

# Evaluation of a protocol combining applications of Ophytrium-based shampoo and mousse in dogs with sensitive, itchy and irritated skin

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## Research Article

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# Abstract

Topical formulations are key when managing skin conditions as they bring ingredients directly to the target organ. The objective of the study was to evaluate the effect of a protocol combining applications of Ophytrium-based shampoo and mousse (DOUXO® S3 CALM) in atopic dogs presenting with a skin flare. Thirty-four atopic dogs were included in the analysis of this prospective, multicentric European and US field study. Dogs received a shampoo application on D0 then mousse applications every 48–72 hours for three weeks. CADESI-04 scores were assessed by a dermatologist and pruritus by the owners (Pruritus Visual Analogue Scale score (PVAS)) on a weekly basis. On D21, veterinarian and pet owner evaluations were collected through questionnaires. Mean CADESI-04 score decreased significantly from 24.2 on D0 to 11.9 on D21 (49.4% mean improvement,  $P < 0.001$  at all timepoints), and 61.8% of dogs achieved a  $\geq 50\%$  decrease of their CADESI-04 score on D21. Mean PVAS scores also decreased significantly between D0 and D21 (30.8% mean improvement,  $P < 0.001$  at all timepoints) and 44.1% of the dogs showed a  $\geq 50\%$  PVAS decrease on D21. Veterinarians considered the improvement as satisfactory, good or excellent in 66.7% of cases. Pet owners were satisfied with the performance of the protocol in 72.7% of cases. In conclusion, the protocol combining applications of Ophytrium-based shampoo and mousse resulted in a quick and significant improvement in both skin health and pruritus in atopic dogs presenting with a skin flare with a high level of pet owner satisfaction.

## Introduction (459 Mots)

Canine atopic dermatitis (AD) is a common, relapsing, inflammatory and pruritic skin disorder with a genetic predisposition. It often involves immunoglobulin E (IgE) directed against environmental allergens (Hensel et al. 2015). The estimated prevalence of atopic dermatitis in the worldwide dog population is 15% (Hillier and Griffin 2001). The pathogenesis of canine AD is not fully understood but there is evidence of changes to the mechanical (Marsella et al. 2011; Olivry 2011) and immunological skin barriers with an increase in Th2 cytokine release (Olivry et al. 1999; Schlotter et al. 2011), as well as to the microbiological skin barrier with a lower diversity of both bacteria and fungi (Rodrigues Hoffmann et al. 2014; Meason-Smith et al. 2015; Bradley et al. 2016) and an increase in the relative abundance of the *Staphylococcus* genus. This dysbiosis is likely to favour or enhance the intensity of atopic flares.

Current guidelines and recommendations for the treatment of canine AD emphasize the need for a multimodal approach including allergen avoidance, use of topical products, use of drugs to reduce pruritus and skin lesions and allergen-specific immunotherapy (Olivry et al. 2015; Olivry and Banovic 2019). The use of non-steroidal topical agents that contribute to improve skin barrier function and soothe irritation with limited side effects may be beneficial during a flare as well as providing proactive measures to limit and delay relapses (Bourdeau et al. 2007; Bensignor et al. 2013; Olivry et al. 2015; Bensignor and Fabriès 2018; Olivry and Banovic 2019).

There are many topical formulations available on the market with various active ingredients. Ophytrium is a specific purified extract from *Ophiopogon japonicus*, shown to have a threefold action on the skin

barrier. It strengthens the mechanical skin barrier for supple and durably hydrated skin with an action on ceramides, filaggrin, and Natural Moisturizing Factor content. It limits water loss (Ollivier et al. 2019a) and increases tight junction expression. Ophytrium reduces skin permeability, as shown with the Lucifer yellow test, where the dye passed through the skin when stressed with proinflammatory cytokines, but no penetration was seen in the presence of Ophytrium (Ollivier et al. 2019a). Ophytrium restores the balance of the protective microbial flora, limiting the adhesion, and as a consequence biofilm formation, of *Staphylococcus* species (proven on *Staphylococcus aureus* and *Staphylococcus pseudintermedius* (Ollivier et al. 2019b)). It also reduces skin irritation through a decreased release of pro-inflammatory cytokines (TSLP, IL-8 (Ollivier et al. 2019a) and IL-13).

As atopic dermatitis is a lifelong disease, pet owner compliance with an easy-to-use protocol and pleasant-to-apply products are needed because regular application or administration is often necessary. Among the available formulations, shampoos are the most commonly used in veterinary medicine but baths require adequate facilities and are time-consuming. While a shampoo mainly cleans the skin and fur with active ingredients in contact with the skin for a relatively short time, mousses offer a complementary formulation that brings ingredients directly to the skin, allowing good skin absorption as well as optimal and prolonged action. In addition, mousses are applied through massage that encourages frequent use: pet owners share a pleasant moment with their pet, which is expected to favour compliance. The performance of a protocol combining applications of phytosphingosine-based shampoo and mousse has previously been demonstrated (Bensignor et al. 2013; Fantini et al. 2015) in atopic dogs presenting with a skin flare.

The objective of this study was to evaluate the performance of a new protocol combining one initial shampoo application followed by mousse applications every 48–72 hours for three weeks in atopic dogs presenting with a skin flare. Both formulations contained Ophytrium (DOUXO® S3 CALM Shampoo and Mousse, Ceva Santé Animale, Libourne, France).

## Materials And Methods (1239)

### Inclusion and exclusion criteria

Client owned dogs of any sex and breed, aged between 1 and 12 years old, with documented history of atopic dermatitis and previously diagnosed according to accepted criteria (Favrot et al. 2010) were recruited from 12 veterinary clinics in France, Spain and the USA. To be included in the study, dogs had to present with a skin flare, defined as a Canine Atopic Dermatitis Extent and Severity Index (Olivry et al. 2014) (CADESI-04) >10/180 and a pruritus score on a 10 cm visual analogue scale (Rybníček et al. 2009) (PVAS) between 2 and 8 (score given by the pet owner). The dogs also had to be clear of ectoparasites as well as secondary bacterial and fungal infections.

Exclusion criteria comprised of pregnant and lactating dogs, dogs with flea allergic dermatitis, skin infections, dermatophytosis, endocrine or immune-mediated disease, and dogs with evidence of

demodicosis within the past 6 months. Any major disease or clinical signs that may interact with the study were also forbidden. To avoid recurrence of clinical signs linked to a food intolerance, dogs with any change in their food regimen (including fatty acid supplementation) within the last 8 weeks were also excluded. Dogs with lesions in the auricular canal that required beginning the application of a topical treatment containing antibiotics and/or anti-fungals and/or glucocorticoids were also excluded from the study.

Concurrent treatment was allowed on the condition that it had been stable for several weeks and remained unchanged during the whole study. Thus, dogs fulfilling at least one of the following conditions were excluded from the study:

- Initiation or modification (including dose change) to long-term immunomodulatory treatment (cyclosporine, oclacitinib) within 8 weeks before inclusion
- Initiation or modification of ASIT (Allergen Specific Immunotherapy) protocols within the last 9 months
- Initiation or modification (including dose change) of oral glucocorticoid treatment within 4 weeks before inclusion
- Initiation or modification (including dose change) of topical corticoids or calcineurin inhibitor treatments or ear cleaner within 4 weeks prior to inclusion
- Initiation or modification of anti-histamine treatment within 1 week prior to inclusion
- Lokivetmab injection within 2 months prior to inclusion and during the study.

To avoid a residual effect during the study, some other treatments were also subject to restrictions:

- Treatment with antimicrobial or antifungal therapies within 4 weeks prior to inclusion
- Injection with parenteral long-acting glucocorticoids within 8 weeks prior to inclusion
- Application of topical antiseptic(s) (e.g. chlorhexidine, diluted bleach bath) within 2 weeks prior to inclusion
- Application of any of the following topical products: lotions, sprays, shampoos within 7 days prior to inclusion.

## **Application protocol and study schedule**

All included dogs received the same application protocol combining applications of Ophytrium-based Shampoo and Mousse (DOUXO® S3 CALM Shampoo and Mousse, Ceva Santé Animale, Libourne, France). The products were provided in a neutral packaging. The application protocol consisted in one Shampoo application and 2 Mousse applications during the first week and 3 Mousse applications weekly

during the 2 following weeks (Figure 1). A minimum interval of 2 days and a maximum of 3 days had to be respected between each topical application. The Shampoo and Mousse were applied on the whole body according to the manufacturer's recommendations. The number of pumps of the Mousse to be applied to the dog was estimated on Day 0 according to the dog's bodyweight, hair length and hair density. If necessary, this dose could be adjusted by the owner at home in order to cover the whole body surface.

Each dog was examined four times by the veterinarian: at the inclusion visit on day 0, and at follow-up visits on day 7, day 14 and day 21 (Figure 1).

## Evaluated parameters

During each weekly visit, veterinarians evaluated dogs' skin health using the CADESI-04 (Olivry et al. 2014). The effect of the protocol on this parameter was the primary outcome. The percentage of dogs with a final  $\geq 50\%$  or  $\geq 70\%$  CADESI-04 decrease and the percentage of dogs with a final CADESI-04  $< 10$  were secondary outcomes. As suggested by a recent publication for short-term studies (shorter than 6 weeks) (Olivry 2019), CADESI4-E score (erythema from CADESI-04 score) was also evaluated. Also the veterinarian evaluated the status of the skin condition from back to normal to very severe.

At each veterinary visit, owners evaluated their dog's pruritus using a validated 10-cm visual analogue scale (PVAS), taking into account their dog's pruritus in the previous 24 hours. Mean evolution of this parameter was assessed, as well as the percentage of dogs with a final  $\geq 30\%$  or  $\geq 50\%$  PVAS decrease and the percentage of dogs with a final PVAS  $< 2$ . A decrease of at least 2 cm on the PVAS between the beginning and the end of study was already used as a success criterion (Cosgrove et al. 2013).

At the end of the study, both pet owner and veterinarian overall assessment and satisfaction were collected through questionnaires. The veterinarian was asked to appraise the clinical evolution at D21 compared to D0 (absent or worse, weak, satisfactory, good, excellent). The owners were asked to assess the performance and the practicality of the protocol using a 4-point scale: totally disagree, somewhat disagree, somewhat agree, totally agree. They were also asked to evaluate the overall response to the protocol (no response, a poor response, a fair response, a good response, an excellent response) (OGATE) (Olivry et al. 2018).

As suggested by Olivry et al. in 2018 to allow some standardization of clinical trial results, the COSCAD'18 was evaluated, considering the OGATE, the number of dogs with a PVAS  $< 2$ , and number of dogs with a CADESI-04  $< 10$  at the end of the study.

## Data analysis

Data were analysed using the SAS software (V.9.4; SAS Institute) and were two-tailed with a type one error of 5%. Parameters were analysed using descriptive statistics. Quantitative variables were analysed

using mean and standard deviations, categorical variables were analysed using frequency counts and percentages.

The dogs which were prematurely withdrawn for “lack of efficacy” were used in the analysis. The “LOCF” (Last Observation Carried Forward) imputation method was used to use to not advantage the protocol. Other premature terminations which were not linked to AD were excluded from efficacy analyses and kept in the safety analysis.

The evolution of the CADESI-04 score and PVAS were assessed with linear mixed models. The covariates were the study visit and the baseline as fixed effects. Additional covariates like coat length, ectoparasite treatments, coat density or treatment for atopic dermatitis were tested in the statistical model and removed if p-values were above 5%.

An order 1 autoregressive was used as the covariance matrix in the CADESI-04 model and the dog id was used as a random effect in the PVAS analysis.

Least-square means with their 95% confidence intervals were calculated to see significant differences between visits.

A linear mixed model was used to analyze the evolution through time of the CADESI-04 and the VAS. The time and the CADESI-04 at D0 or PVAS at D0 were used as fixed effects. To take into account the repeated measures, different correlation structures (No correlation, component symmetry, variance component and order 1 autoregressive) were tested with the AIC. The best correlation structure retained was an order 1 autoregressive for the CADESI-04 analysis and no correlation in the PVAS analysis. A random effect was also tested but retained only on the PVAS analysis. To compare the different visits (D14 vs D0 and D21 vs D0), the Least Square means (LS-Means) from the linear mixed model were computed and tested if they were significantly different from 0.

## Results (1139 Mots)

### Demographics and baseline characteristics

Thirty-eight atopic dogs with a skin flare due to AD were included in the study and in the safety analysis. Four of them were removed from the efficacy analysis: three dogs for which the vet prescribed a forbidden treatment and one dog whose owner removed his consent. The study population had a mean body weight of 17.8 kg and comprised of a majority of female dogs (20/34, 58.8%). The average age of the dogs was 5.6 years (Standard Deviation (SD):3.4 years) and the French bulldog was the most represented breed (12/34). Most dogs had a short and thin coat (19/34, 55.9%), 6 dogs (17.6%) had a short and dense coat, and 6 had a long and dense coat.

All dogs were regularly treated against external parasites, most often with tablets (24/34, 70.6%) rather than spot-ons or collars (10/34, 29.4%).

At baseline, the CADESI-04 score was 24.2/180 in mean (SEM: 1.6) and ranged from 11 to 51, corresponding to mild (30/34 dogs, 88.2%) or moderate intensity (4/34 dogs, 11.8%). Even if most dogs were classified as mild intensity according to their CADESI-04 score at D0, most dogs were considered as moderately affected by the veterinarians (22/34, 64.7%), 4/34 dogs (11.8%) were considered as severe, and only 8/34 (23.6%) were considered as mild. Affected body areas are presented in Fig. 3. All regions were affected in more than 40% of dogs with the highest prevalence on the front paws. No correlation was found between intensity of the flare and the time of onset of the clinical manifestation-visit to the veterinarian.

The initial pruritus score ranged from 2.1 to 7.7, corresponding to very mild to quite severe pruritus, with a mean of 5.3/10 (SEM: 0.3).

Most dogs (19/34, 55.9%) did receive any treatment for atopic dermatitis. Others received regular systemic and/or ear medication as described in Table 1. The presence (or not) of concurrent treatment did not have any impact on any of the results presented thereafter.

Table 1  
Concurrent treatment given during the study for atopic dermatitis

Treatment	Molecule	Number (%)
Systemic	Oclacitinib	6/34 (17.6%)
	Prednisolone	3/34 (8.8%)
	Allergen Specific ImmunoTherapy	4/34 (11.8%)
Auricular	Hydrocortisone aceponate	3/34 (8.8%)
	Triamcinolone	1/34 (2.9%)

## Evolution of the CADESI-04 score

There was a quick and significant decrease in the CADESI-04 score: mean CADESI-04 score decreased from 24.2 on D0 to 15.1 as soon as D7 (41.8% mean decrease,  $P < 0.001$ ), down to 11.9 on D21 (49.4% mean improvement compared to D0,  $P < 0.001$ ) (Fig. 2). At the end of the study, 21/34 dogs (61.8%) achieved a  $\geq 50\%$  decrease of their initial CADESI-04 score and 16/34 (47.1%) achieved a  $\geq 70\%$  decrease of their initial CADESI-04 score. The same percentage of dogs (47.1%) reached a score below the target score of 10 (same as for a normal dog), and 20/34 dogs (58.8%) had a CADESI-04 score  $\leq 10$  at D21. An improvement was seen in all CADESI-04 categories, even in dogs that were more severely affected (Table 2). The presence of previous treatment for atopic dermatitis and the country of origin did not significantly influence the CADESI change ( $p = 0.89$  and  $P = 0.17$  respectively).

Table 2  
Distribution of dogs according to their CADESI-04 score in categories

CADESI-04 score categories	Day 0	Day 21
Target (0–9)	0/34 (0.0%)	16/34 (47.1%)
Mild (10–34)	30/34 (88.2%)	17/34 (50.0%)
Moderate (35–59)	4/34 (11.8%)	1/34 (2.9%)
Severe (> 60)	0/34 (0.0%)	0/34 (0.0%)

On average, the CADESI4-E score significantly improved by 52.7% between D0 and D21 ( $P < 0.001$  at all timepoints) with similar kinetics as the CADESI-04 score.

Looking at body locations affected at D21 compared to D0, a noticeable improvement was observed in all body areas: all body locations were less frequently affected after 21 days of product application (Fig. 3).

## Evolution of pruritus

A quick and significant decrease of the PVAS score was observed during the study. The mean PVAS score decreased from 5.3 on D0 to 3.9 as soon as D7 (20.2% mean improvement,  $P < 0.001$ ). At the end of the study, the mean PVAS score was 3.6, with a mean improvement of 30.8% ( $P < 0.001$ ) (Fig. 4). An improvement was observed even in cases with quite severe itching. From D14 until D21, 11/34 dogs (32.4%) presented with a PVAS score  $< 2$ , corresponding to the PVAS score of a normal dog. At the end of the study, 18/34 dogs (52.9%) achieved a  $\geq 30\%$  decrease, 15/34 (44.1%) achieved a  $\geq 50\%$  decrease, and 15/34 dogs (44.1%) had achieved a  $\geq 2\text{cm}$  decrease of their initial pruritus score.

The presence of previous treatment for atopic dermatitis and the country of origin did not significantly influence the CADESI change ( $p = 0.89$  and  $P = 0.17$  respectively).

Again other parameters were tested, the presence of previous treatment for atopic dermatitis did not significantly influence the pruritus change ( $p = 0.71$ ) and the something for the country ( $p = 0.26$ ).

## Veterinarian overall assessment and satisfaction

Regarding the status of the skin condition after 21 days, 9/34 dogs (26.5%) were considered as being back to normal, and 15/34 (44.1%) as having mild intensity signs. Most dogs (22/34, 64.7%) were in an improved category at D21 compared to D0.

At the end of the study compared to D0, veterinarians considered the improvement as satisfactory, good or excellent in 66.7% of cases (30.3%, 30.3%, 6.1%, respectively). The response to the protocol was considered to be minimal in 24.2% of cases and absent or worse in the remaining cases.

## Pet owner overall assessment and satisfaction

After 21 days, pet owners considered the protocol to be effective in 72.7% of cases and practical in 100% of cases. Overall, they highlighted a fair, good or excellent response to the protocol (OGATE) in 72.7% of cases (24.2%, 36.4%, 12.1% respectively).

## Core Outcome Set for Canine Atopic Dermatitis (COSCAD'18)

The results on the parameters included in COSCAD'18 after using the product for 21 days are presented in Table 3.

Table 3  
Core outcome set for canine AD (COSCAD)  
results after 21 days of product application (n  
= 34)

Parameter	n (%)
PVAS < 2	11/34 (32.4%)
CADESI-04 < 10	16/34 (47.1%)
CADESI-04 ≤ 10	20/34 (58.8%)
OGATE (good-to-excellent)	16/34 (47.1%)

## Adverse events

In total, nine adverse events involving eight dogs were reported during the study. The majority were of mild to moderate intensity. Three dogs presented with superficial pyoderma as a secondary complication due to atopic dermatitis. These dogs were withdrawn from the study to receive antibiotics as this was not allowed by the study protocol. Two dogs were excluded from the study because of worsening of their skin status and/or pruritus but kept in the analysis (the last values for CADESI-04 and PVAS score were imputed up to the end of the study). One dog presented with erythema and papules after shampoo application that did not worsen after mousse application. This dog was withdrawn from the study. The

remaining four adverse events did not lead to study discontinuation: one superficial eye ulcer started on D4, one puncture behind an ear that could have been due to an opossum bite wound, and gastrointestinal signs (diarrhoea and emesis) that spontaneously recovered in one day.

## Discussion (1160 Mots)

This study included 34 atopic dogs that presented with a skin flare. The protocol using Ophytrium-based products (combination of one shampoo and subsequent mousse applications for three weeks) significantly improved both CADESI-04 score and pruritus. The results illustrate the clinically soothing properties of Ophytrium which have already been demonstrated *in vitro* by the decreased secretion of pro-inflammatory cytokines (IL-8, TSLP (Ollivier et al. 2019a), IL-13).

The CADESI-04 score was the primary outcome of the study. CADESI-04 significantly improved by 47.1% on average after 21 days of application. In published reports, the lack of harmonisation of evaluated parameters makes the comparison between the studies difficult, especially regarding skin health. This is why Olivry proposed the COSCAD'18 in 2018, allowing some standardization of clinical results (Olivry et al. 2018) and suggesting parameters of interest. A shampoo containing linoleic acid, gamma-linoleic acid, mono and oligosaccharides, Vitamin E and piroctone olamine applied three times weekly allowed a 69% decrease in mean CADESI-03 score in dogs with mild manifestations of AD or Flea Allergy Dermatitis (FAD) (Bensignor et al. 2013). However, the population included FAD dogs treated with a parasiticide at D0, so it is difficult to compare with our population which included only atopic dogs. The same shampoo applied once a week in chronically pruritic dogs showed no significant difference with the control group on the CADESI score (Löflath et al. 2007). In a cross-over study, a mousse containing plant extracts showed a 41.9% reduction of the mean Canine Atopic Dermatitis Lesion Index (CADLI), another validated scoring index (Plant et al. 2012), (from 19.38 to 11.25) after 14 days (Bensignor and Fabriès 2018). During the same period of time, Ophytrium-based products showed a decrease in the mean CADESI-04 score of 43% (from 24.2 to 13.8), and dogs kept on improving during the following week. Nonetheless, it is difficult to draw conclusions from the published cross-over study: the number of dogs is very low (8 dogs), values of each parameter at the beginning of each period are not shown so the validity of the cross-over design cannot be checked. More information would have been useful to appreciate the improvement (e.g. the number of dogs receiving a concurrent treatment during the study, and number of dogs reaching  $\geq 50\%$  improvement of the CADLI or reaching a CADLI  $< 5$  at the end of the study). Several studies lasting 4 to 8 weeks aimed at evaluating the performance of lipid-based formulas, either essential oils, fatty acids, sphingolipids, palmitoylethanolamide or ceramides (more or less in combination with other active ingredients), on skin status and/or pruritus (Fujimura et al. 2011; Tretter and Mueller 2011; Marsella et al. 2013, 2017, 2020; Blaskovic et al. 2014; Noli et al. 2015). Regarding skin status parameters (either CADLI or CADESI), improvement was higher with Ophytrium-based products after 3 weeks than with any of the above mentioned formulations (bar one) when used for 4 or more weeks. Improvement of the mean scores ranged from 30.2–79.3% (Fujimura et al. 2011; Tretter and Mueller 2011; Marsella et al. 2013; Blaskovic et al. 2014; Noli et al. 2015) or were not significant compared to D0 and/or the control group (Marsella et al. 2017, 2020). When reported, the percentage of dogs with a score decrease  $\geq 50\%$

(either CADESI or CADLI) ranged from 35 to 37% (Fujimura et al. 2011; Tretter and Mueller 2011; Marsella et al. 2013; Blaskovic et al. 2014; Noli et al. 2015), which is lower than in this study (61.8%).

The effect of DOUXO® S3 CALM formulas on pruritus was also significant, with a mean 30.8% improvement and 15/34 dogs (44.1%) having a  $\geq 50\%$  decrease after 21 days. Among the previously published studies, some showed no significant improvement in pruritus *versus* control group (Fujimura et al. 2011; Marsella et al. 2013, 2017, 2020; Blaskovic et al. 2014). In some others, the percentage of dogs whose pruritus improved by more than 50% ranged from 23–50% after 4 weeks, 1 month or 8 weeks (when results at 4 weeks were not available) (Fujimura et al. 2011; Blaskovic et al. 2014; Noli et al. 2015). Published reduction in the mean pruritus score ranged from 25–43.5% (Tretter and Mueller 2011; Blaskovic et al. 2014; Noli et al. 2015). The results obtained in this study are in the same order of magnitude.

The results in this study were not significantly different whether the dogs received concurrent treatment or not, suggesting that topical products can effectively be used either alone or as part of multimodal approach without interference with systemic drugs.

In the absence of specific regulations for non-drug veterinary topical products, no guidelines are available for the tolerance and safety evaluation of such products, in consequence the safety assessment was also essential in this study. On the 38 dogs included only one present an adverse event clearly in relation with the product, the event seems to be an allergic reaction which is difficult to prevent completely, especially in dogs with sensitive skin. Indeed, a particular care on the ingredient selection and a previous study have been performed to evaluate the safety associated with those products. The safety study used an original design with increased frequency and dose of application compared to recommended use (Kolasa and al. 2019 poster at SCIVAC Dermatology Congress) and did not provide any indication of possible reaction with DOUXO® S3 CALM use as it could be observed for a large majority in this study.

A control group was not included in the present study. Nevertheless, in the literature review, control or vehicle groups did not substantially improve after 4 weeks, either regarding irritation or pruritus (Steffan et al. 2005; Horvath-Ungerboeck et al. 2009; Cosgrove et al. 2013; Marsella et al. 2019). This observation is confirmed in the majority of longer studies (Ferguson et al. 2006; Puigdemont et al. 2013; Marsella et al. 2017, 2020).

Topical formulations are useful to improve skin health as they bring ingredients directly to the target organ. Ingredients with beneficial properties on the three skin barriers (mechanical, microbiological and immunological) which are altered in atopic dermatitis, are of particular interest. Shampoo and mousse formulations are complementary: the shampoo mainly cleans the skin and the fur as well as removed dirt, debris, scales or allergens present on the skin surface, and the mousse allows the ingredients longer direct skin contact for prolonged action. The shampoo is not always accepted by pets who are quite often reluctant to be restrained or wet. In addition, it usually requires adequate facilities and time (stand time is usually 5 to 10 minutes). This can be demotivating to owners on a long-term basis. An American retrospective study showed that 5 years after the diagnosis of atopic dermatitis, only 18% of pet owners

kept on washing their dogs at least once a week (Dell et al. 2012). A study carried out in 2010 showed that 32% of pet owners with an atopic dog felt that their dog's treatment was a major burden (Linek et al. 2010). Not only is long-term use considered a constraint by pet owners: Bensignor stated in 2013 that 56/68 pet owners (82%) considered a twice weekly shampoo a constraint, difficult or impossible (Bensignor et al. 2013). A lower frequency of application can alter the performance of a product (Löflath et al. 2007). Mousse formulations are applied through massage into haired skin, which is a pleasant moment for both pet and owner. This leave-on formulation is efficient, convenient, pleasant to use and time-sparing for pet owners. It is expected to encourage compliance which is key in the management of lifelong skin disorders.

## **Conclusion**

The protocol combining applications of an initial shampoo followed by mousse containing Ophytrium, a specific purified extract from *Ophiopogon japonicus* (DOUXO® S3 CALM Shampoo and Mousse, Ceva Santé Animale, Libourne, France), quickly and significantly improved both CADESI-04 and pruritus in atopic dogs presenting with a skin flare. The products had previously demonstrated their safety even under acute conditions of exposure. The shampoo/mousse combination can be used in dogs with or without concurrent treatment without any impact on performance. This protocol is expected to maximise performance: the shampoo mainly cleans the skin and the fur while the leave-on Mousse provides longer lasting direct skin contact with the ingredients. These factors are expected to favour compliance which is of major importance when dealing with lifelong skin disorders such as atopic dermatitis. This study demonstrated the performance of Ophytrium-based products in atopic dogs presenting with a skin flare. Further studies would be of interest to evaluate their response when used proactively to delay the recurrence of skin flares.

## **Declarations**

### **Conflict of Interest**

MG, RK, EO, TB, CZ and XDJ are employees of Ceva Santé Animale.

The other authors were study investigators and received fees from Ceva Santé Animale for the follow-up of their recruited cases.

### **Ethics statement**

The animal study was reviewed and approved by Avogadro Ethics Committee. Written informed consent was obtained from the owners for the participation of their animals in the study.

### **Authors Contributions**

EO, RK, CZ and XDJ contributed to conception and design of the study. PF, DG, EI, JFJ, GM, VM, LO, MP, WR, MW and CY recruited dogs for the study and performed their follow-up examinations. TB participated in results elaboration. MG, XDJ and TB wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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## Data availability statement

The data from this study are not publicly available because they contain proprietary information of a recently commercialized veterinary product. Requests to access the data sets should be directed to Xavier De Jaeger: [xavier.de-jaeger@ceva.com](mailto:xavier.de-jaeger@ceva.com)

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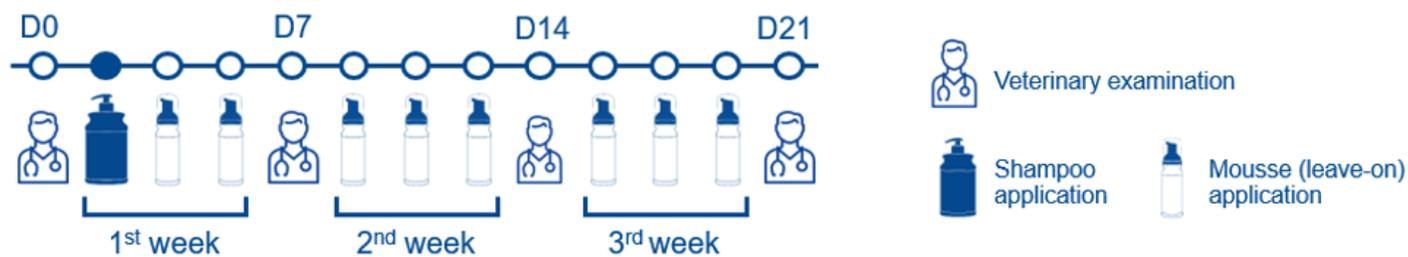
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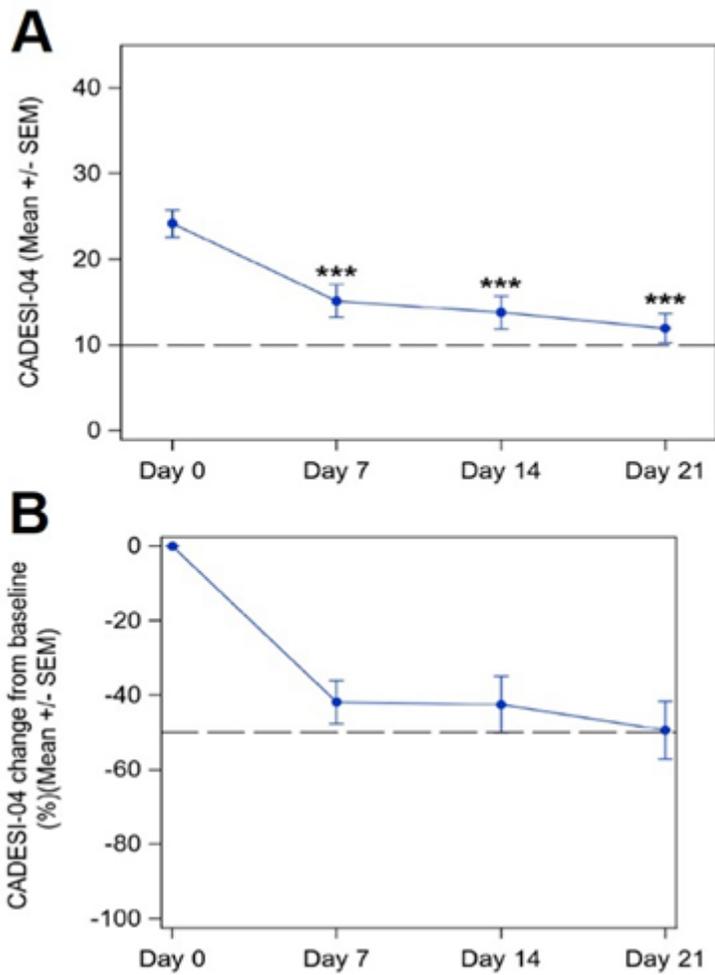
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## Figures



**Figure 1**

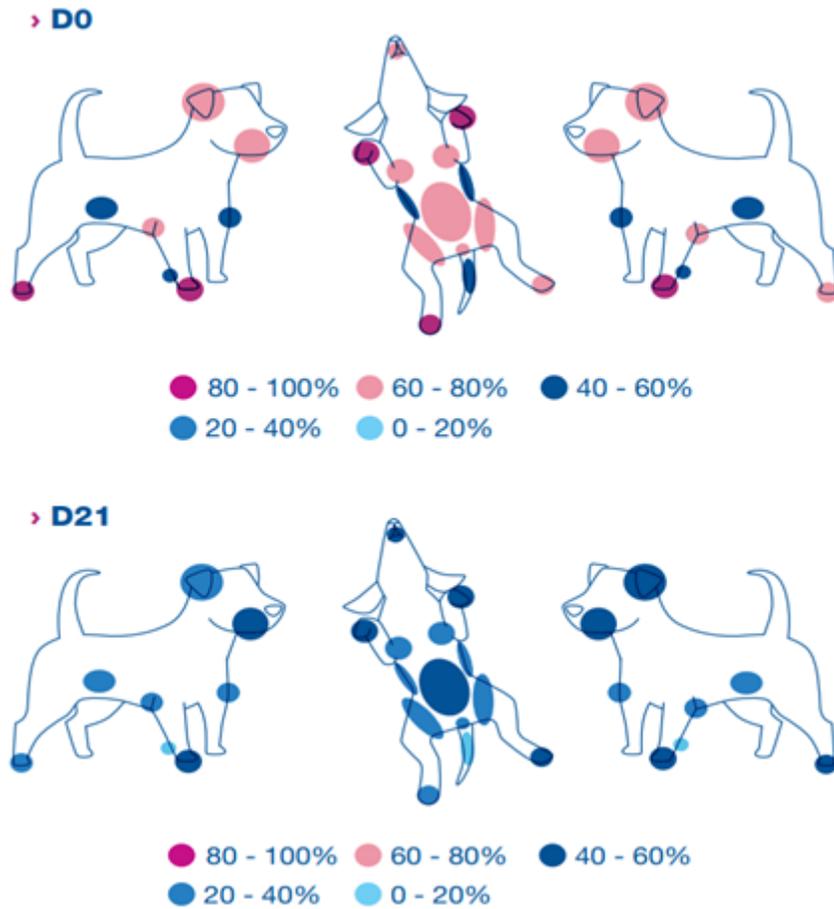
Study design



**Figure 2**

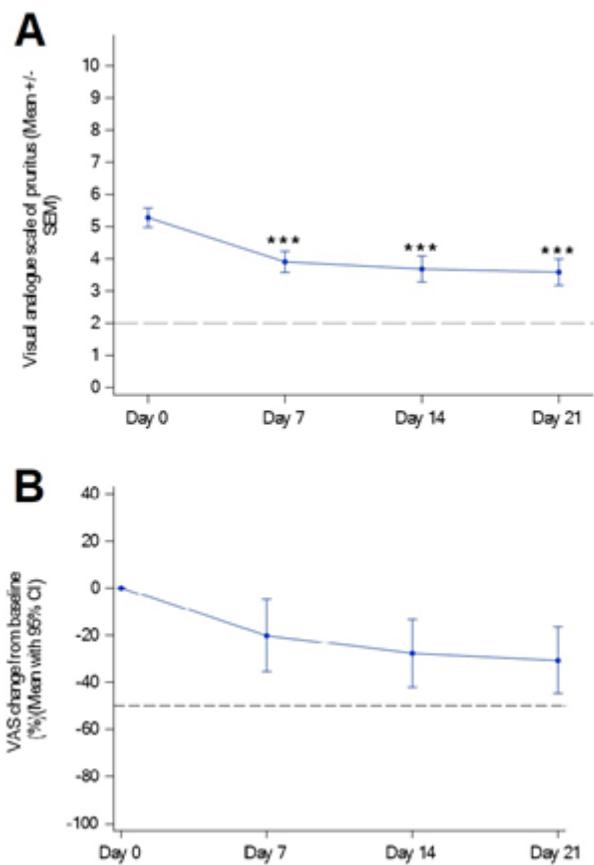
A. Evolution of the mean CADESI-04 score in absolute values. B. Mean evolution of the CADESI-04 score compared to D0

\*\*\* results are significantly different from D0 ( $P < 0.001$ )



**Figure 3**

Percentage of dogs affected on each body location affected at D0 and D21



**Figure 4**

A. Evolution of the mean PVAS score in absolute values. B. Mean evolution of the PVAS score compared to D0.

\*\*\* results are significantly different from D0 ( $P < 0.001$ )