

Barazone decreases skin lesions and pruritus and increases quality of life in dogs with atopic dermatitis: a randomized, blinded, placebo-controlled trial

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A randomized, blinded, placebo-controlled study was conducted to assess the efficacy of a new 0.025% budesonide leave-on-conditioner (Barazone) in controlling the clinical signs of canine atopic dermatitis (AD). Twenty-nine dogs with AD were randomly allocated to receive 3 weeks of once-weekly treatment with either Barazone or Placebo and then were crossed-over to receive the alternative treatment for a further 3 weeks. At the start and end of each treatment phase, referring veterinarians performed a dermatological and general physical examination on each dog, assigned a *Lesional Score*, collected blood for haematological and biochemical analyses and rated the dog's overall tolerance to the preceding treatment. Owners assessed their dog's level of pruritus and quality of life (QoL) daily, using visual analogue scales labelled with behavioural descriptors. Barazone improved skin lesions ($P = 0.02$) and QoL ($P < 0.001$) and reduced pruritus ($P \leq 0.002$) compared with treatment with Placebo. There were no significant differences in the tolerance scores and only minor differences in the general physical examination findings and haematological and biochemical parameters between dogs receiving Barazone or Placebo. This study demonstrated that Barazone, applied once weekly at 1 g/kg for 3 weeks, was an efficacious treatment for the control of the clinical signs of AD in dogs.

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INTRODUCTION

Canine atopic dermatitis (AD) is one of the most common skin diseases affecting dogs, although the prevalence (Hillier & Griffin, 2001) and pathogenesis (DeBoer & Marsella, 2001; Halliwell & DeBoer, 2001; Hill & Olivry, 2001; Marsella & Olivry, 2001; Marsella & Sousa, 2001; Sousa & Marsella, 2001) of this disease remain unclear. The definition of AD has been progressively refined over the last four decades, as our understanding of the disease has improved. The definition revised by the *International Task Force on Canine AD* describes the disease as 'a genetically predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features associated with IgE antibodies most commonly directed against environmental allergens' (Halliwell, 2006).

There is no definitive way to diagnose AD (DeBoer & Hillier, 2001), thus diagnosis relies on the fulfillment of clinical criteria that are strongly associated with the disease, and exclusion of differential diagnoses. As the clinical criteria used to diagnose AD

have varied over time and between investigators, the *ACVD Task Force on AD* reviewed the literature and compiled a list of typical clinical manifestations, including:

- pruritus, manifested as excessive scratching, rubbing, chewing or biting of the skin (beginning at a young age),
- nonseasonal, seasonal or seasonally exacerbated clinical signs of disease (depending on the allergens involved),
- the observation of lesions at specific body sites, such as areas of friction and flexure (including folds on the ventral body surface and on the face and feet).

As the aetiology of AD is not fully understood, no curative treatments are currently available. Instead, the goal of treatment is to manage the symptoms of disease, which often requires a combination of interventions (Olivry & Sousa, 2001a). An important intervention is to minimise a dog's exposure to known environmental allergens by eliminating or avoiding them whenever possible. Removal of surface allergens from the skin

by bathing and the application of topical preparations formulated to act as 'allergen barriers' have been proposed to minimize epidermal absorption of environmental allergens, although there is currently no evidence to support these interventions in atopic dogs (Olivry & Sousa, 2001a).

The most effective pharmacological therapies for the management of AD are systemically administered anti-inflammatories, specifically glucocorticoids and cyclosporine (Steffan *et al.*, 2006). While efficacious at controlling pruritus, both glucocorticoids and cyclosporine work by suppressing the immune system and consequently can produce significant adverse effects (Olivry & Sousa, 2001b; Steffan *et al.*, 2006). In consideration of these potential side effects, topically applied glucocorticoids are the standard treatment in human patients with atopic dermatitis and systemic glucocorticoids are reserved for short-term therapy of recalcitrant cases (Sibbury & Hanifin, 2000). The topical application of glucocorticoids is favoured over oral or injectable routes because it allows direct application of drug to the target organ, thus reducing the total dose required and the incidence of systemic side effects (Brazzini & Pimpinelli, 2002; Mills & Cross, 2006). Despite this, systemically administered glucocorticoids remain the most common pharmacological intervention in dogs (Olivry & Sousa, 2001b).

The majority of bases used in the topical glucocorticoid formulations registered for use on dogs were initially developed for application to human skin. Consequently, many topical glucocorticoid products are unsuitable for application on hairy skin as they tend to adhere to and matt the coat, attracting dirt, impairing transdermal penetration and leaving smear marks on surrounding surfaces. An important further consideration in veterinary medicine is that many topical glucocorticoids require frequent application, which is time consuming and contributes to poor owner compliance (Rosenkrantz, 2006). Topical glucocorticoids suitable for widespread use on dogs such as shampoos, conditioners, sprays and gels are becoming available, although some have obvious limitations and there are few published reports documenting their efficacy. A study that assessed treatment efficacy demonstrated that a topical spray containing triamcinolone (Genesis® Topical Spray; Virbac, Fort Worth, TX, USA; triamcinolone acetonide 0.015%) significantly improved pruritus, erythema, skin eruption and overall clinical scores in 52 dogs with known or suspected allergic skin disease (DeBoer *et al.*, 2002). More recently, the efficacy of a hydrocortisone spray (Cortavance®; Virbac SA, Carros, France; hydrocortisone aceponate 0.0584%) was evaluated in 15 dogs with AD, and found to have significantly improved skin lesions and reduced pruritus, with owners reporting significantly higher satisfaction with Cortavance treatment compared with placebo (Nuttall *et al.*, 2009). While efficacious, both sprays require regular application to control the clinical signs of AD, which necessitates significant commitment by owners. Another potential drawback of the triamcinolone spray is its alcohol content, which may irritate the inflamed skin of atopic dogs.

A topical budesonide treatment (Barazone; Dermcare-Vet; budesonide 0.025%) has been developed to overcome some of

the limitations of the topical glucocorticoids currently available to veterinarians. Budesonide is a moderately active glucocorticoid which has been formulated as a nongreasy, leave-on-conditioner which absorbs into the skin without matting the hair or leaving a cosmetically unacceptable residue on the coat. In addition, Barazone only requires once weekly application and so is likely to improve owner compliance. The primary objective of this study was to test the null hypothesis that there was no difference between the efficacy of Barazone and Placebo in treating the clinical signs of AD, using the following outcome measures: (i) extent and severity of AD skin lesions (ii) pruritus and (iii) quality of life (QoL). The secondary objective of this study was to assess the safety of 3 weeks of Barazone treatment in atopic dogs, using two outcome measures: (i) the incidence of adverse effects and (ii) haematology and biochemistry parameters.

MATERIALS AND METHODS

Study design

This multicentre study was designed as a prospective, randomized, blinded, placebo-controlled, cross-over clinical trial of six weeks duration. General practice veterinarians ($n = 20$) from veterinary clinics ($n = 13$; A.M.) in mainly eastern Australia participated in this study (see Table 1 and Fig. 1). Over 140 days (from April to September 2007), 29 dogs with AD were progressively inducted into the trial and randomly allocated to begin treatment with Barazone or Placebo. Following 3 weeks of treatment with Barazone or Placebo, the dogs were crossed-over to receive the alternative treatment for the remaining 3 weeks without an intervening wash-out period. The study schedule was applied to each dog independently. Approval to conduct this study was obtained from the University of Queensland Animal Ethics Committee, approval number SVS/265/06/ Dermcare. This study was conducted and documented in accordance with the principles of Good Clinical Practice (GCP), as detailed in the VICH guidance document (GL9, June 2000).

Sample size estimation

A minimum of 11 dogs per treatment group was determined to be necessary to provide 95% power to detect a 50% improvement from baseline *Lesional Scores* following 3 weeks of treatment ($SD = 30\%$; $\alpha = 0.05$; $\beta = 0.05$). A reduction in AD lesions of 50% from baseline has been proposed to represent good clinical efficacy of the test product (Olivry & Mueller, 2003).

Training of veterinarians and owners

Using the *Veterinary Instructions and Training Brochure*, veterinarians were trained by the study investigator either in person ($n = 13$) or over the phone ($n = 7$), to perform the dermatological examinations (including *Lesional Score* assessments), apply

Table 1. Information on study dogs

Dog	Grp	Breed	Sex ^a	Age (year)	Weight ^b (kg)	Vet	Clinic	Baseline (day 0)			Location ^c
								Lesional Score	Pruritus Score	QoL Score	
001	1	Large Munsterlander	F ⁻	4.5	25.6	1	A	119	4	8	Brisbane
002	2	Large Munsterlander	M ⁺	4.5	29.2	1	A	36	4	9	Brisbane
003	2	Jack Russell Terrier X	F ⁻	2.0	7.5	2	B	117	8	2	Mackay
004	1	Fox Terrier X	M ⁻	2.0	4.7	2	B	95	6	6	Mackay
005	2	Jack Russell Terrier X	F ⁻	6.0	7.0	3	C	107	7	7	Townsville
006	1	Beagle	M ⁻	1.3	19.7	4	D	48	5	6	Townsville
007	2	Staffordshire Bull Terrier	M ⁻	3.0	20.4	5	E	132	7	7	Rockhampton
008	1	Staffordshire Bull Terrier	F ⁻	1.0	13.5	6	B	113	9	2	Mackay
009	2	Bull Arab	M ⁺	1.2	39.5	7	F	264	10	4	Emerald
010	1	Staffordshire Bull Terrier X	M ⁻	7.0	21.1	8, 9	G	59	6	7	Cairns
011	1	German Shorthaired Pointer	M ⁻	1.5	28.3	9	G	149	7	7	Cairns
012	2	Jack Russell Terrier X	F ⁻	9.0	13.2	10	H	183	2	9	Cairns
013	2	Jack Russell Terrier	F ⁻	2.0	5.4	11	C	102	5	10	Townsville
014	1	Irish Wolfhound X	F ⁻	2.3	34.0	12	C	93	8	3	Townsville
015	2	Staffordshire Bull Terrier	F ⁺	1.5	14.1	13	I	217	8	9	Sydney
016	1	Fox Terrier X	F ⁻	6.0	4.8	14	B	187	10	0	Mackay
017	2	Staffordshire Bull Terrier	F ⁻	3.0	20.8	12	C	113	10	10	Townsville
019	1	Golden Retriever X	M ⁻	1.3	31.9	10	H	83	5	10	Cairns
020	2	Australian Silky Terrier X	F ⁻	5.0	3.3	15	J	57	0	10	Cairns
021	1	Rhodesian Ridgeback	F ⁺	1.6	39.8	7	F	178	9	7	Emerald
022	2	Bull Terrier	M ⁻	1.5	23.3	16	K	117	8	8	Mackay
023	2	Fox Terrier	M ⁻	NR	7.8	7	F	97	8	7	Emerald
025	1	Staffordshire Bull Terrier	F ⁻	9.5	22.1	17	L	230	7	5	Cannonvale
026	2	Labrador Retriever X	F ⁻	4.2	26.5	18	J	253	10	2	Cairns
027	2	Golden Retriever	M ⁻	1.0	32.3	19	M	17	0	10	Adelaide
028	1	Labrador Retriever	M ⁻	2.0	34.4	7	F	84	8	7	Emerald
029	1	Staffordshire Bull Terrier	M ⁺	3.0	24.9	16	K	94	8	7	Mackay
030	2	Terrier X	M ⁻	7.0	12.9	15	J	130	8	6	Cairns
031	2	Fox Terrier X	M ⁻	1.5	5.1	18	J	436	9	6	Cairns

^aDesexed (-) or entire (+); ^bweight recorded at trial induction; ^cnearest city; NR, not recorded. The three dogs (023, 027 and 031) whose identification numbers are bounded by asterisks were withdrawn from the trial. There were two identification numbers (018 and 024) that were allocated to dogs that were not enrolled into the trial.

treatments, assess pruritus and QoL, train the owners and record data. Veterinarians then used the *Owner Instructions and Training Brochure* to teach the owners how to assess their dog's pruritus

and QoL, apply treatments, observe side effects and record data. The study investigator communicated frequently with the veterinarians to ensure their compliance with the study protocol.

Animal source

Twenty-nine client-owned dogs with AD were enrolled in the study, with random allocation of 13 to begin treatment with Barazone (Group 1) and 16 to begin treatment with Placebo (Group 2) (see Table 1). Three dogs were subsequently withdrawn from Group 2. Informed consent for the dogs' participation in the study was given by all owners prior to trial commencement.

Inclusion criteria

All dogs were diagnosed with AD, according to criteria slightly modified from those described by Willemse and Prélud (Willemse, 1988; Prélud *et al.*, 1998) and reviewed by the *ACVD Task Force on AD* (DeBoer & Hillier, 2001). Criteria essential to a diagnosis of AD included:



Fig. 1. Geographical location of study sites. The numerals in brackets represent the number of study dogs within each region.

- the presence of pruritus,
- clinical signs of an allergic dermatitis, with pruritus and erythema of the feet,
- chronic or relapsing dermatitis over two or more seasons,
- an onset of symptoms before three years of age,
- a positive response to treatment with glucocorticoids or cyclosporine (if ever given).

The dogs did not have flea allergy dermatitis, fleas or clinical signs of secondary skin infections with bacteria, *Malassezia*, *Demodex canis* or *Sarcoptes scabiei* at the time of enrolment. In addition, the dogs were not pregnant, lactating or concurrently enrolled in another clinical trial and their baseline total Lesional Scores were greater than 10. Furthermore, the dogs were in good general health apart from AD, with normal blood parameters and no clinical signs of excess systemic glucocorticoids. The performance of intradermal or serological allergen-specific testing were not essential for inclusion in the trial.

Randomization and blinding

To allocate the dogs to a treatment sequence, a blocked randomization technique was selected, using blocks of two sequentially enrolled dogs (i.e. sequentially enrolled dogs were not necessarily enrolled at the same veterinary clinic). A coin toss determined the treatment sequence of the first dog in the block (e.g. an outcome of tails resulted in allocation to Group 1, with a treatment sequence of Barazone, then Placebo). The second dog in the block was automatically assigned to the alternative treatment sequence. The Quality Assurance Officer at Dermcare-Vet established this treatment sequence prior to trial commencement, and was the only person with knowledge of and access to the group allocation of individual dogs. The study investigator coordinated the enrollment of dogs from 13 veterinary clinics, assigned a unique identification number to each dog on enrollment (identification numbers were assigned sequentially from 001 to 031) and allocated the treatment packages according to identification number. Throughout the trial the study investigator, veterinarians and owners were blinded to the dogs' treatment sequences. To guarantee adequate blinding, the consistency, opacity, colour, odour, container and dosing volume and frequency were identical for Barazone and Placebo. No situations arose during the study to necessitate the un-blinding of any study participant.

Diet, housing and management

The owners maintained their dog's usual diet, management and environment, without change, for the duration of the study.

Medications administered before and during the study

Anti-inflammatory drugs were discontinued for variable periods before beginning the trial, depending on the duration of drug action (1 week for anti-histamines, 2 weeks for cyclosporine,

3 weeks for oral and topical glucocorticoids, 4 weeks for short or medium acting glucocorticoids and 8 weeks for long-acting glucocorticoids). Dogs that had received allergen-specific immunotherapy (ASIT) were eligible for the trial with the following restrictions: (i) the last ASIT injection was administered over 4 months before trial commencement; or (ii) the last ASIT injection was administered over 1 week before trial commencement, but the dog had received ASIT for more than nine months and the pruritus and skin lesions had failed to respond even partially to this treatment. Anti-inflammatory drugs were prohibited during the trial, as were medicated shampoos, soaps or rinses apart from the test articles. There was no restriction on the administration of external parasiticides, anthelmintics, heartworm prophylactics or vaccinations.

Withdrawn dogs

Three dogs were withdrawn from this study. One dog (023) was withdrawn on the second day of the trial, when clinical pathology results revealed significant abnormalities in the baseline blood sample. The second dog (027) was withdrawn from the trial following detection of a significant change in its environment and management which was likely to have compromised the study results. The third dog (031) was withdrawn due to poor owner compliance including their failure to return the data recording forms and test articles for dose validation.

Test products

Barazone consisted of 0.025% budesonide in a leave-on conditioner base. The Placebo consisted of the leave-on conditioner base without the active drug. Treatments (either Barazone or Placebo) were applied once weekly for 3 weeks at a dose of 1 g (treatment) per kg (body weight) to all dogs, irrespective of their body surface area, hair length and extent or severity of clinical signs. Dogs were bathed using nonmedicated shampoo (Natural Shampoo; Dermcare-Vet), rinsed with water, allowed to shake and then lightly towelled dry. While the coat was still damp, the treatment (either Barazone or Placebo) was progressively dispensed, a few pumps at a time, to the AD affected areas and massaged into the coat so that it reached the skin. After the affected areas were preferentially treated, any remaining dose was applied to the adjacent areas. Dogs that attempted to lick themselves after application were distracted for at least 30 min to prevent ingestion of treatment.

Assessment of owner compliance

Owner compliance with the study protocol was assessed during weekly phone calls and veterinary assessments performed at the beginning and end of each treatment phase. Veterinary staff reviewed the owners' data recording forms and dog management practices. In addition, treatment bottles were weighed before and after treatment phases, to verify that the correct dose was dispensed.

Outcome measures

Assessment of skin lesions. Veterinarians scored the dogs' skin lesions at the beginning and end of each treatment phase (day 0 and day 21), using a modification of the Canine Atopic Dermatitis Extent and Severity Index (CADESI-03) recommended by the *International Task Force on AD* for the quantitative assessment of the extent and severity of cutaneous clinical signs of AD (Olivry *et al.*, 2007). This revised score was named the *Lesional Score*. The veterinarians assessed the severity of four clinical signs of AD at 32 sites on the body that are typically affected by this disease. The clinical signs evaluated were: (i) erythema (including erythematous macules and papules), as a sign of acute skin inflammation; (ii) lichenification (including pigmentation and thickening of the skin), as a sign of chronic cutaneous inflammation; (iii) excoriations and crusts (damage to the skin as a result of self-trauma included rubbing, chewing and bleeding), as signs of underlying pruritus; (iv) self-induced alopecia (including damage to the hair as a result of self-trauma), as another sign of pruritus. The severity of each clinical sign at each of the 32 regions of the body was scored from 0 to 5 (0 = none; 1 = mild; 2 = mild to moderate; 3 = moderate; 4 = moderate to severe; 5 = severe). Therefore, the maximum Lesional Score possible was 640 (5 (severity) $\times$ 4 (clinical signs) $\times$ 32 (regions)).

Assessment of pruritus. Owners made daily assessments of their dog's pruritus over the previous 24 h, by selecting a score on the Pruritus Visual Analogue Scale (PVAS). The PVAS consisted of a line with 11 equally spaced graduations labelled from 0 (not itchy: no scratching, licking, chewing, scooting or rubbing observed) to 10 (extremely itchy: constant scratching, licking, chewing, scooting or rubbing observed). A mean *Pruritus Score* was calculated for each dog, on each treatment, as follows:

$$\begin{aligned} &\text{Mean Pruritus Score on treatment } x \\ &= \frac{\sum \text{Daily Pruritus Scores on treatment } x}{\text{Number of daily Pruritus Scores recorded}} \\ &\quad \text{during treatment with } x \end{aligned}$$

Assessment of QoL. Owners made daily assessments of their dog's demeanour, behaviour and general contentedness over the previous 24 h, by choosing a score on the QoL Measure. This measure consisted of a line with 11 equally spaced graduations labelled from 0 (distracted, not usual self, uncomfortable, unhappy, not playing or attentive) to 10 (bright, alert, comfortable, happy, attentive, playful, responsive to family). A mean QoL Score was calculated for each dog, on each treatment, as follows:

$$\begin{aligned} &\text{Mean QoL Score on treatment } x \\ &= \frac{\sum \text{Daily QoL Scores on treatment } x}{\text{Number of daily QoL Scores recorded}} \\ &\quad \text{during treatment with } x \end{aligned}$$

Assessment of tolerance to the treatments. Tolerance was defined as the dog's ability to receive the treatment without side-effects, and was assessed by the dog's veterinarian and owner at the end of

each treatment phase (day 21). The dog's overall tolerance to the treatment applied over the preceding 3 weeks was rated on a scale consisting of a line with six equally spaced graduations labelled from 0 (poor tolerance with many side-effects) to 5 (excellent tolerance with no side-effects). The significance of any side-effects observed were then ranked on a scale from 0 to 3 (0 = absent; 1 = mild; 2 = moderate; 3 = severe). The side effects specifically evaluated were those known to be associated with glucocorticoid use, namely: polydipsia (PD); polyphagia (PP); skin thinning (ST); and vesicles, stretch marks and ulcerations (VSU). At the beginning and end of each treatment phase, the dogs also received a general physical examination and had blood collected for routine haematology and biochemistry analyses.

Statistical analyses

Intention-to-treat analyses were performed, to include the data pertaining to all 29 dogs that were enrolled in the trial. Data was missing for two (023 and 027) of the three withdrawn dogs and the 'last-value-carry-forward' rule was followed. Statistical significance was set at $\alpha = 0.05$. SAS version 9.1 (Cary, NC) was used for all statistical analyses. To determine if treatment sequence (Barazone or Placebo first) affected the outcome of treatment on skin lesions, the difference between the change in *Lesional Scores* on treatment with Barazone and Placebo for Groups 1 and 2 were compared using the Wilcoxon rank sum test. To determine if treatment sequence affected the outcome of treatment on pruritus and QoL, the differences between the mean scores on treatment with Barazone and Placebo for Groups 1 and 2 were compared using a *t*-test. Where it was found that there was no effect of treatment sequence, the data for Groups 1 and 2 was pooled and the effect of treatment was determined on the study population ($n = 29$). To determine if there was an effect of treatment (i.e. a difference between Barazone and Placebo) on skin lesions, the dogs' mean change in *Lesional Score* on treatment with Barazone and Placebo were compared using a paired *t*-test. To determine if there was an effect of treatment on pruritus and QoL, the dogs' mean scores on treatment with Barazone and Placebo were compared using a paired *t*-test. The mean scores for overall tolerance, PD, PP, ST and VSU for dogs on treatment with Barazone or Placebo were compared using the Wilcoxon signed rank test. To determine if there was an effect of treatment on any clinical pathology parameter, the difference between the changes in each parameter on treatment with Barazone or Placebo was compared with 0 using a *t*-test. The results of physical examinations were described for each group on each treatment.

RESULTS

Verification of owner compliance

The owner of dog 027 failed to return data recording forms and treatment bottles for dose validation at the completion of the

trial, resulting in this dog's withdrawal from the study. All other owners were deemed to have complied with the study protocol. Dose validation suggested that all owners correctly followed their dosing instructions, as the average ($\pm 95\%$ CI) amount of treatment dispensed was 102.3% (100.4–104.3%) and 101.0% (98.8–103.0%) of the prescribed dose for Barazone and Placebo, respectively.

Treatment effect on Lesional Scores

There was no effect of treatment order on *Lesional Scores* ($P = 0.23$), which allowed the data of both groups to be combined. The change in *Lesional Scores* was greater on treatment with Barazone than Placebo ($P = 0.02$; see Tables 2 and 3 and Fig. 2). Overall, the *Lesional Scores* of the dogs decreased by 51% (95% CI, 35–68%) on treatment with Barazone and increased by 150% (95% CI, 6–293%) on treatment with Placebo. Twenty out of 29 dogs experienced a 50% or greater reduction in *Lesional Score* on treatment with Barazone (compared with their score at the start of Barazone treatment; see Table 4). Of these 20 dogs, eight experienced a 75–90% reduction and three experienced a 90% or greater reduction in *Lesional Score* on treatment with Barazone.

Treatment effect on Pruritus Scores

Treatment significantly affected the mean *Pruritus Scores* of dogs in Group 1 ($n = 13$; $P = 0.002$) and Group 2 ($n = 16$; $P < 0.001$), with lower scores on treatment with Barazone (see Table 5 and Fig. 3). However, the difference between mean *Pruritus Scores* on treatment with Barazone and Placebo was significantly greater ($P < 0.05$) for the dogs in Group 2 than Group 1, which suggested that treatment order affected this outcome and precluded pooling of the groups' data. Group 2 dogs were twice as pruritic on treatment with Placebo than on treatment with Barazone, whereas Group 1 dogs were only 1.5 times as pruritic on Placebo compared to Barazone treatment.

Treatment effect on QoL Scores

Treatment sequence had no effect on *QoL Scores* ($P = 0.13$), which allowed the data of both groups to be combined. The quality of the dogs' lives was significantly higher on treatment with Barazone than on treatment with Placebo ($P < 0.001$; see Table 6 and Fig. 3).

Table 2. Median *Lesional Scores* of all dogs ($n = 29$) before (day 0) and after (day 21) treatment with Barazone or Placebo

Day	Treatment		<i>P</i> -value
	Barazone	Placebo	
0	96 (83–160)	64 (24–117)	0.18
21	31 (17–58)	89 (45–114)	0.006
<i>P</i> -value	<0.001		1.0

Median values are followed by the inter-quartile range.

Table 3. Mean change in *Lesional Scores* of all dogs ($n = 29$) on treatment with Barazone or Placebo

	Treatment		<i>P</i> -value
	Barazone	Placebo	
Change in <i>Lesional Score</i>	–73.2 (–111.5, –34.8)	8.3 (–25.7, 42.4)	0.02

Negative values indicate a reduction and positive values indicate an increase in mean *Lesional Score*. Mean values are followed by the 95% confidence interval

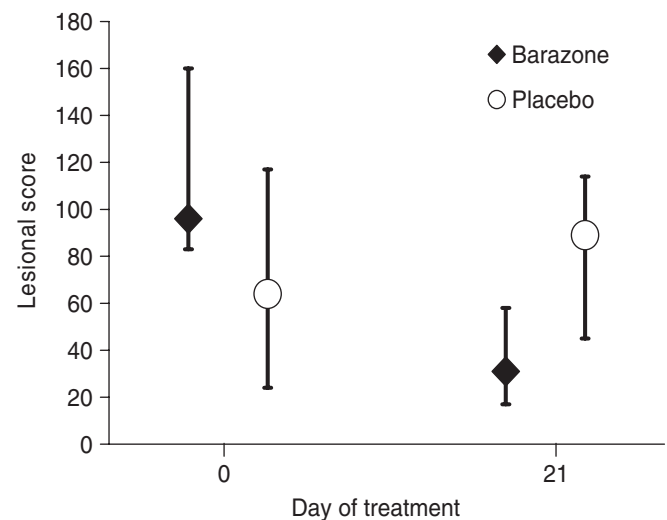


Fig. 2. Median *Lesional Scores* of all dogs ($n = 29$) before (d 0) and after (d 21) treatment with Barazone or Placebo. The vertical error bars represent the interquartile range.

Table 4. The number of dogs that experienced a reduction of ≥ 50 , 75 or 90% from their baseline *Lesional Score* following 21 days of treatment with Barazone or Placebo

Treatment	Reduction from baseline <i>Lesional Score</i> ^a (%)		
	≥ 50	≥ 75	≥ 90
Barazone	20	11	3
Placebo	5	0	0

^aThe *Lesional Score* of the dog at the beginning (d 0) of that treatment

Table 5. Mean *Pruritus Scores* of dogs in Groups 1 ($n = 13$) and 2 ($n = 16$) on treatment with Barazone or Placebo

Group	Pruritus Score		<i>P</i> -value
	Barazone	Placebo	
1	3.0 (2.3–3.7)	4.6 (3.5–5.7)	0.002
2	3.1 (1.8–4.3)	6.3 (4.8–7.8)	<0.001

Pruritus Score of 0 = no pruritus and 10 = worst pruritus. Mean values are followed by the 95% confidence interval

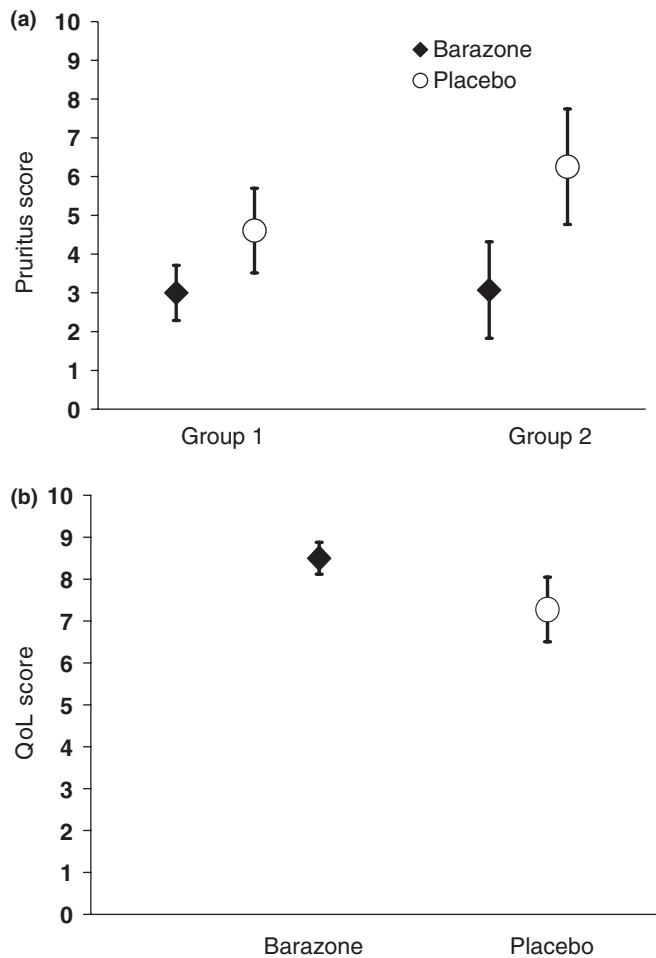


Fig. 3. Mean (a) Pruritus and (b) QoL Scores on treatment with Barazone and Placebo (Pruritus Score of: 0 = no pruritus, 10 = worst pruritus; QoL Score of: 0 = poorest QoL, 10 = best QoL). The vertical error bars represent the 95% confidence intervals.

Treatment effect on tolerance scores

There were no differences between the scores assigned for overall tolerance ($P = 1.0$), PD ($P = 0.3$), PP ($P = 1.0$), ST (mean score = 0.04 on both Barazone and Placebo) or VSU (mean score = 0 on both Barazone and Placebo) when dogs were treated with Barazone or Placebo. Following 3 weeks of treatment with Barazone, all dogs were given an overall tolerance score of either 4 (2/28) or 5 (26/28), to indicate very good or excellent tolerance to treatment.

Treatment effect on general physical examination results

The most common abnormalities reported in dogs throughout the study regardless of treatment were otitis externa and conjunctivitis, both of which are common features of AD (Griffin & DeBoer, 2001). At the final general physical examination of one dog (028), slow regrowth of hair was observed on its dorsal forearm where it had been clipped for venipuncture. Apart from this finding, the general physical examinations did not reveal

Table 6. Mean QoL Scores of all dogs ($n = 29$) on treatment with Barazone or Placebo

	Treatment		P-value
	Barazone	Placebo	
QoL Score	8.5 (8.1 – 8.9)	7.3 (6.5–8.1)	<0.001

QoL Score of 0 = poorest QoL and 10 = best QoL. Mean values are followed by the 95% confidence interval.

any major clinical problems related to Barazone or Placebo. Furthermore, there were no obvious differences in the general physical health of the dogs receiving either treatment.

Treatment effect on clinical pathology

Treatment with Barazone significantly reduced five (mean corpuscular haemoglobin; mean corpuscular haemoglobin concentration; white blood cell, neutrophil and monocyte counts) of the 13 haematology parameters assessed and increased one (urea) of the 16 measured serum chemistry parameters. However, the mean values for all haematology and biochemistry parameters remained within normal ranges¹ for both groups of dogs throughout the study.

DISCUSSION

This study has demonstrated that Barazone is a more efficacious treatment than Placebo for reducing the clinical signs of AD in dogs. Treatment with Barazone reduced the extent and severity of the dogs' AD skin lesions and improved their pruritus and QoL compared to Placebo. Furthermore, there were only minor differences in the clinical pathology results and physical examination findings and no differences in the tolerance scores between dogs receiving Barazone or Placebo.

The lower median Lesional Score of dogs at the commencement of treatment with Placebo compared to Barazone (64 vs. 96; see Fig. 2) was because of the improvement in skin lesions experienced by the Group 1 dogs on treatment with Barazone in the first 3 weeks of the study. At the start of treatment with Placebo, Group 1 dogs had significantly less extensive and severe lesions than the dogs in Group 2, who were allocated to receive Placebo first and thus, had not received an effective intervention in the previous weeks. Despite this difference, no effect of treatment order was detected on this outcome measure. The order of treatments did affect the Pruritus Scores of the groups, as the dogs in Group 1 were less pruritic on treatment with Placebo than the dogs in Group 2 (see Fig. 3). As the Group 1 dogs received Barazone for 3 weeks immediately prior to beginning treatment with Placebo, their reduced Pruritus Scores on Placebo were likely to be because of a carry-over effect from the Barazone treatment.

¹According to the reference ranges provided by the veterinary pathology laboratory that analysed all samples.

The higher mean serum urea concentrations observed following treatment with Barazone may be explained by the effect of glucocorticoids, which increase protein catabolism and subsequent hepatic synthesis of urea (Latimer *et al.*, 2003). Treatment was also found to have significantly affected the mean white blood cell count of Group 1 dogs, who exhibited a mild leukopaenia (because of neutropaenia and monocytopenia) on treatment with Barazone instead of the leukocytosis typically induced by glucocorticoids. However, the mean serum urea concentrations and white blood cell counts of both groups remained within their normal ranges at all times, suggesting that these changes were of minor clinical importance.

Although the dogs in the trial were selected from the canine population affected with AD, those with milder disease may have been over-represented in this study. The treatment restrictions preceding and during the trial, including three weeks on placebo with no effective treatment discouraged the inclusion of severely affected dogs. In consideration of the dogs' welfare, a wash-out period between treatment phases was omitted, to minimise the participating dogs' time without treatment and reduce the likelihood of patient withdrawals. Additionally, a cross-over design was selected as it was considered unethical to assign diseased dogs to a placebo treatment for the entire duration of the trial, and furthermore, fewer dogs would be required to demonstrate efficacy in a cross-over compared to a parallel study. A potential limitation of this cross-over design was the obvious effectiveness of Barazone compared to Placebo in many dogs. This could have alerted owners and veterinarians to the identity of the treatments, introducing a bias into their assessment of outcome measures.

The *Lesional Score* used as an outcome measure of treatment efficacy in this study was a modification of the CADESI-03 recommended by the *International Task Force on AD* as a validated tool for the assessment of disease severity of AD (Olivry *et al.*, 2007). This index was modified to increase its ease of use by grouping some body regions together, thus reducing the number of discrete regions assessed from 62 to 32. Additionally, crusts were included as an evaluated lesion within the excoriation score and an assessment of hyperpigmentation was included within the lichenification score of the CADESI-03. Atopic dermatitis can severely affect the quality of human patients' lives and consequently QoL is frequently assessed in clinical trials to determine the efficacy of interventions for this disease. Similarly, improving dogs' QoL is a fundamental goal of AD therapy, albeit no tool to measure this outcome in dogs has yet been validated (Hewson *et al.*, 2007). Nevertheless, a QoL measure consisting of a visual analogue scale with descriptions of the dog's behaviour at the extremities was designed for and used daily by owners in this study. As pruritus is the most significant symptom of AD, an estimation of its intensity is an important indicator of treatment efficacy. Owners were trained in using a visual analogue scale to estimate their dog's pruritus, which they then did daily during the study in order to capture relapses or remissions, as recommended by Olivry (Olivry *et al.*, 2007). Although this

method relied on the owners' opinions rather than an objective measure of pruritus, similar scales have been validated for the assessment of pruritus in dogs (Plant, 2007; Rybníček *et al.*, 2009).

The efficacy of once weekly application of Barazone in reducing skin lesions and pruritus in atopic dogs is comparable to that of two glucocorticoid sprays (Genesis and Cortavance) evaluated in similar studies (DeBoer *et al.*, 2002; Nuttall *et al.*, 2009). Like Barazone, Genesis and Cortavance are very low-concentration topical glucocorticoid formulations, designed to be applied to and left on the skin. However, the frequency of application in efficacy studies was higher for both Genesis and Cortavance than for Barazone, with Cortavance applied once daily and Genesis applied twice daily for 1 week, then once daily for 1 week, and then on alternate days for 2 weeks. The efficacy of Genesis in reducing the clinical signs of pruritic skin disease in 103 pruritic dogs was evaluated in a randomized, double-blinded, placebo-controlled, parallel trial, with dogs assigned to receive either Genesis or Placebo (DeBoer *et al.*, 2002). In accordance with our results for Barazone, there were significant improvements in all veterinarian assessed outcome measures (pruritus, erythema, eruptions and overall clinical score) on treatment with Genesis. Specifically, the mean scores for pruritus, erythema and eruptions more than halved in the Genesis treated dogs and treatment success (defined as an improvement of at least two of six grades in overall clinical score) was achieved in 35 of 52 (67%) Genesis treated dogs and 12 of 51 (24%) placebo treated dogs. Treatment efficacy was also verified by owners, with Genesis receiving a significantly higher overall effectiveness rating than placebo (DeBoer *et al.*, 2002). The efficacy of Cortavance in the management of AD was similarly demonstrated in 28 atopic dogs, using a randomized, double-blinded, placebo-controlled, parallel trial, with dogs assigned to receive either Cortavance or Placebo (Nuttall *et al.*, 2009). After 28 days of treatment, significant differences in veterinarian assessed outcome measures (CADESI-03) and owner assessed outcome measures (pruritus and owner satisfaction) were observed between treatment groups. Cortavance treated dogs experienced reductions in CADESI and pruritus of 61.4 and 38.8%, respectively, whereas placebo treated dogs experienced a reduction in CADESI of 13.4% and an increase in pruritus of 57.6%. Correspondingly, owner satisfaction with treatment was also significantly higher for the Cortavance treated dogs (Nuttall *et al.*, 2009).

The absence of adverse effects or clinically relevant changes in haematology and serum chemistry parameters on treatment with Barazone compared to Placebo, support the findings of previous studies into the safety of topical glucocorticoids formulated for widespread use on dogs. For example, DeBoer *et al.* found no differences in the haematology and biochemistry parameters, or in the incidence of adverse effects in the dogs treated 28 times with Placebo or Genesis over four weeks (DeBoer *et al.*, 2002). Similarly, there were no significant changes in haematology and biochemistry parameters, no evidence of abnormal adrenal function and no adverse events

in the dogs treated daily with Cortavance over four weeks (Nuttall *et al.*, 2009). A 1% hydrocortisone leave-on conditioner applied twice weekly for six weeks in seven pruritic dogs and eight normal dogs produced no adverse effects and only minor changes in blood parameters. All dogs retained normal adrenal function during the study, although the pruritic dogs had significantly lower post-ACTH cortisol concentrations than the normal dogs (Thomas *et al.*, 1999), demonstrating the importance of evaluating the safety of a novel topical glucocorticoid formulation in its intended patient population. The safety of a 0.01% fluocinolone shampoo used twice weekly for six months was established in nine dogs with allergic dermatitis. Again, treatment had no effect on the adrenal-pituitary axes, serum chemistries or complete blood counts of the study dogs (Beale *et al.*, 2000). The importance of assessing the hypothalamic-pituitary-adrenal axes of dogs treated with topical glucocorticoids was highlighted in a study that demonstrated their reversible suppression of the adrenal function of healthy dogs (Zenoble & Kemppainen, 1987). However, as the primary objective of the current study was to determine the efficacy of Barazone, the adrenal function of the study dogs was not evaluated.

Topical glucocorticoids have been associated with a number of grossly evident, cutaneous, adverse effects including comedo formation (Gross *et al.*, 1992, 1997), pigmentation changes (Gross *et al.*, 1992; Kimura & Doi, 1999), ulceration (Kimura & Doi, 1999), skin blistering (Gross *et al.*, 1997), alopecia (Gross *et al.*, 1992; Zerbe & MacDonald, 1993), slow hair growth (Kimura & Doi, 1999) and calcinosis cutis (Gross & Ihrke, 1992). Of these potential effects, only slow re-growth of clipped hair was observed during this study, in one dog (028). This was likely to have resulted from Barazone treatment, as glucocorticoids interfere with the hair growth cycle (Latimer *et al.*, 2003). A tolerance study of longer duration is necessary to investigate the incidence of this side effect with longer-term Barazone treatment. Histologically evident, cutaneous changes induced by topical glucocorticoids include epidermal atrophy (Kimura & Doi, 1999; Beale *et al.*, 2000), sebaceous gland atrophy (Beale *et al.*, 2000), a reduction in dermal thickness and elasticity (Kimura & Doi, 1999; Beale *et al.*, 2000) and vascular changes (Zerbe & MacDonald, 1993). Skin biopsies were not collected in this study, thus the incidence of such changes in response to Barazone is unknown.

This study has shown that Barazone applied once-weekly at 1 g/kg is a more efficacious treatment than Placebo for reducing the clinical signs of AD in dogs. Treatment with Barazone reduced the extent and severity of skin lesions, reduced pruritus and improved QoL in atopic dogs within three weeks. Additionally, there were no differences in the tolerance scores and only minor differences in the physical examination findings and the haematology and biochemistry parameters of the dogs when treated with Barazone compared to Placebo. Further research is needed to determine the efficacy and safety of longer-term treatment with Barazone and in a study population including dogs with a wider-range of AD severities.

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