinical examination and cytological evaluation of impression smears at each visit. If a skin infection occurred during the study, routine antimicrobial therapy was administered. Antibiotics were discontinued during the last 3 weeks of the study to avoid influence on clinical and pruritus scores. All patients received a spot-on treatment for flea control containing pyriprole (Prac-Tic, Novartis) once monthly.

Statistics

Primary outcome measure was the change of the daily dose of cyclosporine, which was compared before and after the trial in both groups. The dose of cyclosporine at the end of the trial was the dose the dog would have gone home with if the trial were to be continued. The secondary outcome measures were CADESI-03, pruritus score, quality of life score, coat quality and the global assessment after compared to before the trial. The changes in the treatment group (week 0–week 12) were compared with the corresponding changes in the control group using a Mann–Whitney test. A $P < 0.05$ was considered significant. For the comparison of cyclosporine dose changes the P value was one-sided, for all other comparisons a two-sided P -value was chosen. In addition to a per-protocol analysis, an intention-to-treat with the last value carried forward analysis was performed.

Results

Patients

Thirty-six dogs were enrolled in the study. There were 20 dogs in the active group and 16 in the placebo group. Of these, 34 completed the trial. One dog in the placebo group was euthanased due to an intervertebral disc prolapse, while another in the same group failed to attend the final study visit for unspecified reasons.

The dogs' ages ranged from 12 months to 13 years with a mean of 6 years. Twenty-one dogs were female (nine neutered) and 15 were male (one neutered, 14 intact). Twenty-one different pure breeds and five mixed breeds were represented. The mean bodyweight was 19.3 kg (range 5.5–54.6 kg). Of the 36 atopic dogs, 22 were solely allergic to environmental allergens, while 14 dogs also indicated signs of food allergy and were therefore on a special diet. All dogs showed allergy symptoms throughout the year.

For detailed information on concurrent medications, see Appendix A: Supplementary material. There were no significant differences between the active and placebo groups in age, weight and dose of cyclosporine of the study population.

Clinical evaluation

The median cyclosporine dosage in the active group was 4.1 mg/kg/day at the beginning of the study, and 2.6 mg/kg/day at the end of the study. In the placebo group, the cyclosporine dose was 3.5 mg/kg/day at inclusion and 3.3 mg/kg/day at completion. The mean and median decreases in the cyclosporine dosage in the active were 0.85 mg/kg and 0.32 mg/kg, respectively. For the placebo group the respective values were 0.06 mg/kg and 0.0 mg/kg. As the statistical evaluation of the intention-to-treat analysis and the per-protocol analysis were similar, only the former results are shown. The difference in the cyclosporine dosage between the two groups at inclusion compared to completion was significant (Mann–Whitney test, $P = 0.009$). For the complete data set, see Appendix A: Supplementary material.

¹ In other countries, also known as Omevio, Novartis Animal Health.

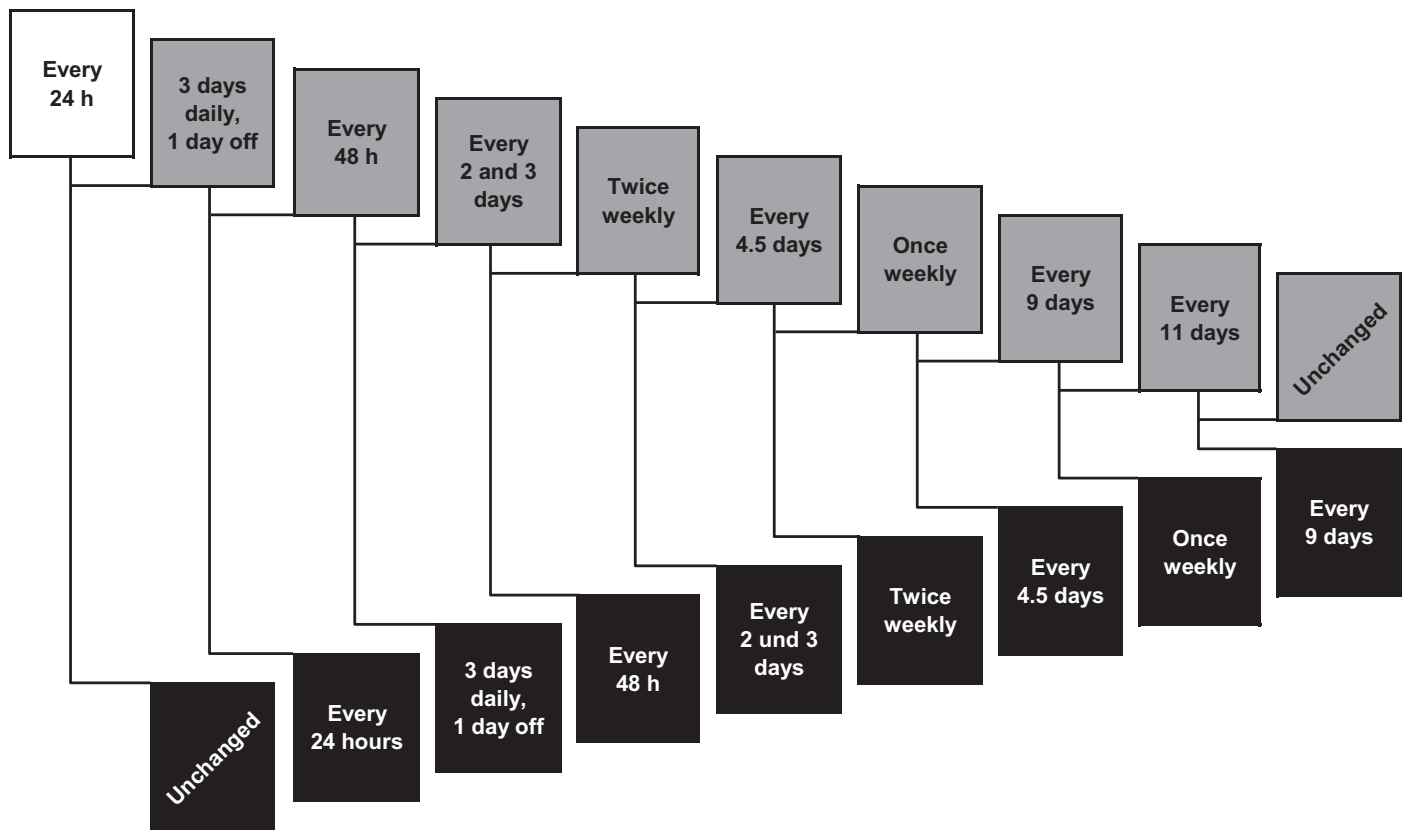


Fig. 1. Dose adjustments of cyclosporine were dependant on changes in the Canine Atopic Dermatitis Extent and Severity Index (CADESI-03) and/or pruritus. Each dog's individual cyclosporine dosing interval at study start was chosen from the top fields. From each of these fields, one line leads to a grey and one to a black field. If the scores for CADESI and/or pruritus at the second visit were decreased by at least 25% compared with the previous visit, the dose of the adjoining grey field was administered, with immediate effect. In the case of equivalent increased values, the dose of the adjoining black field was followed. The dose was maintained if there was no change in CADESI and pruritus. This procedure was pursued at every visit.

The median CADESI-03 score in the active group at the beginning of the study was 15, compared to 6 at the end of the study. In nine of the dogs in this group the CADESI-03 score improved by more than 50%. For the placebo group, the median CADESI-03 was 19 before and after the study. There was no difference in CADESI-03 changes between the two groups ($P = 0.39$). A reduction by more than 50% was observed in only two dogs in the placebo group.

The median pruritus score in the active group at the beginning of the study was 4.2, compared to 3.0 at the final visit. For the placebo group, the median pruritus score increased from 3.2 to 5.3. Pruritus was significantly improved in the active group compared to the placebo group ($P = 0.04$).

The median quality of life score decreased in the active group from 14 to 11, and from 14 to 10 in the placebo group. The median coat score stayed the same at 1 in both groups. There was no significant difference in the changes of these scores between groups ($P = 0.86$ and $P = 0.20$ respectively). The median global condition score increased from 0 to 1 in the active group as well as in the placebo group ($P = 0.22$).

Adverse events

In 10/20 dogs in the active group and 9/16 dogs in the placebo group, owners reported adverse events over the course of the study. In 5/20 dogs of the active and in 6/16 dogs of the placebo group, these events had been evident prior to the start of the study, including diarrhoea or soft stools, hypertrichosis, drowsiness, nausea, flatulence, vomiting, hypersalivation and cutaneous papillomatosis. One dog in the placebo group had an interdigital subcutaneous

mass, for which the owner declined further diagnostics. One dog in the active group had a benign testicular neoplasm surgically removed during the study, as part of a castration. In two dogs in the placebo group, previously existing flatulence worsened during the study.

In 6/20 dogs in the active group and 4/16 dogs in the placebo group, new adverse events occurred during the study, including diarrhoea or soft stools, vomiting, nausea, drowsiness, hair loss, weight loss and seborrhoea. Most adverse events were self-limiting and none were life-threatening. One was considered as moderate to severe (flatulence).

Discussion

A significant reduction in the dose of cyclosporine was achieved when PUFA were administered concurrently compared to placebo in atopic dogs. PUFA have been reported to reduce clinical signs of CAD; n-3 fatty acids such as EPA and DHA (Mueller et al., 2004), n-6 fatty acids such as GLA (Scarff and Lloyd, 1992), as well as a combination of both (Harvey, 1999). Also, special diets with a defined omega-6/omega-3 ratio (Scott et al., 1997) led to a reduction in pruritus and lesions.

Cyclosporine, originally an immunosuppressive agent used for humans (Calne et al., 1998) is highly efficacious in the therapy of CAD (Steffan et al., 2006). It is a calcineurin inhibitor and inhibits the activation of T-cells and other immune cells, as well as production of proinflammatory cytokines (Palmeiro, 2013). Adverse effects, especially gastrointestinal signs, are commonly seen in

cyclosporine-treated dogs, although most are mild and of short duration (Steffan et al., 2006).

To decrease the costs of cyclosporine therapy in dogs, imidazole antifungal drugs such as ketoconazole have been administered concurrently (Patricelli et al., 2002). Ketoconazole inhibits the activity of the cyclosporine degrading cytochrome P450 enzymes and thus leads to increased blood cyclosporine concentrations. The cyclosporine dosage can be reduced up to 75% with concurrent ketoconazole administration (Dahlinger et al., 1998). However, adverse effects occur frequently with this drug combination. A further risk is severe idiosyncratic hepatotoxicity (Mayer et al., 2008). Therefore, safer alternatives are required.

Although PUFA are not adequate for monotherapy in CAD (Olivry et al., 2010), a medication-sparing effect has been shown in several studies. Two of these reported a reduction in antihistamine dosage when administering concurrent PUFA (Paradis et al., 1991; Paterson, 1995). The other two studies, one of which was a placebo-controlled, double-blinded study (Sævik et al., 2004), reported a prednisolone-sparing effect of a mixed omega-3/omega-6 PUFA supplementation as well as a reduction in lesion and pruritus scores (Bond and Lloyd, 1994; Sævik et al., 2004).

The present study suggests a cyclosporine-sparing effect of PUFA supplementation in atopic dogs. This effect was anticipated due to previous anecdotal evidence and the medication-sparing effect of PUFA on the dose of prednisolone and antihistamines (Paradis et al., 1991; Bond and Lloyd, 1994; Paterson, 1995; Sævik et al., 2004). This also was the reason to choose a one-sided *P* value for statistical comparison between the two groups.

The omega-6:3 ratio of the supplement (1:3.75) differed to the study of Sævik et al. (2004) which was 17.88:1. Lower omega-6:3 ratios, as in the present study, were more effective in increasing production of the anti-inflammatory eicosanoid leukotriene (LT)_{B5} and decreasing the pro-inflammatory LT_{B4} in dog neutrophils (Vaughn et al., 1994). These changes may result in reduced cutaneous inflammation. However, at this time, there is no definitive information or clear consensus about the ideal PUFA dosage or the optimal omega-6:3 ratio for treatment of CAD (Olivry and Sousa, 2001; Olivry et al., 2001, 2010; Mueller et al., 2004).

When comparing the changes of scores between the two groups in this study, only pruritus was significantly more improved with PUFA compared to placebo. Neither CADESI-03 nor quality of life or coat quality score changes were significantly different between groups. Yet, if an unjustified dose reduction had occurred in this group (in contrast to the actual findings) a deterioration of clinical parameters would have been expected. Further support to warrant a decrease in cyclosporine dose after PUFA supplementation was the finding that CADESI and pruritus scores decreased concurrently, albeit not significantly.

One shortcoming of the present study is the lack of diet standardisation, such that PUFA intake was variable between dogs. However, as each dog served as its own control and changes in scores and doses were compared between groups, this should not have influenced the results. In addition, the study aimed to provide information for general practitioners and a diet standardisation for all atopic dogs is not possible in practice.

The improvement of some of the parameters in the placebo group could be attributed to the placebo effect. Cyclosporine is a lipophilic substance and is thus also soluble in the placebo product paraffin oil. Thus, a further reason for the improvements in the placebo group could be an increased bioavailability of cyclosporine in both groups over and above the reported bioavailability of the ultramicronised cyclosporine formulation of approximately 35% (Guaguere et al., 2004). As the cyclosporine blood concentration was not monitored during the study, this hypothesis of an increased cyclosporine bioavailability requires further study. The 12-week study period corresponds to established recommendations (Olivry et al.,

2001) based on the time required for PUFA plasma alteration (Campbell and Dorn, 1992; Campbell et al., 1995).

Some dogs had adverse events that were not present prior to the study, mainly gastrointestinal disturbances that partly resolved over the study course. The supplementation did not need to be withdrawn in any dog, so the combination of PUFA supplementation and cyclosporine was deemed a safe treatment for CAD.

Conclusions

The results of this study indicate a significant reduction of the cyclosporine dose can be achieved by PUFA supplementation in atopic dogs. As fatty acid supplementation is also safe and inexpensive, it may be considered for atopic dogs receiving cyclosporine therapy.

Conflict of interest statement

MRM was financially supported by Novartis. In the past 5 years, RM has performed studies, lectured and acted as a consultant for a number of companies manufacturing products for CAD, amongst them Novartis. ML has also performed studies and lectured for a number of such companies including Novartis. KD and KT are employees of Novartis Animal Health. Novartis and WDT, the study sponsors, played no role in the study design, in the collection, analysis and interpretation of data, or in the manuscript writing or submission for publication. None of the authors has any other financial or personal relationships that could inappropriately influence or bias the content of the paper.

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Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.tvjl.2015.11.012.

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Supplementary Table 1: Overview on concurrent medication of the participating dogs during the study.

Dog number	Group	Shampoo	Antihistamines	Ear cleanser	Systemic glucocorticoids	Topical glucocorticoids	ASIT ^a	Nurturing spray
1.	P	x			x	x		
2.	V				x			
3.	V							
4.	P	x						
5.	P	x					x	
6.	V	x			x			
7.	P					x		
8.	P	x						
9.	P	x						
10.	V	x		x		x	x	x
11.	P							
12.	V			x		x		
13.	V	x		x		x		
14.	P	x						
15.	P				x			
16.	V	x				x	x	
17.	V	x				x		
18.	V							
19.	V		x		x			
20.	V							
21.	V							
22.	P							
23.	V	x						
24.	P							
25.	P	x						
26.	V	x				x		
27.	V		x					x
28.	V	x						
29.	V			x		x		
30.	V			x				
31.	P	x		x		x	x	
32.	V	x				x		
33.	V	x						
34.	P	x	x					
35.	P	x						
36.	P		x					

^a Allergen-specific immunotherapy

Supplementary Table 2: Values for CADESI-03, pruritus and cyclosporine dose in the verum group at week 0 and week 12

Dog number	CAD ^a 0 ^b	CAD 12 ^c	Pru ^d 0	Pru 12	Cs ^e 0	Cs 12
2.	31	12	5.4	5.3	4.35	4.35
3.	15	2	0.0	1.5	1.59	1.27
6.	54	78	7.7	7.6	3.85	3.85
10.	49	35	5.2	5.7	6.25	4.69
12.	33	12	3.2	3.0	1.94	1.45
13.	0	0	3.1	3.0	0.89	0.89
16.	2	0	5.8	0.0	2.27	1.14
17.	24	20	4.3	3.6	4.59	3.44
18.	0	0	0.0	0.0	4.55	1.82
19.	13	4	4.3	3.5	1.43	1.11
20.	0	0	7.1	0.0	2.08	2.08
21.	12	0	1.8	0.0	7.00	3.00
23.	65	7	4.1	1.1	1.79	1.79
26.	32	36	6.3	7.4	4.88	4.88
27.	15	14	0.4	1.6	4.84	3.63
28.	4	4	6.3	5.0	4.72	3.54
29.	15	52	0.0	6.7	4.55	4.54
30.	13	7	0.0	2.0	2.17	2.17
32.	14	4	6.3	5.5	3.29	3.29
33.	14	5	2.1	0.0	4.88	1.95

^a CADESI-03

^b Week 0

^c Week 12

^d Pruritus

^e Cyclosporine dose in mg/kg/day

Supplementary Table 3: Values for CADESI-03, pruritus and cyclosporine dose in the placebo group at week 0 and week 12

Dog number	CAD ^a 0 ^b	CAD 12 ^c	Pru ^d 0	Pru 12	Cs ^e 0	Cs 12
1	11	10	3.3	5.3	5.13	5.13
4	2	14	3.0	6.6	4.55	4.55
5	50	110	8.5	9.0	3.66	3.66
7	0	0	1.3	5.3	1.00	1.67
8	21		5.7		4.35	
9	17	24	1.3	1.5	3.53	3.53
11	89	14	3.0	1.7	4.44	2.22
14	2	2	3.2	7.3	1.92	1.92
15	12	6	5.1	7.3	1.30	3.03
22	65	47	1.7	1.7	5.00	5.00
24	105	105	7.6	7.7	2.86	2.86
25	166		5.3		4.29	
31	30	26	4.9	5.2	4.00	4.0
34	46	4	2.2	0.3	0.99	0.63
35	16	26	1.2	1.5	3.47	2.78
36	17	50	3.1	3.2	4.17	4.17

^a CADESI-03

^b Week 0

^c Week 12

^d Pruritus

^e Cyclosporine dose in mg/kg/day