

Intradermal injection of heat-killed *Mycobacterium vaccae* in dogs with atopic dermatitis: a multicentre pilot study

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What is known about the topic of this paper

- *Mycobacterium vaccae* was used in children with atopic dermatitis with some success.
- *M. vaccae* has not been used in dogs with allergic disease.

What this paper adds to the field of veterinary dermatology

- Possible new treatment for atopic dermatitis.
 - Use of *M. vaccae* in dogs.
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Abstract

Canine atopic dermatitis (cAD) is a common disease with a multifactorial aetiology associated with impaired immunoregulation. The immunopathogenesis has similarities to that of human atopic dermatitis. Clinical signs of allergic disease in humans and mice are reduced by administration of saprophytic mycobacteria that amplify regulatory cytokines and hence the effect of *Mycobacterium vaccae* on the clinical severity of cAD was investigated. Sixty-two dogs with cAD, selected according to strict criteria, were treated with a single intradermal injection and evaluated monthly for 3 months in a placebo-controlled double-blind clinical

trial. Clinical severity was quantified using standardized scores and by owner assessment of pruritus. A single injection of a heat-killed suspension of *M. vaccae* was found to be well tolerated and effective in treating mild to moderate cases of cAD demonstrable for 3 months, but was insignificant in more severely affected dogs.

Accepted 19 January 2007

Introduction

Atopic dermatitis (AD) is one of the most common pruritic skin diseases in dogs; only flea allergic dermatitis is more common.¹ Because of the lack of reliable epidemiological data, the true prevalence and incidence of canine AD (cAD) are unknown¹ but it remains an important disease in veterinary dermatology. It is not known whether there is a marked rise in incidence of allergic diseases in dogs, comparable to that recently seen in human beings. This rise in humans has been linked to environmental changes associated with a western lifestyle.² This alteration in environment may also apply to dogs since, unlike 50 years ago, dogs now live mostly indoors in an urbanized situation, are regularly vaccinated and dewormed, and receive commercial diets.

cAD is described as a genetically predisposed inflammatory and pruritic allergic skin disease frequently associated with immunoglobulin E (IgE) antibodies to environmental allergens.³ Studies in beagles with high IgE levels and in dogs with spontaneous cAD have revealed increased Th2 type cytokines, involvement of Langerhans' cells and induction of an inflammatory reaction after atopy patch testing (APT) with house dust mites.^{4–7} APT in 'high-IgE' beagles induced a similar biphasic pattern of cytokine production to that in human patients.^{5,8}

The main treatments currently used for cAD include glucocorticoids, cyclosporin A and allergen-specific immunotherapy (ASIT). Following oral treatment with cyclosporin A or glucocorticoids, the extent and severity of the lesions are expected to decrease by at least 50% up to 85% in dogs.⁹ Glucocorticoids and cyclosporin A are both effective. However, glucocorticoids produce undesirable side-effects such as polyuria, polydipsia, polyphagia, iatrogenic hyperadrenocorticism, diabetes mellitus and hypotrichosis. Cyclosporin A can induce diarrhoea, vomiting and gingival hyperplasia and costs may prohibit its use in large breeds.¹⁰

The clinical benefits of ASIT in dogs are variable. Although ASIT rarely shows side-effects only 50% improvement occurs in 20–70% of animals depending on

Some of the results were presented at the 21st annual Congress of the ESVD-ECVD in Lisbon, Portugal in September 2006.

Editors Note: Antimicrobial therapies should always be carefully used. Even though there were no clinical signs of pyoderma, the use of antibiotics in all of the dogs was considered to be a necessary, acceptable and specific requirement of this trial. Such antibiotic use would not ordinarily be recommended by the authors or editors for the management of canine atopic dermatitis.

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the individual study.¹¹ Most dogs require management with at least one of the above therapies over many years and consequently there is a need for effective new therapies with fewer undesirable side-effects.

The characteristic clinical phenotype of human AD is the product of complex interactions among susceptible genes, the host's environment, skin barrier defects, and systemic and local immunological responses.¹²

In the western world, the prevalence of atopic diseases in humans has doubled in the last 50 years,^{13,14} a massive increase that cannot be explained on the basis of enhanced genetic predisposition alone and is more likely to be related to environmental changes.² One explanation for the increased prevalence, which has gained much support, is the 'hygiene hypothesis', which suggests a correlation with increased hygiene, fewer childhood infections and increased antibiotic use.¹³ For example epidemiological studies have demonstrated a negative correlation between childhood incidence of allergic symptoms and both the time spent in the cowshed and the presence of endotoxin in the house.^{15,16} Similarly, exposure to *Mycobacterium tuberculosis* or to environmental mycobacteria was considered to protect from atopy,¹⁷ but an inverse relationship between tuberculin responses and atopy has not been confirmed.¹⁸

The aberrant immune response in AD is mediated through an important effector cell, the T helper (Th) type 2 cell, and an associated cytokine pattern including interleukin (IL)-4, IL-5, and IL-13. An initial Th2 type cytokine response, found in both uninvolved and acute lesional skin, develops into a more mixed Th1/Th2 type reaction with an increase in IL-5, granulocyte-macrophage colony stimulating factor (GM-CSF), IL-12, and interferon (IFN)- γ -expressing cells.⁸ The local skin reaction is further complicated by the production of chemokines and proinflammatory cytokines by keratinocytes, which direct the immune response.¹⁹

An optimal balance between the Th1 and the Th2 responses is essential for the maintenance of a healthy skin on exposure to potential allergens. This balance is under the control of regulatory networks, an essential component of which are regulatory T cells (Treg).²⁰

Harmless saprophytic mycobacteria are recognized by the innate immune system as so called 'old friends' and are known to induce Treg.²¹ The induction of this network was demonstrated by the development of CD4⁺ regulatory T cells.²² Specific inhibition was found to reside within the CD45RB^{low} subset and was mediated through IL-10 and transforming growth factor β (TGF- β). Moreover, increased levels of IL-10, TGF- β and IFN- α mRNA were also detected in pulmonary CD11c⁺ cells from *Mycobacterium vaccae*-treated allergic mice (which function as antigen-presenting cells²³), and were associated with disease prevention.²⁴ Additionally, recent studies have demonstrated induction of Treg, IL-10 and TGF- β through allergen-specific immunotherapy in humans, and these cytokines play a role in tolerance towards environmental allergens in healthy individuals.^{24,25}

Further encouraging results supporting the immunomodulatory potential of *M. vaccae* were obtained in allergic children with moderate to severe AD who showed a remarkable improvement after one injection with heat-killed *M. vaccae*.²⁶ Moreover, in a small double-blind randomised study in patients with atopic asthma, serum

IgE levels and *in vitro* production of IL-5 were reduced after a single injection of *M. vaccae*.²⁷

Immunomodulation by *M. vaccae* has thus shown promising results, in both animal models of allergic disease and human clinical studies of asthma and AD. Hence, it might also be of value in treating cAD as the clinical signs and immunological aspects of cAD are very similar.^{28,29} Therefore, the aim of this pilot study was to investigate the effectiveness of a single injection of *M. vaccae* in reducing or controlling clinical signs in dogs suffering from cAD.

Materials and methods

Study design

A double-blind, placebo-controlled multicentre pilot study was undertaken on dogs with clinically established cAD. Dogs were examined monthly for 3 months for improvement or progression of disease following an initial intradermal injection of either inactivated *M. vaccae* or a buffer solution (placebo).

Approval for the protocol was obtained from the Swiss Ethics Committee for the use and care of animals prior to commencing the study.

Patients

A total of 64 client-owned dogs (33 males and 31 females) diagnosed with cAD were enrolled but two were later excluded because of protocol violation. Consequently, data from a total of 62 dogs (29 treated and 33 placebo) were used.

The dogs were selected from 12 dermatology referral centres in Belgium, France, and Switzerland, and cAD was diagnosed using accepted criteria and the exclusion of other pruritic diseases (Table 1).^{30–32} A minimum canine atopic dermatitis extension and severity index (CADESI) score was not required for entry to enable the trial population to represent the full spectrum of cAD and not just severe or very chronic cases.

Prior to enrolment and during the study, all dogs and other dogs and cats in the household, underwent stringent monthly flea control with lufenuron at approximately 10 mg kg⁻¹ (Program®, Novartis Animal Health Inc., Basel, Switzerland) and fipronil at 7–10 mg kg⁻¹ (Frontline®, Merial SA, Toulouse, France).

To prevent early drop out as a result of the development of secondary pyoderma, all dogs received oral cefalexin, 20–30 mg kg⁻¹ twice daily for 4 weeks.

Treatment

Each dog received an intradermal injection of 0.1 mL of a 10-mg mL⁻¹ suspension of heat-killed *M. vaccae* (SRL172) or a buffer solution (placebo) when they entered the study (visit 1). This dose was advised by the manufacturer and was based on toxicity and efficacy studies in humans and mice. Treatment and placebo vials were randomised in blocks of four and numbered continuously. Patients were allocated the next available treatment number sequentially. As the placebo was visually different from the *M. vaccae* suspension, the injection was given by an independent person ('medicator'), who at subsequent visits was responsible for examining the injection site and covering it prior to handing the dog over to a designated overall investigator for clinical evaluation. This precaution was taken because in preliminary tolerability studies (M.E. Ricklin Gutzwiller, unpublished data), a transient, nonpainful, single nodular swelling, which healed spontaneously, occurred at the injection site following the intradermal administration of *M. vaccae*.

Clinical assessment

At each visit, a physical examination was performed, and skin lesions were recorded using the CADESI.³³ As pruritus is a very important feature of AD and a major concern for dog owners its assessment, even though necessarily subjective, is nonetheless extremely relevant and owners assessed its intensity [owner's pruritus score

Table 1. Entry criteria

Inclusion criteria

1. Healthy dogs (apart from the skin problems) between 6 months and 8 years old
2. Clinical diagnosis of atopic dermatitis (Willemse,³⁰ Prélud *et al.*,³¹ and DeBoer and Hillier,³²)
3. Lack of success of a novel home-cooked or hydrolysed diet during at least 6 weeks
4. No response to flea control for at least 6 weeks previously
5. Antimicrobial or other shampoos and ear cleaners devoid of corticosteroids were allowed if not used more frequently than once a week and if frequency did not change during the study period
6. Owner's informed consent in writing

Exclusion criteria

1. Clinical evidence of ectoparasite infestation
2. Clinical evidence of active bacterial or fungal cutaneous infection
3. Flea bite hypersensitivity (flea-specific IgE ELISA and/or positive intradermal allergen test or lesions located in the dorso-lumbar region or at the tail base)
4. Oral treatment of steroids or cyclosporin within the previous 4 weeks
5. Use of long-lasting injectable corticosteroids within the previous 6 weeks
6. Topical corticosteroids or steroid ear treatment within the previous 2 weeks
7. Ketoconazole treatment within the previous 2 weeks
8. Unsuccessful immunotherapy (allergen-specific immunotherapy); if there was no improvement: inclusion 6 months after the last therapy
9. Use of traditional Chinese medicines, homeopathy, supplementary essential fatty acids (dietary and topical)
10. If seasonal dermatitis: expected end of symptoms during the study
11. Vaccination within 4 weeks prior to the inclusion date and during the trial
12. Pregnant or lactating females

(OPS)] using a visual analogue scale, where 0 equalled to 'no pruritus' and 10 equalled 'as severe as possible'. The overall response or 'global assessment' (GA) was a joint assessment by the owner and the investigator comparing the status at each visit to baseline (study beginning). Assessment: excellent (0), good (1), fair (2), poor (3), and none (4).

The following support/rescue medication was allowed: one additional course of oral antibiotics or an additional weekly treatment with a chlorhexidine shampoo. The need for such rescue medication was recorded at each visit. This need and drop out from the trial were used as secondary endpoints in the evaluation of efficacy.

Statistical analysis

Summary statistics were calculated for demographic, baseline and efficacy parameters. Demographic and baseline parameters were compared between the two groups, using the nonparametric Mann-Whitney *U*-test. For efficacy parameters, ANOVA with repeated measurements was performed with the following model effects: treatment group, visit number, treatment group by visit number interaction, baseline values and subject as random effect.

Based on initial results, a second analysis was performed using additional model effects, such as degree of disease and degree of disease by treatment interaction. For this second analysis dogs were divided in two groups: those with a CADESI score < 60 and those with a CADESI score ≥ 60. Within each group the treatment group and the placebo group were compared. Based on previous in-house trials (W. Seewald, unpublished data), it was determined that, with 30 dogs in each of the two groups, the statistical power of 95% would be sufficient to detect a true group difference in CADESI scores of at least 30%, assuming a standard deviation of 30% or less. The level of significance was chosen to be 0.05.

A paired-sample Wilcoxon test was used to compare data at the end of the study with baseline values for all animals. As the main goal of this pilot study was to assess clinical efficacy 3 months after injection, no time-point analyses were undertaken for visits 2 and 3.

Dogs that did not complete the trial were still included in the analysis and their values used as long as data were available. For summary statistics and graphics, data were extrapolated (last observation carried forward) after drop out.

As the distribution of the CADESI scores was skewed, logarithmic transformation was applied, to provide a more symmetric distribution and better approximation to normality, therefore facilitating analysis. All calculations were carried out using SAS®, version 8.2 (SAS Institute, Cary, NC, USA).

Results

Patients

The trial was undertaken between February 2004 and April 2005 with, for the final analysis, 29 dogs in the treatment group and 33 in the placebo group. The composition of the two groups at baseline (visit 1 – Table 2) shows a good match regarding sex, age at onset of the disease, duration of signs, OPS and CADESI score.

Clinical scores

First analysis of all 62 dogs

At the end of the study (visit 4) the means of the parameters assessed, i.e. CADESI score, OPS and global assessment (GA), were lower in the treatment group than in the placebo

Table 2. Baseline characteristics of canine atopic dermatitis dogs

Characteristic	SRL 172	Placebo	P-value
Dogs included in analysis	29	33	
Dogs with intention-to-treat data	9	13	0.60
Age at onset of disease (years); mean ± SD	2.24 ± 1.86	2.2 ± 1.36	0.84
Male, <i>n</i> (%)	15 (52)	17 (52)	1
Duration of signs at study begin (years); mean ± SD	2.02 ± 1.25	2.23 ± 1.36	0.7
Owner's pruritus score; mean ± SD	5.93 ± 2.87	5.37 ± 2.53	0.4
CADESI score; mean ± SD	58.38 ± 44.83	55.52 ± 37.36	0.9

Table 3. Means and standard deviations at baseline and at the end of the study. *P*-values demonstrate the differences between the two groups

Response		<i>M. vaccae</i> : mean (SD)		Placebo: mean (SD)		<i>P</i> -value‡
		Visit 1	Visit 4	Visit 1	Visit 4	
CADESI	All dogs	58.38 (44.83)	39.38 (40.55)	55.52 (37.36)	43.61 (41.49)	0.13
	< 60*	33.26 (13.28)	21.42 (22.23)	35.41 (14.68)	30.14 (22.23)	0.008
	= 60†	106.1 (45.14)	73.5 (46.41)	95.73 (36.68)	70.55 (57.21)	0.29
OPS	All dogs	5.93 (2.87)	3.95 (3.24)	5.37 (2.53)	4.83 (3.48)	0.43
	< 60*	5.18 (2.85)	3.01 (2.79)	5.58 (2.63)	5.38 (3.54)	0.04
	= 60†	7.35 (2.44)	5.75 (3.4)	4.92 (2.36)	3.62 (3.21)	0.12
GA	All dogs		2.11 (1.05)		2.67 (1.14)	0.047
	< 60*		1.88 (0.99)		2.77 (1.15)	0.001
	= 60†		2.5 (1.08)		2.45 (1.13)	0.32

*Dogs with a baseline CADESI of below 60.

†Dogs with a baseline CADESI of 60 or higher.

‡Significant *P*-values are in bold.

CADESI, canine atopic dermatitis extension and severity index, GA, global assessment; OPS, owner's pruritus scores.

group (Table 3). However, the difference between the two groups at the end of the study was only significant for the GA group (ANOVA, $P = 0.047$). The use of rescue medication did not show a significant difference between the treated and the placebo group ($P = 0.266$, Wilcoxon); 14 treated and 15 placebo dogs needed rescue medication. Sex of the dogs did not have an influence on the model.

By visit 4 the CADESI scores in the treatment and placebo groups had decreased by 33% and 22%, respectively, compared to visit 1. Both of these reductions were significant with *P*-values (Wilcoxon) of 0.0002 and 0.0087, respectively.

The OPS in the treated dogs also decreased from a mean of 5.93 at baseline to a mean of 3.95 at the end of the study, statistically a significant reduction by 33% ($P = 0.0043$, Wilcoxon). In the placebo group OPS decreased by 10% only, which is a nonsignificant reduction ($P = 0.44$).

Second analysis

Results for dogs with an initial CADESI of < 60

Forty-one of the 62 dogs enrolled in the study showed mild to moderate clinical signs (19 treated and 22 placebos) with an initial CADESI score of below 60.

At visit 4, all efficacy parameters (CADESI, OPS and GA) were significantly improved in the treatment group compared to the placebo group (ANOVA, $P = 0.008$, 0.04 and 0.001, respectively, Table 3). Between visits 1 and 4 the treated dogs improved by 36% ($P = 0.004$, Wilcoxon) in CADESI scores, whereas the placebo groups improved by 15% only ($P = 0.082$, Wilcoxon).

The OPS decreased by 42% in the treated dogs compared to baseline ($P = 0.016$, Wilcoxon). For comparison, the decrease in the placebo dogs was only 3.6% ($P = 0.11$, Wilcoxon).

In the treatment group, eight dogs needed rescue medication, compared to nine in the placebo group. This difference is not significant ($P = 0.419$, Wilcoxon).

Results for dogs with an initial CADESI score of = 60

This subpopulation comprised 21 dogs with moderate to severe clinical signs. In this subset of data, there were no significant differences between the treatment and the placebo groups (ANOVA, Table 3).

When the scores at the end of the study were compared to the baseline values, only the CADESI score of the treatment group was significantly reduced by 30% ($P = 0.037$,

Wilcoxon), while the corresponding decrease of 26% in the control group was not significant ($P = 0.067$, Wilcoxon).

At visit 4, the OPS had not decreased significantly compared to baseline in either the treated or the placebo dog groups ($P = 0.23$ and 0.19, respectively, Wilcoxon). The use of rescue medication was not significantly different between the two groups ($P = 0.567$, Wilcoxon).

Adverse reactions

In five dogs of the treatment group, a slight erythema and nonpainful swelling was seen at visit 2 at the injection site; this healed without treatment. No other adverse reactions related to the injection procedure were observed during the course of the study.

Discussion

In mild to moderate cases of canine atopic dermatitis, a single injection of a heat-killed *M. vaccae* suspension resulted in statistically significant improvement in CADESI scores, OPS and GA 3 months later; the pertinent scores of the treated dogs were always lower than those of controls. This treatment did not, however, have any significant effect on severely affected dogs, a difference that could be due to more complex local inflammatory skin reactions triggered by self-trauma and secondary microbial colonization in this latter group.

Treatment with *M. vaccae* can be considered as safe and free of side-effects at the dose used; toxicity and dose titration studies in dogs, rats and mice demonstrated increased electrolyte secretion and reduced spontaneous locomotor activity, but only at very high concentrations (100 times the dose used). There was no information available on the optimum dose for dogs, and dose titration was well outside the scope of this study. The dogs were observed for 3 months after a single injection because a similar study in children with AD reported significant improvement at 1 and 3 months,²⁶ although a later study that measured the effects up to 6 months found no significant improvement compared to the placebo group.³⁴ A higher or lower dose of *M. vaccae* and repeated treatment could have been more efficacious and further studies are required to investigate this issue.

The strong placebo effect was unexpected and might be explained by, either the strict flea control measures, or the administration of oral antibiotics during the first

4 weeks of the study. There is clinical and experimental evidence that secondary bacterial infections can induce, increase and maintain the clinical signs of cAD.^{35–37}

Therefore antibiotic treatment might be responsible for the observed placebo effect, although how long any beneficial effect of such therapy may have persisted and how it may have influenced the inflammatory reaction in the skin are unknown. Although only dogs without clinical signs of secondary infections (bacterial or yeast infections) were enrolled, an effect of antibiotic administration on microbial colonization cannot be excluded. Antibiotic administration was permitted because results from trials in children²⁶ and in mice³⁸ indicated that this therapy could take up to 1 month to be effective. As this was a long time to leave dogs untreated, they were given antibiotics to prevent recurrence of bacterial overgrowth and exacerbation of clinical signs. Indeed bacterial overgrowth may remain unidentified as the clinical signs are those of cAD and do not include pustules and collarettes as in pyoderma. A strong placebo effect was also observed in young children with AD,³⁴ where a substantial part of this effect was claimed to be because of spontaneous resolution, a phenomenon frequently seen in children.³⁹ Thus spontaneous healing cannot be excluded in dogs, albeit that age-related healing is not a recognized feature of cAD.

The CADESI scoring system is widely used in veterinary medicine.^{33,40} Recent validation of this index showed an acceptable degree of reproducibility⁴¹ although investigator differences were observed. No such differences occurred in the present study.

The 36% reduction in mean CADESI score in mild to moderate cases was lower than the improvement reported in children.²⁶ However, a direct comparison with the human studies is difficult because of procedural differences in the assessment of disease severity.

The mechanism behind the improvement in mildly affected dogs is still unknown.

However, oral administration of specific antigens to allergy-prone puppies induced IL-10 and TGF- β expression and prevented allergy.⁴² This limited information suggests that dogs may also possess a regulatory network, involving the regulatory T cells (Treg) described in the introduction, that could be responsible for the benefit observed.

The reported improvement is less than that seen with standard corticosteroid, cyclosporin and ASIT treatments but the only side-effect in this instance was a transient reaction at the injection site in five of 64 dogs that healed uneventfully. This is a significant benefit as corticosteroids and cyclosporin can both have undesirable side-effects and require daily administration, and ASIT provides variable results from a rather complicated therapeutic programme.

In conclusion, the benefit reported here, though modest, is clinically significant, can be elicited by a single injection, is demonstrable for 3 months and has potential for treatment of mild to moderate cases of cAD. The effects of more frequent injections and a longer treatment period remain to be explored.

Acknowledgements

The authors are grateful to Rolf Steiger for critically reading the manuscript and to SR Pharma for support and for kindly providing the product samples.

They thank the following veterinarians for their collaboration: Luc Beco, Emmanuel Bensignor, Beat Bigler, Claude Favrot, Jacques Fontaine, Eric Guaguère, Dominique Heripret, Martin Keiser, Didier Pin, Pascal Prélaud, Christine Rivierre and Marc Vingerhoets. Grateful thanks also to Fabienne Brovedani, Catherine Rudaz and Sibyll Buob for their excellent technical support.

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Résumé La dermatite atopique canine (cAD) est une maladie fréquente dont l'étiologie est multifactorielle, associée à une immunorégulation anormale. Sa pathogénie a des ressemblances avec celle de la dermatite atopique de l'homme. Les signes cliniques des maladies allergiques chez l'homme et la souris sont diminués par l'administration de mycobactéries saprophytes qui amplifient les cytokines régulatrices et c'est pourquoi l'effet de *Mycobacterium vaccae* (*M. vaccae*) a été testé sur la sévérité de la cAD. Soixante deux chiens à cAD, sélectionnés selon des critères stricts ont été traités avec une seule injection intradermique et évalués tous les mois pendant trois mois dans un essai en aveugle contrôlé par placebo. La sévérité clinique a été jugée en utilisant des scores standardisés et en se basant sur l'évaluation du prurit par le propriétaire. Une simple injection de la suspension de *Mycobacterium vaccae* s'est avérée bien tolérée et efficace pour traiter les cas peu évolués, mais inefficace pour les chiens plus sévèrement atteints.

Resumen La dermatitis atópica canina es una enfermedad bastante frecuente con una etiología multifactorial asociada con una inmunoregulación alterada. La inmunopatogenesis tiene similitudes con la dermatitis atópica humana. Los signos clínicos de la enfermedad alérgica en humanos y ratones se reducen tras la administración de micobacterias saprofíticas, las cuales amplifican la producción de citoquinas reguladoras, y por ello investigamos el efecto de *Mycobacterium vaccae* en la intensidad clínica de la dermatitis atópica canina. Sesenta y dos perros con dermatitis atópica, seleccionados siguiendo estrictos criterios, se trataron con una sola inyección intradérmica y se evaluaron mensualmente durante tres meses en una prueba doble ciego controlada por placebo. La severidad de los signos clínicos se cuantificó usando métodos de puntuación estandarizados así como mediante la evaluación del prurito por el dueño. Una sola inyección de una suspensión de *M. vaccae* destruidas por calor fue bien tolerada y efectiva en el tratamiento de casos leves a moderados en los tres meses de estudio, pero no tuvo ningún efecto en los perros con un proceso muy severo.

Zusammenfassung Die canine atopische Dermatitis (cAD) ist eine häufige Erkrankung mit multifaktoriellen Ursachen, die mit einer beeinträchtigten Immunregulierung einhergeht. Die Immunpathogenese zeigt Ähnlichkeiten zu jener der humanen atopischen Dermatitis. Die klinischen Zeichen der allergischen Erkrankung werden beim Menschen und bei Mäusen durch die Verabreichung von saprophytischen Mykobakterien, welche die regulatorischen Zytokine vervielfältigen, reduziert. Daher wurde die Auswirkung von *Mycobacterium vaccae* (*M. vaccae*) auf den klinischen Schweregrad von cAD untersucht. Zweiundsechzig Hunde mit cAD, welche nach strikten Kriterien ausgesucht worden waren, wurden mit einer einzigen intradermalen Injektion behandelt und monatlich für die Dauer von 3 Monaten in einer Placebo-kontrollierten klinischen Doppelblindstudie evaluiert. Die klinischen Schweregrade wurden mittels standardisierten Werten quantifiziert sowie durch die Beurteilung des Juckreizes durch den Besitzer. Es wurde festgestellt, dass eine einzige Injektion einer durch Hitze getöteten Suspension von *Mycobacterium vaccae* gut toleriert wurde und bei der Behandlung von milden bis moderaten Fällen von cAD innerhalb von 3 Monaten wirksam war, allerdings bei stärker betroffenen Hunden eine nicht signifikante Wirksamkeit zeigte.