

# Effect of a mixture of essential oils and a plant-based extract for the management of localized superficial pyoderma in dogs: An open-label clinical trial

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## Abstract

A mixture of essential oils and a plant-based extract in the form of shampoo and spot-on were shown to have *in vitro* antimicrobial activity against *Staphylococcus pseudintermedius*. The purpose of this open-label clinical trial was to evaluate the efficacy of these two products for the management of canine localized superficial pyoderma. Twenty dogs diagnosed with 3-4 localized pyoderma lesions were enrolled. The dogs were bathed weekly for 8 weeks with shampoo and 48 hours later, spot-on was applied near the lesions. Clinical lesions and pruritus scores were subjectively assessed using a clinical improvement score system (1-5) and a pruritic visual analog scale (0-10) on days 0, 14, 28, 42, and 56. Wilcoxon Signed-Rank Test was used to compare the two parameters between day 0 and days 14, 28, 42, and 56. Sixteen dogs completed the study and four discontinued prematurely due to worsening of clinical signs. The average clinical improvement scores on day 14 ( $2.6 \pm 0.5$ ), day 28 ( $2.05 \pm 0.6$ ), day 42 ( $1.63 \pm 0.7$ ) and day 56 ( $1.13 \pm 0.5$ ) were significantly better than day 0 (5) (P value  $< 0.0001$ ). The average pruritus scores on day 14 ( $3.75 \pm 1.37$ ), day 28 ( $3.15 \pm 2.01$ ), day 42 ( $2.26 \pm 2.45$ ) and day 56 ( $0.81 \pm 1.56$ ) were significantly better than day 0 ( $4.90 \pm 1.74$ ) (P value  $< 0.007$ ). In conclusion, the weekly bath with shampoo and topical spot-on may be an option to manage localized superficial pyoderma. A randomized, double-blinded, placebo-controlled study should be conducted to support the results.

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**Keywords:** essential oil and plant-based extract shampoo, spot-on, superficial pyoderma, dog

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## Introduction

Pyoderma is a very common skin disease of dogs and is typically secondary to underlying disorders such as allergies (atopic dermatitis, flea allergic dermatitis, cutaneous adverse food reaction), ectoparasitosis (demodectic, sarcoptic mange) and endocrinopathy (hyperadrenocorticism, hypothyroidism) (Hill et al., 2006; Miller et al., 2013). *Staphylococcus pseudintermedius* is considered to be the most common cutaneous pathogen of canine bacterial skin disease (Bannoehr and Guardabassi, 2012; Miller et al., 2013).

Due to the increased incidence of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) and multidrug-resistant strains (MDR), investigations into topical antimicrobial shampoo to manage canine superficial pyoderma have been conducted in order to avoid using systemic antimicrobial drugs (Kloos et al., 2013; Murayama et al., 2010; Young et al., 2011; Valentine et al., 2012). A major advantage of using topical antimicrobial products is their ability to attain high local concentration on corneocytes with minimal systemic absorption and to decrease the risk of systemic adverse effects. They also physically remove crusts, debris, and bacterial from the skin surface (Mueller et al., 2012; Seltzer et al., 2010).

An *in vivo* study in which a combination of 4% chlorhexidine shampoo and solution was used for 4 weeks demonstrated the same efficacy with oral antibiotic for treatment of canine superficial pyoderma (Borio et al., 2015). Twice-weekly shampoo with 2% chlorhexidine as a sole therapy also showed improvement in five out of eight dogs diagnosed with MRSP (Murayama et al., 2010). Two studies compared the efficacy between two shampoos as a monotherapy for dogs with superficial pyoderma. The first study compared three-times weekly bath with either 2.5% Benzoyl peroxide shampoo (n=10) or ethyl lactate shampoo (n=30) for 4 weeks; 90% and 70% of dogs had excellent or good response, respectively (Ascher et al., 1990). The second study demonstrated no significant difference between twice-weekly bath with 2.5% benzoyl peroxide shampoo and 3% chlorhexidine shampoo, but the fastest response was observed in dogs treated with the chlorhexidine shampoo (Loeffler et al., 2011). The *in vitro* study of chlorhexidine shampoo supported the *in vivo* study, which shows that this product is considered the most effective topical biocide. However, the *in vitro* study of benzoyl peroxide and ethyl lactate shampoos did not replicate antimicrobial efficacy as demonstrated in the *in vivo* study (Young et al., 2011; Kloos et al., 2013). In order to consider a topical shampoo as a sole therapy for pyoderma, the product should be studied both *in vitro* and *in vivo*.

Two preliminary *in vitro* studies of the antimicrobial activity of two commercial products in the forms of shampoo and spot-on, containing a mixture of essential oils and a specific plant-based natural complex against *Staphylococcus pseudintermedius* and *Malassezia pachydermatis* suggested that these products could be used as adjunctive cares for the treatment and prevention of pyoderma and *Malassezia* dermatitis (Bensignor et al.,

2012, 2015; Bergvall and Varjonen, 2013). Both shampoo and spot-on contained essential fatty acids from a vegetable source combined with a natural complex of plant extracts and essential oils with antimicrobial properties, called PhytoC-2®. This combination of active ingredients is specific for each formulation and based on essential oils of manuka, lavender, eucalyptus, savory, rosemary, oregano, palmarosa, associated with propolis, neem and ajowan plant extracts. A recent study of a topical spray containing similar essential oils and plant extracts demonstrated its efficacy to speed the resolution of superficial pyoderma when used concurrent with systemic antimicrobial (Bensignor et al., 2016).

The aim of this open-label clinical trial was to evaluate the *in vivo* efficacy of a shampoo and a spot-on containing essential oils and a specific plant-based natural complex as an alternative option for the management of canine localized superficial pyoderma.

## Materials and Methods

**Study design:** This was an open-label, uncontrolled clinical trial.

**Animals:** Client-owned dogs, of any age, sex and breed were enrolled between October 2014 and March 2015. Written consent was required for the study participation.

**Inclusion criteria:** Included dogs must be diagnosed with localized superficial pyoderma. Superficial pyoderma was diagnosed based on the clinical lesions of papules, pustules, epidermal collarettes and scales. The criteria of localized lesion were determined as the dogs should not have superficial pyoderma lesions on more than 4 areas on the body. The infected areas were confirmed to have bacterial skin infection by skin cytology and bacterial culture. At initial visit, deep skin scrapings or trichograms were performed to check for demodex mange, and hair samples were collected for dermatophyte culture (Dermatophyte Test Media: Fisher Scientific, 300 Industry Drive, Pittsburgh) in order to rule out other causes of folliculitis. There was no limitation for ingredients of diet for participating dogs.

**Exclusion criteria:** Dogs were excluded from the trial if (1) they had received topical or systemic antimicrobial treatments within 14 days prior to the enrollment date, (2) dermatophyte culture showed positive results, (3) deep skin scrapings or trichograms showed positive result for demodectic mange, and (4) dogs had evidence of other systemic infections and required to be treated with systemic antibiotic.

**Withdrawn criteria:** A dog could be withdrawn from the study if the skin infection worsened >50% (based on the investigator's clinical scoring system) or per owner's request.

**Treatment:** The owners were instructed to bathe the dogs with a shampoo. The owner was recommended to lather the shampoo, starting with the infected area, then the other parts of the body, for 10 minutes before

rinsing it off. Forty-eight hours later, a spot-on was applied around the infected lesions. After application on the skin, the spot-on would be stored in the sebaceous glands before being progressively released and distributed along the skin layer in order to replenish the hydrolipidic film of the epidermis layer. The 48 hours after bath period was recommended to allow sufficient sebum for an efficient bio-diffusion of the spot-on all over the epidermis layer. The treatment was performed weekly for a total of 8 weeks. No other systemic therapies were permitted. Dogs were allowed to continue with their external parasite control and heart worm prophylaxis and thyroid treatment. After completing the study (day 56), the owners were recommended to continue with the weekly bath and application of spot-on every 2 weeks based on the manufacture's recommendation. There was no follow-up scheduled after 56 days.

**Cytological examination:** Cytology samples were collected at skin lesions on days 0, 14, 28, 42, and 56. The samples were collected by impression smear using glass slides at exudate ruptured by a sterile 25-gauge needle from papular or pustular lesions. For epidermal collarette lesion, samples were collected under crusts and/or at the margin of lesions. Impression slides were air-dried, stained with a DipQuick stain (Jorgensen Laboratories, Loveland CO, USA) and microscopic examination was performed under oil immersion (X1000). The average number of intracellular bacteria and inflammatory cells observed per oil immersion field on each slide was assigned a score as previously described (Budach and Mueller, 2012) from 0 to 4 (0: no organisms, 1: less than 1-2 organisms per field, 2: 2-5 organisms per field, 3: 5-20 organisms per field and 4: greater than 20 organisms per field).

**Bacteriological culture:** A bacteriological culture was taken at lesion sites from each dog on day 0 and day 56. A sterile culturette (BBL™ Culture swab, Copan™, Sparks, MD) was gently rolled across the exudate ruptured by a sterile 25-gauge needle from papular or pustular lesions. For epidermal collarette lesion, samples were collected under crusts and/or at the margin of lesions. The samples were submitted to the diagnostic laboratory for aerobic bacterial culture and antibiotic susceptibility testing. Susceptibility testing to antimicrobial agents was determined by disc diffusion using Kirby-Bauer disc. Report was classified as susceptible, intermediate or resistant according to the clinical breakpoints described by the Clinical and Laboratory Standard Institute (CLSI).

**Investigator's assessment of treatment efficacy:** Improvement in clinical lesions including papules, pustules, epidermal collarettes, scales and skin cytology were subjectively evaluated on days 0, 14, 28, 42, and 56 by the same investigator. The improvement in clinical lesions was determined by resolution of clinical signs and skin cytology. Parameters of clinical improvement were recorded by using a score system (5: no improvement, 4: <25%, 3: 25-49%, 2: 50-74%, and 1: 75%-100% improvement). Pictures of affected areas were taken at each visit to objectively document progress.

**Owner's assessment of treatment efficacy:** Owners were asked to evaluate the pruritus score by using a visual analog scale (Hills et al., 2001) (0=no pruritus, 10=severe pruritus) on days 0, 14, 28, 42, and 56.

**Data analysis:** Descriptive statistics were used to describe the demographics and baseline characteristic data. Percentage of the treated dogs that achieved >75-100% of clinical improvement was reported. Wilcoxon Signed-Rank Test was used to assess changes in clinical improvement and pruritus scores from baseline (day 0) throughout the trial. The clinical improvement and pruritus scores from each dog on days 14, 28, 42, and 56 were compared with day 0. Statistical significance was declared when P value <0.05.

## Results

The study consisted of 20 dogs, including 13 female dogs (65%) and 7 male dogs (35%), with an average age of 6.75 years old (SD=4.09) and an average weight of 8.04 Kg (SD= 5.55). There were 12 breeds of dogs including 5 small breeds, which were Poodles (2), Pomeranian (2), Chihuahua (2), Miniature Pincher (4) and Yorkshire Terrier (2); 5 medium breeds, which were Dachshund (1), Beagle (1), French Bulldog (1), Scottish Terrier (1) and Pug (1); one large breed, which was Siberian Husky (1); and 2 cross-bred dogs (Table 1). Sixteen dogs (80%) completed the study while four dogs discontinued prematurely due to worsening of clinical signs. One dog discontinued after day 28 (5%) and three dogs discontinued after day 42 (15%). One out of the 16 dogs which completed the study did not show any improvement on day 56, whereas the remaining fifteen dogs showed 75-100% improvement (15 out of 20 dogs: 75%). At the initial visit (day 0), all twenty dogs (100%) had superficial bacterial folliculitis and the skin culture revealed *Staphylococcus* spp.

Two parameters (clinical improvement and pruritus visual analog scores: PVAS) were compared between baseline (day 0) and days 14, 28, 42, and 56. Clinical improvement was analyzed using Wilcoxon Signed-Rank Test for comparison of clinical improvement scores between day 0: 5 (SD: 0), day 14: 2.6 (SD: 0.50), day 28: 2.05 (SD: 0.60), day 42: 1.63 (SD: 0.68) and day 56: 1.13 (SD: 0.50). The comparison between days 0, 14, 28, 42 and 56 revealed significant improvement (P value <0.0001) (Table 2 and Figure 1).

Pruritus scores were also analyzed using Wilcoxon Signed-Rank Test, comparing scores between day 0: 4.90 (SD: 1.74), day 14: 3.75 (SD: 1.37), day 28: 3.15 (SD: 2.01) day 42: 2.26 (SD: 2.45) and day 56: 0.81 (SD: 1.56). The comparison of pruritus scores between day 0 and days 14, 28, 42 and 56 revealed significant improvement (P value <0.007) (Table 3 and Figure 2). Figures 3 to 7 show pre-treatment (day 0) and post-treatment (days 14, 28, 42, and 56) of one participating dog.

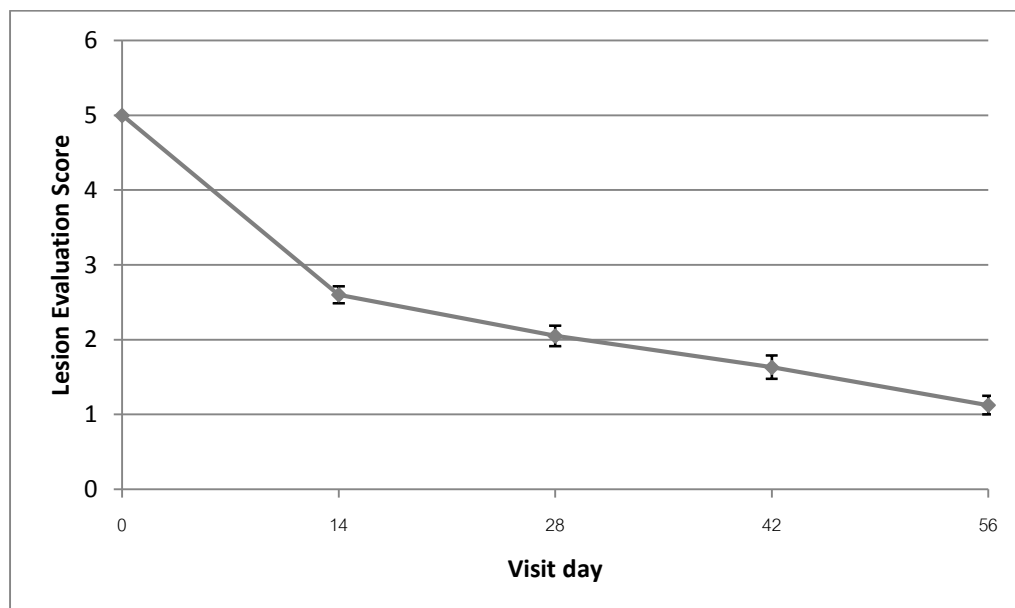
Skin cytology was compared between day 0 and day 56 using Wilcoxon Signed-Rank Test. The means of day 0 and day 56 were 2.25 (SD: 1.25) and 0.85 (SD: 1.46) (P value <0.0024), respectively.

**Table 1** List of participating dogs

N° of dog	Age (y)	Sex	Breed	Weight (kg)	Finished Yes or No
1	10	F	Poodle	5.7	Yes
2	6	M	Pomeranian	2.8	Yes
3	3	F	Siberian Husky	20	Yes
4	11	F	Miniature pinscher	4.68	Yes
5	10	M	Miniature pinscher	5	Yes
6	13	M	Miniature pinscher	6.5	Yes
7	5	M	Pomeranian	6	Yes
8	15	F	Cross Breed	22	Yes
9	6	M	Chihuahua	3.1	Yes but not better
10	6	F	Cross Breed	12	Yes
11	2	F	Dachshund	8.3	Yes
12	8	F	Beagle	11.1	No (after D42)
13	1	F	Yorkshire Terrier	2.44	Yes
14	1	F	Chihuahua	2	Yes
15	6	M	Poodle	6.7	No (after D42)
16	11	F	Yorkshire Terrier	2	No (after D42)
17	2	M	French Bulldog	13	No (after D28)
18	4	F	Scottish Terrier	10	Yes
19	5	F	Pug	7.5	Yes
20	10	F	Miniature pincher	10	Yes

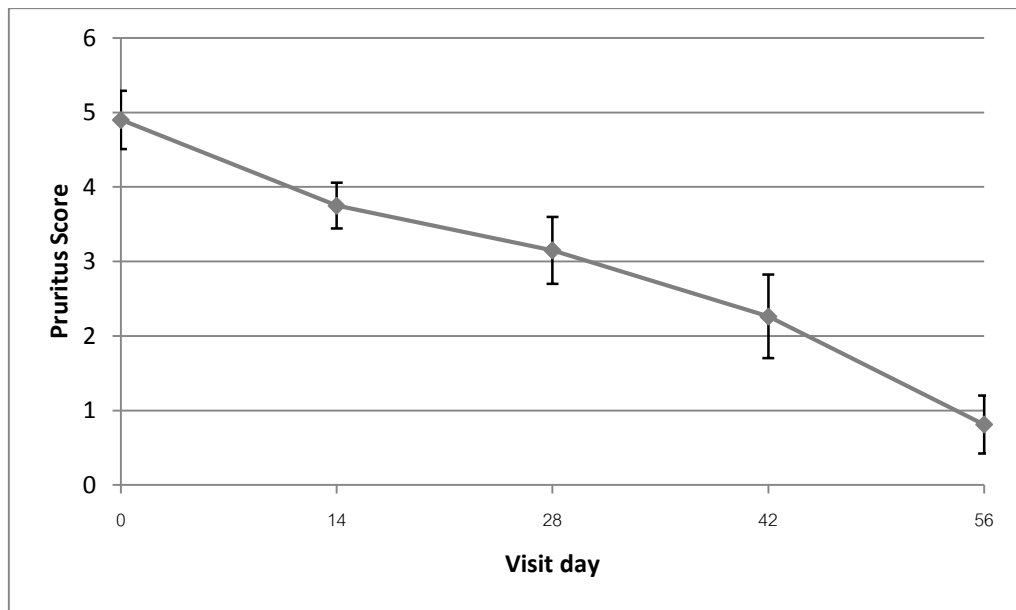
**Table 2** Summary of lesion evaluation scores by visit (only 16 dogs completed all visits)

Visit day	Number of cases	Lesion evaluation scores (P value <0.0001)					
		Mean	Standard deviation	Standard error	Maximum	Median	Minimum
0	20	5	0	0	5	5	5
14	20	2.60	0.50	0.11	3	3	2
28	20	2.05	0.60	0.14	3	2	1
42	19	1.63	0.68	0.16	3	2	1
56	16	1.13	0.50	0.12	3	1	1

**Figure 1** Mean lesion evaluation score over time  $\pm$  SE (P value <0.0001)

**Table 3** Pruritus score over time

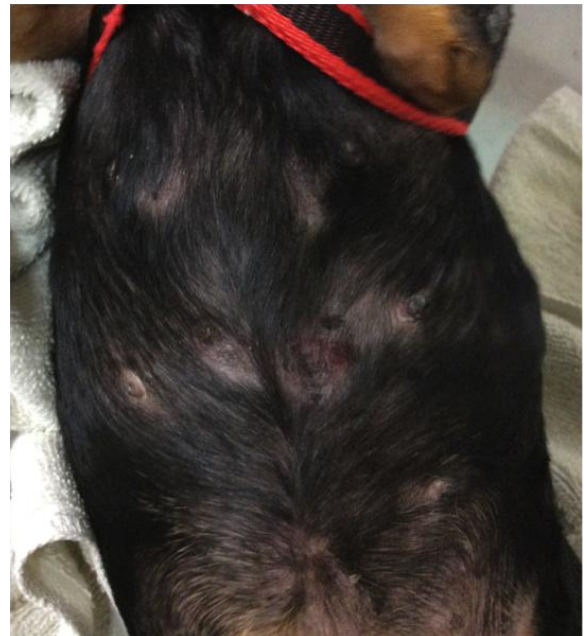
Visit day	Number of cases	Pruritus scores (P value <0.007)					
		Mean	Standard deviation	Standard error	Maximum	Median	Minimum
0	20	4.9	1.74	0.39	8	5	2
14	20	3.75	1.37	0.31	8	4	2
28	20	3.15	2.01	0.45	8	2.5	0
42	19	2.26	2.45	0.56	8	2	0
56	16	0.81	1.56	0.39	5	0	0



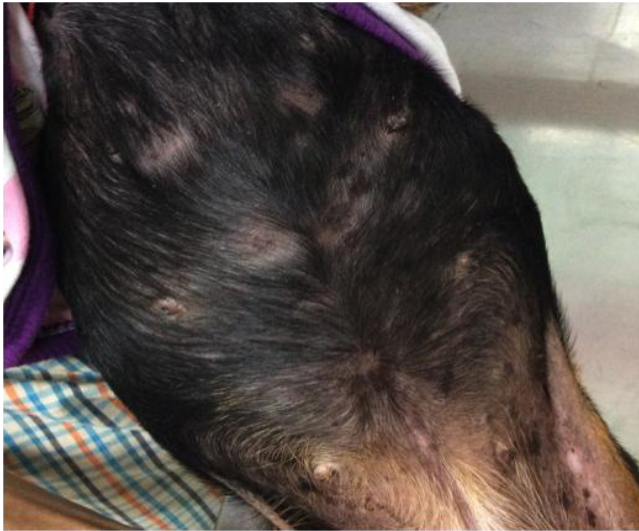
**Figure 2** Pruritus score over time ± SE (P value <0.007)



**Figure 3** Picture on day 0: pre-treatment



**Figure 4** Picture on day 14: post-treatment



**Figure 5** Picture on day 28: post-treatment



**Figure 6** Picture on day 42: post-treatment



**Figure 7** Picture on day 56: post-treatment

### ***Discussion***

The results of this study support our hypothesis that weekly bath with a shampoo containing specific essential oils and plant extracts followed by topical application of a spot-on could be considered as an alternative option for the management of localized superficial pyoderma. Fifteen dogs among twenty dogs (75%) showed 75-100% improvement from clinical improvement and pruritus scores over 8 weeks of treatment. Bacterial culture and susceptibility of pyoderma lesions on each dog were performed on day 0 prior to starting the topical management in order to confirm the causative pathogenic bacteria. All dogs had skin cultures positive for *Staphylococcus* spp.

Both shampoo and spot-on were based on

natural plant extracts and essential oils specifically selected for their antimicrobial properties. The essential oil of manuka in the shampoo and the essential oil of eucalyptus in the spot-on formulation are demonstrated to contain high activity *in vitro* against Gram-positive bacteria and to be potent inhibitors of *Staphylococcus pseudintermedius* biofilm formation (Harkenthal et al., 1999; Song et al., 2013). Neem and ajowan plant extracts are known for their antimicrobial properties (Boskabady et al., 2014; Raut et al., 2014) and propolis is effective against *Malassezia pachydermatis* and *Staphylococcus pseudintermedius* (Cardoso et al., 2010). Essential fatty acids from hemp seed oil in both shampoo and spot-on help to reinforce the skin barrier as it is essential components of this structure (Cerrato et al., 2013). It has been reported that defective skin barrier in atopic dogs and human can

predispose them to have bacteria adherence and prone to get recurrent pyoderma (Marsella et al., 2011; Santoro et al., 2015).

In order to monitor the efficacy of these two topical products, two criteria were used, the clinical improvement and the pruritic scores, which were determined between day 0 and days 14, 28, 42 and 56. Superficial pyoderma was diagnosed by using a combination of clinical signs, semi-quantitative number of bacteria from cutaneous cytology, bacterial culture and exclusion of other diseases including dermatophytosis and ectoparasites.

For the clinical improvement score, our study used the investigator's interpretation by evaluation of the improvement in clinical lesions such as papules, pustules, epidermal collarettes, together with skin cytology results on days 14, 28, 42, and 56 after topical management compared with day 0. The clinical evaluation score and cytology were subjectively performed by the same investigator in order to minimize potential variability between clinicians. The pruritic score was subjectively evaluated by the owner. However, parameters such as pruritus have also been notoriously difficult to access due to the lack of a standardized scoring system (Hill et al., 2007). In this study, the owner's assessment on pruritus scores correlated well with the clinical improvement. The improvement in pruritus scores was possibly due to the clinical resolution of superficial pyoderma. It was suspected that the dogs from this study had underlying causes in forms of allergies (food, flea or environment) due to the history of recurrent superficial pyoderma. The definitive diagnosis of allergies has not been concluded in eleven dogs. Five dogs were better by food elimination trial, but the owners refused to perform provocative challenge. Two dogs had intradermal skin test and had strong positive reaction to house dust mite and were treated with allergen specific immunotherapy. One dog was confirmed with a hypothyroid condition and one dog had flea-bite allergy.

The eight-week period of this study was designed based on the company's recommendation to apply the spot-on weekly for 8 weeks as a protocol for adjunctive management with systemic antibiotic treatment. According to previous studies, a topical therapy with twice-weekly bath for 4 weeks with an antimicrobial shampoo was demonstrated to improve clinical signs of pyoderma (Murayama et al., 2010; Loeffler et al., 2011; Borio et al., 2015). The present study chose to perform a weekly bath with the shampoo. However, in order to improve the outcome of the management of superficial pyoderma, twice-weekly shampoo combined with weekly application of the spot-on could be considered. Based on the clinical improvement score in this study, which significantly rose on day 28, the duration of the treatment could be decreased to 4 weeks in order to improve owner's compliance. Moreover, the owner should be informed about giving a minimum of 2 days after bath prior to the spot-on application.

The satisfactory results from this study could also be due to the careful recruitment of participating dogs, which were mainly short-haired, suffering from recurrent superficial pyoderma. Owner compliance

was also another important factor to achieve good results from topical management. The dog owners of this study were well informed of increased risk of bacterial resistance after repetitive treatments with systemic antibiotics (Eckholm et al., 2013). They were willing to try topical management in order to avoid using systemic antimicrobial. The follow-up after day 56 was not decided for this study because the owners were recommended to continue with the weekly bath and application of spot-on every 2 weeks based on the manufacturer's recommendation to prevent the recurrence of pyoderma. According to the literature of Hillier et al. (2014), weekly bath with antimicrobial shampoo should be able to decrease amount of bacterial overgrowth of the skin and prevent recurrence of clinical lesions when the underlying causes of superficial pyoderma are controlled.

To date, there are no published data showing the occurrence of chlorhexidine resistance in *Staphylococcus pseudintermedius*. An increasing number of studies also suggested the development of reduced susceptibility to chlorhexidine in human *Staphylococcus aureus* isolates due to efflux pump resistance linked to *qacA* gene mutation (Horner et al., 2012). The efficacy of a mixture of essential oils and a complex of plant extracts in the form of shampoo and spot-on should be investigated in clinical trials to prove them suitable as an alternative topical option for the management of localized superficial pyoderma besides chlorhexidine shampoo.

The limitation of this study is the fact that the results were obtained through an open-label clinical trial with a relatively small number of dogs. Localized superficial pyoderma was preferred to test the *in vivo* efficacy of these two topical products. After obtaining the satisfactory outcome from this clinical trial, a randomized double-blinded, placebo-controlled study could be performed in order to confirm the efficacy of these two topical products for generalized superficial pyoderma.

## Conclusion

The combination of weekly bath with a shampoo and topical application of a spot-on containing a specific mixture of essential oils and plant extracts may be considered as an alternative option to manage localized superficial pyoderma. A randomized, double-blinded, placebo-controlled study could be conducted to provide more information.

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## References

- Ascher F, Maynard L, Laurent J et al. 1990. Controlled trial of ethyl lactate and benzoyl peroxide shampoo in the management of canine surface and superficial pyoderma. In: von Tscharner C, Haliwell REW, eds. *Advanced in Veterinary*

- Dermatology. Volume 1. London: Bailliere Tindall. P. 375-382.
- Bannoehr J and Guardabassi L. 2012. *Staphylococcus pseudintermedius* in the dog: taxonomy, diagnosis, ecology, epidermiology and pathogenicity. *Vet dermatol*:23(4):253-266.
- Bensignor E, Fabries L, and Baileus L 2016. A split-body, randomized, blinded study to evaluate the efficacy of a topical spray composed of essential oils and essential fatty acids from plant extracts with antimicrobial properties. *Vet Dermatol*. 27(2): 464-e123.
- Bensignor E, Fabries L and Martin-Vo 2012. *In vitro* antimicrobial activity of a spot-on containing a mixture of essential oils and a plant extract against *Staphylococcus pseudintermedius* and *Malassezia pachydermatis* In: Proceeding of the seventh World Congress of Veterinary Dermatology. WCVD, Vancouver, Canada.
- Bensignor E, Fabries L and Martin-Vo 2015. *In vitro* antimicrobial activity of a product range containing plant antimicrobials against *Staphylococcus pseudintermedius* and *Malassezia pachydermatis* In: Proceedings of the American Academy of Veterinary Dermatology & American College of Veterinary Dermatology. ACVD, Nashville, TN.
- Bergvall K and Varjonen K 2013. The *in vitro* antimicrobial activity of a spot on containing a mixture of essential oils and a plant extract against *Staphylococcus pseudintermedius* and *Malassezia pachydermatis*. (Lionel Fabriès). *Advances in Veterinary Dermatology* vol.7 Wiley-Blackwell. p. 325-326.
- Borio S, Colombo S, La Rosa G, De Lucia M, Damborg P and Guardabassi L 2015. Effectiveness of a combined (4% Chlorhexidine digluconate shampoo and solution) protocol in MRS and non-MRS canine superficial pyoderma: a randomized, blinded, antibiotic controlled study. *Vet dermatol*. 26(5): 339-344.
- Boskabady MH, Alitaneh S and Alavinezhad A 2014. *Carum copticum* L.: *Carum copticum* L: a herbal medicine with various pharmacological effects. *BioMed Research International*, vol. 2014, 11 p.
- Budach S and Mueller R 2012. Reproducibility of a semiquantitative method to assess cutaneous cytology. *Vet dermatol*. 23(5):426-e80.
- Cardoso RL, Maboni F, Gustavo M, Hartz A and Agueda C 2010. Antimicrobial activity of propolis extract against *Staphylococcus* coagulase positive and *Malassezia pachydermatis* of canine otitis. *Veterinary Microbiology*, vol. 142(3-4): 432-434.
- Cerrato S, Ramió-Lluch L, Fondevila D, Rodes D, Brazis P and Puigdemont A 2013. Effects of Essential Oils and Polyunsaturated Fatty Acids on Canine Skin Equivalents: Skin Lipid Assessment and Morphological Evaluation, *Journal of Veterinary Medicine*. e231526.
- Eckholm N, Outerbridge C, White S and Sykes J 2013. Prevalence and risk factors for isolation of methicillin resistant *Staphylococcus* spp. form dogs with pyoderma in northern California, USA, *Vet Dermatol*. 24(11): 154-161.
- Harkenthal M, Reichling J, Geiss HK et al 1999. Comparative study on the *in vitro* antibacterial activity of Australian tea tree oil, cajuput oil, niaouli oil, manuka oil, kanuka oil and eucalyptus oil. *Pharmazie*. 54(6): 460-463.
- Hill PB, Lau P and Rybnicek J 2007. Development of an owner-assessed scale to measure the severity of pruritus in dogs, *Vet dermatol*. 18(5):301-308.
- Hill PB, Lo A, Eden CA et al 2006. Survey of the prevalence, diagnosis and treatment of dermatological conditions in small animal in general practice. *Vet Rec*. 158(16): 533-539.
- Hillier A, Lloyd D, Weese S, Blondeau J, Boothe D, Breitschwerdt E, Guardabassi L, Papich M, Rankin S, Turnidge J and Sykes J 2014. Guidelines for the diagnosis and antimicrobial therapy for canine superficial bacterial folliculitis (Antimicrobial Guidelines Working Group of the International Society for Companion Animal Infectious Diseases). *Vet Dermatol*. 25(3):163-165.
- Horner C, Mawer D and Wilcox M 2012. Reduced susceptibility to chlorhexidine in *Staphylococci*: is it increasing and does it matter? *J antimicrob Chemoth*. 67(11): 2547-2559.
- Kloos J, Straubinger RK, Werckenthin C and Mueller R 2013. Residual antibacterial activity of dog hairs after therapy with antimicrobial shampoos. *Vet dermatol*. 24: 250-254.
- Loeffler A, Cobb MA and Bond R 2011. Comparison of a chlorhexidine and a benzoyl peroxide shampoo as a sole treatment in canine superficial pyoderma. *Vet Rec*. 169(10): 249.
- Marsella R, Olivry T and Carlotti D 2011. Current evidence of skin barrier dysfunction in human and canine atopic dermatitis, *Vet dermatol*. 22(3): 239-248.
- Miller WH, Griffin CE and Campbell KL. 2013. *Bacterial skin diseases in Muller and Kirk's Small Animal Dermatology* 7<sup>th</sup> edition Elsevier Saunders St. Louis, MO p. 184-222.
- Mueller R, Bergvall K, Bensignor E and Bond R 2012. A review of topical therapy for skin infections with bacteria and yeast. *Vet Dermatol*. 23(4): 330-341.
- Murayama N, Masahiko N, Terada Y, Shibata S and Fukata T 2010. Efficacy of a surgical scrub including 2% chlorhexidine acetate for canine superficial pyoderma. *Vet dermatol*. 21(6): 586-592.
- Raut RR, Sawant AR and Jamge BB 2014. Antimicrobial activity of *Azadirachta indica* (Neem) against pathogenic microorganisms. *Journal of Academia and Industrial Research*, 3(77): 237-329.
- Santoro D, Marsella R, Pucheu-Haston C, EisenschenK M, Nuttal T, and Bizikova P 2015. Review: pathogenesis of canine atopic dermatitis: skin barrier and host-micro-organism interaction, *Vet dermatol*. 26(2): 84-e25.
- Seltzer J, Flynn-Lurie A, Marsella R, and Brennan M 2010. Investigation of the clinical efficacy of 0.2% topical stannous fluoride for the treatment of canine superficial pyoderma: a prospective, randomized, double-blinded, placebo-controlled trial. *Vet Dermatol*. 21(3): 249-258.

- Song SY, Nam E, Park SH and Hwang C 2013. In vitro efficacy of the essential oil from *Leptospermum scoparium* (manuka) on antimicrobial susceptibility and biofilm formation in *Staphylococcus pseudintermedius* and biofilm formation in *Staphylococcus pseudintermedius* isolates from dogs. *Vet Dermatol.* 24(4): 404-408.
- Valentine BK, Dew W, Yu A and Weese JS 2012. In vitro evaluation of topical biocide and antimicrobial susceptibility of *Staphylococcus pseudintermedius* from dogs, *Vet dermatol.* 23(6):493-e950.
- Young R, Buckley L, McEwan N and Nuttal T 2011. Comparative in vitro efficacy of antimicrobial shampoos: a pilot study. *Vet dermatol.* 23: 36-e8.

## บทคัดย่อ

### การศึกษาประสิทธิภาพของแชมพูและยาหยดบนผิวหนังที่มีส่วนประกอบของน้ำมันหอมระเหย และสารสกัดจากพืชในการรักษาสุนัขที่มีการติดเชื้อของผิวหนังแบบไม่กระจายทั่วตัว (localized pyoderma): การศึกษาแบบ open-label clinical trial

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การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาการใช้ผลิตภัณฑ์แชมพู ร่วมกับยาหยดบนผิวหนังที่มีส่วนประกอบของน้ำมันหอมระเหย และสารสกัดจากพืชในการรักษาสุนัขที่มีการติดเชื้อแบคทีเรียของผิวหนังแบบไม่กระจายทั่วตัว (localized pyoderma) การศึกษานี้อ้างอิงจากรายงานประสิทธิภาพในห้องปฏิบัติการของการใช้ผลิตภัณฑ์ทั้งสองชนิดนี้ในการกำจัดเชื้อแบคทีเรียชนิด *Staphylococcus pseudintermedius* สำหรับการศึกษา มีสุนัขที่เข้าร่วมการศึกษาทั้งหมด 20 ตัว โดยสุนัขทุกตัวมีการติดเชื้อแบคทีเรียของผิวหนังแบบไม่กระจายทั่วตัว (localized superficial pyoderma) ในวันแรกของการศึกษา สุนัขที่ผ่านขั้นตอนการคัดเลือกต้องมีรอยโรคที่บ่งชี้ว่ามีการติดเชื้อแบคทีเรีย และได้รับการประเมินปริมาณของจำนวนเชื้อแบคทีเรียของผิวหนัง รวมถึงได้รับการวินิจฉัยเพิ่มเติมว่าไม่ได้เป็นโรครื้อนขุมขนและเชื้อรา สุนัขที่เข้าร่วมการศึกษาต้องไม่ได้รับยาปฏิชีวนะมาเป็นเวลาอย่างน้อย 2 สัปดาห์ เจ้าของทำการอาบน้ำสุนัขด้วยแชมพู และหลังจากอาบน้ำ 48 ชั่วโมง เจ้าของหยอดยาหยดบนผิวหนังที่กลางหลัง หรือรอบๆ บริเวณติดเชื้อ ทำการรักษาซ้ำทุกอาทิตย์เป็นเวลา 8 สัปดาห์ การประเมินประสิทธิภาพของผลิตภัณฑ์สองตัวนี้ในการกำจัดเชื้อแบคทีเรียที่ผิวหนังทำโดยการวิเคราะห์รอยโรคของผิวหนังทุกๆ 2 สัปดาห์ โดยเปรียบเทียบจากวันแรกที่เข้ารับการศึกษา การประเมินทางสถิติใช้ Wilcoxon Signed-Rank Test โดยเปรียบเทียบ clinical improvement score จากวันที่ 0 กับวันที่ 14, 28, 42 และ 56 (คะแนน 1-5) และเจ้าของประเมินระดับความคัน โดยวัดจากระดับ 0-10 ใน การศึกษานี้สุนัข 16 ตัวได้รับการศึกษาจนครบ 8 อาทิตย์ และสุนัข 4 ตัวหยุดการศึกษาเนื่องจากรอยโรคของผิวหนังไม่ดีขึ้น การประเมินผลพบว่า การเปลี่ยนแปลงของรอยโรคที่ผิวหนังในวันที่ 14 (2.6+/-0.5) วันที่ 28 (2.05+/-0.6) วันที่ 42 (1.63+/-0.7) และวันที่ 56 (1.13+/-0.5) ดีขึ้นอย่างมีนัยสำคัญ (P value <0.0001) เมื่อเทียบกับวันที่ 0 (5) ระดับความคันวันที่ 14 (3.75+/-1.37) วันที่ 28 (3.15+/-2.01) วันที่ 42 (2.26+/-2.45) และวันที่ 56 (0.81+/-1.56) ลดลงอย่างมีนัยสำคัญ (P value <0.007) เมื่อเปรียบเทียบกับวันที่ 0 (4.9+/-1.74) จากการศึกษาสรุปได้ว่า ผลิตภัณฑ์แชมพูร่วมกับยาหยดบนผิวหนังที่มีส่วนประกอบของน้ำมันหอมระเหยและสารสกัดจากพืชมีประสิทธิภาพในการกำจัดเชื้อแบคทีเรียจากรอยโรคของผิวหนังที่มีการติดเชื้อชนิดแบบไม่กระจายทั่วตัว (localized pyoderma) การศึกษาครั้งต่อไปควรจะเป็นแบบ double-blinded control เพื่อสนับสนุนผลของการศึกษานี้

**คำสำคัญ:** แชมพูและยาหยดบนผิวหนังที่มีส่วนประกอบของน้ำมันหอมระเหยและสารสกัดจากพืช การติดเชื้อแบคทีเรียของผิวหนังแบบไม่กระจายทั่วตัว สุนัข

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