Epicutaneous immunotherapy as a new hope for canine atopic dermatitis: a proof-of-concept study

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Abstract

Background – Allergen immunotherapy is a well-established treatment for canine atopic dermatitis (CAD), but non-invasive, safe, effective, and easy-to-use home-administration routes that promote owner's compliance are needed. Epicutaneous immunotherapy (EPIT) has been suggested as a promising alternative treatment for human allergies. This study primarily evaluated EPIT's feasibility, effectiveness, and safety for CAD. **Methods** – Sixteen client-owned dogs with spontaneous, nonseasonal, mite-sensitive CAD were enrolled for a 6-month, once-weekly, 12-hour EPIT. A costume-made 3D-printed device was designed to deliver the allergen-based formulation. Primary efficacy outcomes included the owner's assessed pruritus (PVAS10) and treatment efficacy (OGATE), and veterinarian-assessed skin lesions (2D-IGA). Secondary efficacy outcomes were the quality-of-life (QoL) and serological allergen-specific IgE's concentrations. Effectiveness was defined by the success of the primary efficacy outcomes, according to the ICADA's COSCAD'18 recommendations. EPIT was deemed safe in the absence of severe side-effects. **Results** – EPIT effectively improved clinical condition, with a success rate of 73.3% for pruritus, 66.7% for skin lesions, and 93.3% for QoL. A good-to-excellent response to EPIT was rated by 93.3% of owners in OGATE. EPIT significantly improved PVAS10 (p=0.000015), 2D-IGA (p=0.006) and QoL (p=0.000014) scores over six months. A significant difference was evident within one month for PVAS10 (p=0.003) and 2D-IGA (p=0.009) scores. Seven dogs partially desensitised to at least one mite and two fully desensitised to all mites after six months. Severe adverse events were not recorded. **Conclusions** – This pioneer study emphasises EPIT's potential as a novel and promising, non-invasive, feasible, effective, safe, and well-tolerated CAD treatment, supporting further investigation.

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ABSTRACT: 248 words

Background – Allergen immunotherapy is a well-established treatment for canine atopic dermatitis (CAD), but non-invasive, safe, effective, and easy-to-use home-administration routes that promote owner's compliance are needed. Epicutaneous immunotherapy (EPIT) has been suggested as a promising alternative treatment for human allergies. This study primarily evaluated EPIT's feasibility, effectiveness, and safety for CAD.

Methods – Sixteen client-owned dogs with spontaneous, nonseasonal, mite-sensitive CAD were enrolled for a 6-month, once-weekly, 12-hour EPIT. A costume-made 3D-printed device was designed to deliver the allergen-based formulation. Primary efficacy outcomes included the owner's assessed pruritus (PVAS10) and treatment efficacy (OGATE), and veterinarian-assessed skin lesions (2D-IGA). Secondary efficacy outcomes were the quality-of-life (QoL) and serological allergen-specific IgE's concentrations. Effectiveness was defined by the success of the primary efficacy outcomes, according to the ICADA's COSCAD'18 recommendations. EPIT was deemed safe in the absence of severe side-effects.

Results – EPIT effectively improved clinical condition, with a success rate of 73.3% for pruritus, 66.7% for skin lesions, and 93.3% for QoL. A good-to-excellent response to EPIT was rated by 93.3% of owners in OGATE. EPIT significantly improved PVAS10 (p=0.000015), 2D-IGA (p=0.006) and QoL (p=0.000014) scores over six months. A significant difference was evident within one month for PVAS10 (p=0.003) and 2D-IGA (p=0.009) scores. Seven dogs partially desensitised to at least one mite and two fully desensitised to all mites after six months. Severe adverse events were not recorded.

Conclusions – This pioneer study emphasises EPIT's potential as a novel and promising, non-invasive, feasible, effective, safe, and well-tolerated CAD treatment, supporting further investigation.

Keywords: Allergen immunotherapy; atopic dermatitis; canine; epicutaneous immunotherapy (EPIT); vaccine.

TEXT: 3500 words

INTRODUCTION

Canine atopic dermatitis (CAD) is one of the most prevalent (20-30%), distressing chronic allergic skin diseases in the developed world, accounting for substantial loss of quality-of-life (QoL)[1,2]. CAD's remarkable

similarities to its human counterpart make it a suitable animal model for human atopic dermatitis (AD)[3]. While being a multifactorial disease, its genetic component renders CAD prevalence distinctly high in certain breeds, notably the French bulldog and Labrador retriever[4]. CAD's complex and heterogeneous nature is often an obstacle to achieving therapeutic success.

Allergen immunotherapy (AIT) is the only aetiological CAD treatment capable of reversing the long-term syndrome's pathogenesis, inducing a clinical remission state, and preventing new sensitizations[5-10]. Ultimately, this targeted therapy aims to improve the patient's clinical condition and QoL, re-educating the immune system into a desensitised/tolerogenic state that should persist after treatment discontinuation[5,6]. AIT value for CAD is supported by several studies, reporting success rates between 50% and 80%[10,11-18]. Moreover, the updated International Committee on Allergic Diseases of Animals' (ICADA) guidelines for treating CAD recognise AIT as an effective and safe therapy, despite the evidence being based on inconsistent patient-oriented uncontrolled studies[19]. Among others, AIT's success depends on the protocol and administration route. Despite the well-established success of subcutaneous immunotherapy (SCIT) for several allergic diseases, human patient and pet-owner compliance is a major challenge[5,20-22]. The large number of injections and, although rare, the risk of severe systemic reactions due to allergen leakage into the bloodstream account for SCIT dropout[5,23,24]. Although with still limited evidence and unclear success rate[5], sublingual immunotherapy (SLIT) has been seen as a convenient patient-friendly alternative route, as it dismisses the use of needles, facilitates administration, and is associated with fewer systemic reactions[5,16,21,22,24-26]. Nevertheless, the lengthy treatment time, delay until clinical improvement, perceived investment, and, for SLIT, the required daily administration justifies the low compliance for SCIT and SLIT, reported in both human and veterinary fields[5,16,21,22,24-26]. There is, therefore, a need to uncover new AIT modalities that address the raised challenges. Accordingly, intralymphatic immunotherapy (ILIT) route has recently emerged for CAD treatment, providing encouraging initial evidence of safety and efficacy[11,17,18]. However, relapse of CAD clinical signs after ILIT discontinuation is frequent, and its procedure requires trained personnel[5,6,10,17].

The skin has become a new direction for vaccination against infectious diseases and cancer in people[27-30]. As a physiological site of allergen encounter, the skin is endowed with robust immune surveillance provided by a dense population of Langerhans cells (LC) and dermal dendritic cells with unique immunological features[30-35]. Moreover, as the epidermis is nonvascularised, this route should carry less risk of systemic allergic side-effects[20,21,31,32]. Epicutaneous immunotherapy (EPIT) has recently regained attention in human medicine as a new promising route of tolerance induction against allergens[31,32,35-38]. The protolerogenic properties of the skin sophisticated immune network underpin EPIT's favourable efficacy and safety profile, which is linked to strong patient compliance, even in children and long-term clinical trials[31,32,35-38].

To the authors' knowledge, this is the first clinical trial addressing EPIT for CAD. Our primary aim was to assess EPIT's feasibility, effectiveness, and safety in dogs with spontaneous, nonseasonal, mite-sensitive CAD over six months. Secondary aims were to ascertain owners' adherence to therapy and to assess whether there was a breed effect in EPIT efficacy.

MATERIAL AND METHODS

Ethics

The project was approved for ethical standards by *Comissão de Ética e Bem-Estar Animal* (016/2020), Faculty of Veterinary Medicine, University of Lisbon, Portugal. All owners gave written informed consent for study participation and were free to withdraw at any time. Owner's confidentially was ensured by a system of assigning a number to each dog, concealing the public disclosure of personal details. All procedures were conducted according to national legislation and animal welfare standards.

Patient enrolment

Client-owned dogs, of two predisposed breeds (French bulldog, Labrador retriever) with spontaneous, non-seasonal, mite-sensitive CAD were enrolled after owner consent. CAD diagnosis was based on compatible history and clinical signs, exclusion of resembling/overlapping skin diseases, and positive allergen-specific

IgE serology for domestic mites (>150EA Units, LETIPharma S.L.U., Barcelona, Spain).

Concomitant positive IgE serology for seasonal allergens was accepted, as the symptomatology was perennial, and, therefore considered minor/subclinical. Dogs with concurrent adverse food reaction were included, if controlled, and the diet was unchanged throughout the study. A veterinarian-approved flea control regimen must have been ongoing. Concomitant antipruritic medications were allowed, including systemic/topical steroids and oclacitinib, if given for at least four weeks, and ciclosporin/lokivetmab, if given for at least eight weeks, at unchanged doses before inclusion. Introduction of new treatments was not allowed throughout the study, except for focal application of topical antibacterial and antiseptic products. Essential fatty acid supplements were permitted if ongoing for at least four weeks before inclusion.

Exclusion criteria included prior treatment with AIT, parasitic infestation, uncontrolled bacterial/yeast infection, concomitant disease unrelated to CAD, or pregnant/lactating dogs.

Study withdrawal was mandatory for unacceptable adverse reactions and poor owner compliance.

Study design

This study was designed as a six-month, proof-of-concept, open clinical trial, comprising a once weekly, 12-hour application of a total of 24 EPIT patches.

Patch design

A 3D-printed allergen-delivery device, comprising a 3x5x1 cm open-sided chamber[31] (1.5 mL volume) was custom-made for this study (figure 1), using Blocks One MKII printer (Blocks, Amadora, Portugal). The 3D prototype design used a cloud-based 3D modelling, computer-aided design and manufacturing software platform, FUSION 360 (Autodesk, Inc., California, U.S.A.). The device's shape and size were tailored to cover a large body surface area, as this proved to increase allergen capture by skin dendritic cells and their migration to lymph nodes[39]. It was also necessary to ensure the feasibility of patch application with respect to the dog's body size and shape and owners' constraints regarding the dog's aesthetics. A 1.75mm polylactic acid filament was used, as it is a biodegradable, eco-friendly, affordable material with favourable biocompatibility and safe degradation products.

Allergen-based formulation

LETIPharma S.L.U. has fully supplied the lyophilised mite-allergen extracts required (*Dermatophagoides farinae* ,*Dermatophagoides pteronyssinus, Acarus siro* , *Tyrophagus putrescentiae* , *Lepidogliphus destructor*). Glycerine was used as a solvent, given its ability to retard allergen potency loss. A tailor-made polyethylene glycol-based vehicle was specially designed for this study, given its biocompatibility, well-established safety, bioavailability, and stabilising, penetration enhancing and controlled-release carrier effects[40]. A dose of 100 HEP/mL was chosen as it induced the strongest therapeutic effect and achieved statistical significance in a double-blind, placebo-controlled, dose-escalation human trial[35]. Nevertheless, a lower dose (50 HEP/mL) was selected for the first application. Allergen-based formulations were custom-produced for each dog every two-weeks, according to their IgE serology panel.

Pre-clinical testing in healthy dogs

A small pre-clinical trial was conducted on four healthy dogs to preliminary assess the feasibility and local tolerability of normal skin to both doses (50 and 100 HEP/mL). We hypothesised that if skin irritation occurred in healthy skin, a more pronounced reaction would be expected in atopic skin, so lower doses would have to be considered. Owners were asked to perform a single 12-hour application of both doses in side-by-side patches, following the protocol described below. Both doses were well-tolerated, and the protocol was deemed feasible.

Trial protocol

At the initial visit, dog-owners were informed about the study protocol, while the first patch was applied in their presence. For subsequent patches, owners were free to choose between home-application or application

by the dermatology team at the study institution. Educational infographics were provided to all owners: a study summary and a detailed 4-step EPIT application guide to ease home-application.

Briefly, an area of skin (about 10x10 cm) from the dog's mid-anterior chest was clipped and tape-stripped 15 times[41], ensuring complete but gentle removal of the *stratum corneum* , which promotes skin allergen penetration and dendritic cells' activation[20,31,32,35,41]. The allergen-containing device was centred on a hypoallergenic adhesive (Bastos Viegas S.A., Penafiel, Portugal) and placed on the tape-stripped skin once weekly, for 12 hours, for six months. Finally, all dogs were clothed with a protective bodysuit to support patch fixation and prevent its removal during routine activities (figure 1).

Clinical evaluations

EPIT safety evaluation considered both local and systemic adverse reactions reported weekly by owners. Primary and secondary efficacy outcomes were established. Figure 2 represents the evaluation timeline.

Primary outcomes:

- Owner-assessed pruritus manifestations (validated pruritus visual analogue scale - PVAS10)[42,43].
- Veterinarian-assessed skin lesions (validated 2D investigator's global assessment graphic - 2D-IGA)[44].
- Owner global assessment of treatment efficacy (OGATE)[45].

Secondary outcomes:

Owner-reported QoL survey[46].

Allergen-specific IgE serology (LETIPharma S.L.U.).

Outcomes' success criteria

As a therapeutic clinical trial, enrolling dogs with AD, EPIT efficacy was based on primary outcomes' success, following the ICADA's COSCAD'18 recommendations[45]. Nevertheless, some considerations were addressed so that the defined criteria covered all dogs' clinical situation. Therefore, for dogs with an initial moderate-to-severe AD, success was defined as the percentage of dogs achieving the normal-to-mild range at six months, and for dogs with an initial mild AD, returning to the normal status[45]. Exceptionally, maintenance of lesional score at six months was considered successful, for dogs with an initial normal lesional status, but a mild-to-severe pruritus. OGATE was successful when rated as good-to-excellent.

Secondary outcomes' success was defined as the percentage of dogs with improved QoL classification at six months (i.e. decreased QoL score), and, for allergen-specific IgE serology, the percentage of dogs that fully or partially desensitised to allergens.

EPIT was deemed safe if no systemic or severe local adverse events occurred.

Owner's adherence considered protocol compliance and attendance at study appointments.

Statistical methods

Data were processed using IBM® SPSS®Statistics (Version 27). A mixed-model ANOVA test was performed to analyse mean PVAS, 2D-IGA and QoL scores' evolution over time. P-values $<0{,}05$ were considered statistically significant.

RESULTS

Study design and partnership agreement were outlined between September 2019 and January 2020. Patients from the study institution were screened for participation between March-August 2020, alongside product design and manufacture. Sixteen dogs fulfilled the inclusion criteria, and the first owner's telephone interview was held in September 2020. Pre-clinical testing in healthy dogs occurred in early October 2020. The clinical trial ran from October 2020 to April 2021. Laboratory determinations and data analysis were subsequently undertaken.

Owner's compliance with EPIT

Sixteen client-owned dogs were recruited (9 French bulldogs and 7 Labrador retrievers). One Labrador retriever was lost to follow-up after one-month EPIT due to owner unavailability to meet protocol requirements, having been excluded from statistical analysis. All remaining 15 dogs and owners carefully complied with the proposed 6-month protocol.

Primary efficacy outcomes

There were no statistical differences between breeds for all the parameters evaluated.

After 6-month EPIT, all dogs had reduced pruritus scores, with a 63.1% mean improvement, and 13/15 dogs improved their severity level, while two dogs maintained their moderate-to-severe level. Of note, 4/8 dogs starting the study with a severe pruritus concluded the 6-month EPIT with a normal status (table 1). Moreover, all dogs showed improved pruritus scores (56.1% mean improvement) and severity level at three months, including the two dogs that maintained their initial moderate-to-severe level at six months, but were normal-to-mild at three months. According to the success criteria, 73.3% of dogs (11/15) succeeded in pruritus evaluation at six months.

Regarding skin lesions' evaluation, 9/15 dogs (60%) improved in severity after EPIT, with a 56.5% mean improvement, whereas 5/15 dogs (33.3%) maintained their initial normal (2/3) or mild (3/6) range. Furthermore, at both three- and six-month timepoints, all but one dog were in the normal-to-mild range, and no dog was severe (table 2). After EPIT, 66.7% of dogs (10/15) succeeded in skin lesion evaluation, considering the defined criteria.

Concerning OGATE, 14/15 owners rated the response to EPIT as good-to-excellent, ranking success as 93.3%, while the remaining owner assessed it as a fair response.

Over six months, EPIT significantly improved mean PVAS10 scores from 5.27±1.81 to 2.15±1.83 (p=0.000015), as that of 2D-IGA from 2.40±1.06 to 1.67±0.62 (p=0.006). Noteworthy, a significant improvement was evident after one month of treatment for both PVAS10 (p=0.003) and 2D-IGA (p=0.009) scores (table 3, figure 3).

Secondary efficacy outcomes

After 6-month EPIT, all but one dog, who maintained the low initial score (8 in 0-45 score range), improved their QoL scores, establishing a QoL success rate of 93.3% (14/15), with a 58.4% mean improvement. If a $>$ 50% QoL improvement is considered, 66.7% of dogs (10/15) reached this target at six months. Moreover, mean QoL scores significantly improved over six months (p=0.000014), being already evident at three months (p=0.000033) (table 3, figure 3). Notably, 13/15 dogs (86.7%) had improved QoL scores at 3-month EPIT (54.6% mean improvement).

Regarding allergen-specific IgE serology, 46.7% of dogs (7/15) were desensitised to at least one mite over six months, whereas 6/15 dogs (40%) had achieved it at three months. Noteworthy, 2/15 dogs (13.3%) fully desensitised to all mites at both 3-month and 6-month evaluations.

Safety profile and adverse reactions

EPIT was well-tolerated, and neither systemic or severe local reactions were recorded. All side-effects were local and self-limiting. Pruritus was the most frequently observed side-effect, reported by five owners. Of note, dogs were not used to wearing cotton bodysuits, so it is unknown whether it interfered with pruritus reaction. Non-pruritic erythematous reactions were observed under the patch in three dogs (two mild, one moderate) following the 50HEP/mL dose, and once in two dogs (both mild) with the 100HEP/mL dose (second application). One dog presented mild-grade pustules under the patch after two consecutive applications. No reactions required therapeutic intervention.

DISCUSSION

To the authors' knowledge, this was the first study to implement an EPIT protocol for allergens in veterinary medicine and to evaluate its impact on CAD treatment.

Inspired by encouraging human reports[27,29,31,32,35-38]and engaged with the idea of finding a practical, needle-free, owner- and pet-friendly AIT route, recognising the skin's unique immunological features, this pilot was designed as a proof-of-concept clinical trial, to primarily investigate the feasibility, effectiveness, and safety of EPIT in spontaneous, nonseasonal, mite-sensitive CAD. Although small and uncontrolled, this study demonstrates the safety and practicability of EPIT in dogs and provides promising initial evidence of its efficacy in CAD management.

Sixteen atopic dogs of two CAD predisposed breeds were recruited for once-weekly, 12-hour patch application over six months. The inclusion of two common breeds with high CAD prevalence was meant to avoid breed-related differences in treatment response that might exist if only one breed were selected. Conversely, including more breeds would require several sample groups and dogs, which at this research stage was not feasible. Therefore, French bulldogs, with an empirically assumed background of poor response to general CAD treatments, and Labrador retrievers, with a perceived good treatment response, were screened (unpublished data). The belief of a breed-related EPIT response was not confirmed as no statistical breed effect was observed for all parameters. As the immune response depends on the allergen contact time with skin's immune cells, and periods of 8 to 48 hours have proven efficacious in EPIT human studies for environmental allergies[31,32,35], 12 hours was deemed sufficient to trigger the desired immune response and plausible for application in dogs. Alike previous EPIT studies[31,32,35], a weekly protocol was applied.

Considering the AIT long treatment duration and delay until clinical improvement (typically three to twelve months)[7,8], six months could be insufficient to show significant effects. Strikingly, this study shows significant clinical improvement within only one month of EPIT, for both pruritus (p=0.003) and skin lesions' scores (p=0.009) (figure 3), supporting EPIT's potential to induce a rapid clinical response. Over six months, both PVAS and 2D-IGA mean scores decreased monthly (table 3), reaching a significant improvement at the study end (PVAS: p=0.000015; 2D-IGA: p=0.006) (figure 3).

Notwithstanding the more stringent outcome measures used in this study, compared with [?] 50% reduction of baseline pruritus/skin lesion scores[45], success rates of 73.3% and 66.7% were achieved, respectively, in the pruritus and skin lesion evaluation, after 6-month EPIT. Although the different outcome measures used in this study do not allow direct comparison with other canine AIT routes[11-18], the results of this study suggest a similar or even better response rate for EPIT, which aligns with previous EPIT human studies[31,32,35-38] and preliminary 3-month EPIT results on CAD[47].

Noteworthy, all dogs showed improved pruritus scores after both three (56.1% mean improvement) and six months (63.1% mean improvement). Additionally, after three months, all dogs had improved pruritus severity level, including the two that were initially moderate-to-severe at enrolment, normal-to-mild at three months, but moderate-to-severe again after six months. As the 6-month observation occurred in spring and one of these dogs was pollen sensitised, the acute CAD flare at six months may have been triggered by a clinical development of a seasonal CAD strand. For the other dog, there was no evidence of reported flare factors.

The success rate of skin lesions' outcome (66.7%) was slightly lower than that for pruritus (73.3%), which is coherent in a clinical context, as complete resolution of skin lesions can be hard to achieve and often requires prolonged treatment. Furthermore, 60% of dogs were normal-to-mild at the study start (table 2) and improving an already mild lesion condition is challenging. Nevertheless, at 6-month EPIT, 93.3% of dogs presented a normal-to-mild status (table 2).

After 6-month EPIT, 93.3% of owners rated the response to EPIT as good-to-excellent, which captures the owner's perceived EPIT benefit. While the protocol was time-consuming and required strong owner's commitment, which for dogs with a less tolerant character may have rendered weekly application more demanding, EPIT improved QoL for 93.3% of dogs and families (58.4% mean improvement), and for 66.7% of them by more than 50%. The significant decrease in mean QoL scores throughout six months (table 3,

figure 3) and the QoL improvement in 86.7% of dogs at 3-month EPIT also mirror the owner's perceived efficacy and reinforces EPIT's potential for rapid clinical response. As CAD meaningfully impacts the well-being of those involved, QoL assessment was a valuable research topic for this study.

EPIT was able to engage the immune system, producing IgE changes already noticeable at three months. Partial desensitisation to at least one mite occurred in 40% of dogs after three months and in 46.7% at 6-month EPIT, while full desensitisation to all mites was achieved by 13.3% of dogs at both timepoints. These findings are consistent with previous human[48,49,50] and canine[16,26,51]studies, reporting decreased serum IgE levels during AIT. Although no further conclusions could be drawn due to the wide IgE values' variation, the observed immune modulation suggests EPIT's objective effect. However, similarly to previous studies[26,51], mite desensitisation and IgE values' changes did not correlate with clinical improvement in this study, possibly due to CAD's complex and multifactorial nature.

EPIT was practicable, well-tolerated, and safe, as no systemic or severe local reactions were observed. Self-limiting local pruritus was the most common side-effect, alike reported in literature[5,6]. Even so, it remains unclear the bodysuit contribution for pruritus. Although recorded as adverse effects, local skin reactions under the patch indicate that EPIT was able to pulse and activate skin dendritic cells for antigen presentation.

Adherence to chronic therapeutics is a critical challenge for clinical research, especially for AIT, owing to high withdrawal rates[5,17,18,22,24]. We report a 93.8% adherence rate for EPIT, which is higher than that published for other routes[14,17,18,22,51]. Despite the encouraging compliance, this study only followed patients for six months. It is possible that longer trials would result in higher dropout rates.

This pioneer approach suggests that a simple, practical innovation as EPIT can be safe, effective, and improve AIT adherence, which may facilitate its future broad application.

Several limitations can be pointed to this study. Firstly, as a pilot trial, it was open-labelled, neither blinded nor placebo-controlled, as other dog studies[11,13,15,17,18,26]. We faced ethical concerns justifying a control group in a six-month study on a distressing and QoL-disruptive syndrome as CAD. Nevertheless, clinical assessment scales were used to minimise potential bias. Secondly, this was a small-scale study, and it is likely that six months cannot show EPIT's full clinical and immunological potential. Therefore, scientifically sound, prospective, larger, and longer-lasting trials are needed to confirm these findings and assess the long-term EPIT benefits. Thirdly, some dogs were on antipruritic medication with considerable effects on the immune system, which is controversial during AIT, despite poorly studied in dogs[6,18]. Medication-limiting criteria were set to minimise this interference in EPIT success evaluation, so changes in posology before and during EPIT or introduction of new generalised treatments were not allowed. Noteworthy, although focal treatment with topical products was allowed, only one dog required such intervention with an antibacterial product, due to localised pyodermitis. Thus, significant clinical improvement is unlikely to be due to concurrent medication. Moreover, we found ethically questionable to stop dogs' supportive medication, as they would suffer unnecessarily, and owner's compliance could be compromised. Finally, this study used a new validated skin lesions' globally assessment tool (2D-IGA), which overcomes certain limitations of other instruments[44]. However, to guide observers in their lesion extent evaluation, a German shepherd dog silhouette is supplied, which is less suitable for brachycephalic breeds as French bulldogs[44].

This proof-of-concept study demonstrates EPIT's feasibility, effectiveness, and safety in CAD treatment. Promising six-month results emphasise EPIT's potential as a non-invasive, safe, effective, compliance-enhancing, and at-home easy-to-use route. Encouraged by this pilot study, a novel, design- and formulation-improved EPIT project is underway.

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TABLES

Table 1. Dogs' distribution among the pruritus severity levels before and after EPIT.

PVAS10	**PVAS10**	**Before EPIT**	**Before EPIT**	**After 6-month EPIT**	**After 6-month EP**
Severity level	Value range	Absolute Frequency	Relative Frequency	Absolute Frequency	Relative Frequency
Normal dog	0.0 – 1.9	0	0%	10	66.7%
Mild CAD	2.0 – 3.5	2	13.3%	1	6.67%
Moderate CAD	3.6 – 5.5	5	33.3%	4	26.7%
Severe CAD	5.6 – 10.0	8	53.3%	0	0%

CAD – Canine Atopic Dermatitis.

EPIT – Epicutaneous Immunotherapy.

PVAS10 – Validated owner-assessed 10-cm Pruritus Visual Analog Scale for pruritus evaluation.

Table 2. Dogs' distribution among the skin lesions' severity levels before and after EPIT.

2D-IGA	2D-IGA	Before EPIT	Before EPIT	After 6-month EPIT	After 6-month EP
Severity level	Value range	Absolute Frequency	Relative Frequency	Absolute Frequency	Relative Frequency
Normal dog	0 or 1	3	20%	6	40%
Mild CAD	2	6	40%	8	53.3%
Moderate CAD	3	3	20%	1	6.67%
Severe CAD	4	3	20%	0	0%

CAD – Canine Atopic Dermatitis.

EPIT – Epicutaneous Immunotherapy.

2D-IGA – Validated 2D Investigator's Global Assessment graphic for grading the extent and severity of skin lesions.

Table 3. Clinical data of dogs at the study start (0 months) and at 1-, 2-, 3-, and 6-month EPIT evaluations.

Clinical evaluation	0 months	1-month EPIT	2-month EPIT	3-month EPIT	6-month EPIT
PVAS10 (mean ± S.D.)	5.27 ± 1.81	3.52 ± 2.15*	2.82 ± 1.50*	2.27 ± 1.34*	2.15 ± 1.83*
2D-IGA (mean ± S.D.)	2.40 ± 1.06	2.00 ± 1.00*	1.60 ± 0.74*	1.53 ± 0.64*	1.67 ± 0.62*
QoL (mean ± S.D.)	16.60 ± 6.23	-	-	8.53 ± 4.03*	6.67 ± 2.74*

PVAS10 – Validated owner-assessed 10-cm Pruritus Visual Analog Scale for pruritus evaluation.

2D-IGA – Validated 2D Investigator's Global Assessment graphic for grading the extent and severity of skin lesions.

QoL – Quality-of-life survey.

Significant difference from Month 0 at *P<0.01, mixed-model ANOVA test.

FIGURE LEGENDS

Figure 1. 3D patch prototype (left) and patch application on an atopic dog (original photos).

Footnote:

Mm – millimetre; cm – centimetre.

Figure 2. Timeline of the clinical assessments performed over the six months.

Footnotes:

PVAS10 – Validated owner-assessed 10-cm Pruritus Visual Analog Scale for pruritus evaluation.

2D-IGA – Validated 2D Investigator's Global Assessment graphic for grading the extent and severity of skin lesions.

QoL survey – Quality-of-life survey.

OGATE – Owner Global Assessment of Treatment Efficacy survey.

Figure 3. Evolution of pruritus (PVAS10), skin lesions (2D-IGA), and QoL throughout the six months (boxplots).

Footnotes:

PVAS10 – Validated owner-assessed 10-cm Pruritus Visual Analog Scale for pruritus evaluation.

2D-IGA – Validated 2D Investigator's Global Assessment graphic for grading the extent and severity of skin lesions.

QoL – Quality-of-life survey.

Significant difference from Month 0 at *P<0.01, mixed-model ANOVA test.