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Original Article

In Vivo efficacy of clove essential oil spray formulation on canine superficial pyoderma

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Abstract

Superficial pyoderma is a common skin disease caused by coagulase-positive staphylococci, especially *Staphylococcus pseudintermedius* and *Staphylococcus aureus*. The therapeutic use of antibiotics has many unwanted impacts, and the incidence of drug-resistant strains is increasing. Therefore, the aims of this non-inferior randomized controlled trial were to determine the effectiveness of 5% w/w clove essential oil lipid-based spray in treating superficial pyoderma in dogs, compared to 2% w/v chlorhexidine gluconate spray, and to evaluate the potential skin irritation of the clove oil spray formulation. The results demonstrated that the therapeutic effect of the clove essential oil spray was non-inferior to chlorhexidine gluconate spray when applied topically 2 times a day for 15 days. The clinical lesion index scores of dogs treated with clove essential oil spray were reduced by 66.87% on day 10 and 83.73% on day 15 after initiating treatment, with no adverse effects. The skin irritation test showed that clove essential oil spray did not induce erythema or edema at the tested sites. This report suggests that a lipid-based clove essential oil spray formulation is a suitable alternative topical treatment for canine superficial pyoderma.

Keywords: canine pyoderma, clinical trial, clove essential oil, spray, topical formulation

1. Introduction

Superficial pyoderma caused by coagulase-positive staphylococci is a common infectious skin disease in dogs and cats. The bacteria *S. pseudintermedius* and *S. aureus* are the primary causative pathogens of this disease and can cause secondary infections in other skin disorders (Gómez-Beltrán *et al.*, 2020; Older, Rodrigues Hoffmann, Hoover, & Banovic, 2020). Affected animals typically exhibit pustules, erythema, peripheral epidermal collarette with crusts, and alopecia (Miller, Griffin, & Campbell, 2013). Both systemic and topical antibacterial therapies are recommended for treatment of superficial infection in small animals. Systemic antibiotics that are recommended as the first-tier treatment for superficial pyoderma include the macrolides clindamycin or lincomycin, cephalosporins (cephalexin, cefadroxil, cefovecin, cefpodoxime), and amoxicillin-clavulanate. However, these antibiotics can cause adverse effects such as drug hypersensitivity, vomiting, diarrhea, and anorexia, and they can affect the kidney and liver (Giguère, Prescott, Baggot, Walker, & Dowling, 2006; Hillier *et al.*, 2014). In addition, many studies have reported the isolation of *Staphylococcus* strains from dogs with superficial pyoderma that are resistant to antibiotics such as cephalexin, oxacillin, amoxicillin-clavulanate, doxycycline, and enrofloxacin (Chaudhary, Kumar, & Shrivastva, 2019; González-Domínguez, Carvajal, Calle-Echeverri, & Chinchilla-Cárdenas, 2020; Silva *et al.*, 2021). The use of topical antimicrobial agents as a first-line treatment for superficial pyoderma has some advantages. Topical application can directly affect the bacteria in the skin lesion; and crusts, scales, and debris from the skin surface can be removed during application. In addition, topical antibiotics have been shown to enhance the therapeutic effects of systemic antibiotics while minimizing adverse effects and

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reducing the risk of developing drug resistant strains (Gortel, 2020; Hillier *et al.*, 2014). There are several topical formulations currently available for superficial pyoderma such as chlorhexidine gluconate and benzoyl peroxide shampoos, povidone iodine and gentamicin sulfate lotions, and clindamycin gels and sprays. However, these products may also cause skin irritation, erythema, a burning sensation, and photosensitivity (Koch, Torres, & Plumb, 2012). Therefore, the development of new effective drug formulas for superficial pyoderma without the associated adverse effects is becoming of interest.

Clove (*Syzygium aromaticum* (L.) Merr & L.M. Perry) is a medicinal plant that has many pharmacological properties including anticancer and anesthetic effects, nematicidal and insecticidal activity, and antioxidant, anti-inflammatory, and broad-spectrum antimicrobial activities (Kaur & Kaushal, 2019). A previous study demonstrated that clove essential oil has potent antibacterial activity against biofilms and planktonic cells of *S. pseudintermedius* isolated from skin lesions of canine superficial pyoderma (Aiemsaard *et al.*, 2020b). In addition, an *in vitro* time-kill kinetic study revealed that a lipid-based 5% w/w clove essential oil spray formulation could reduce the number of viable *S. pseudintermedius* cells by more than 99.9999% within 15 min (Aiemsaard, Kamollerd, Suwannathada, & Thongkham, 2020a). However, the *in vivo* efficacy of this clove spray formulation has not been investigated. Therefore, the current study aimed to investigate the effectiveness of clove essential oil spray in treating superficial pyoderma in dogs and evaluate the potential for skin irritation and other adverse effects.

2. Materials and Methods

2.1 Clove essential oil spray formulation

Clove essential oil extracted from the flower buds of *S. aromaticum* (L.) Merr. & L.M. Perry by steam distillation was purchased from Thai-China Flavors and Fragrances Industry Co., Ltd., Ayutthaya, Thailand, batch no. 6010334-1. This essential oil contained 98.87% eugenol and 1.13% trans-caryophyllene and the lipid-based preparation of 5% clove essential oil showed good *in vitro* antimicrobial activity (Aiemsaard *et al.*, 2020a). The clove essential oil (5% w/w) was mixed thoroughly with absolute ethyl alcohol (10% w/w, Merck, Germany) and polyoxyethylene (20) sorbitan monooleate (10% w/w, Ajax Finechem Pty Ltd., Australia) and the mixture was vortexed in a closed container for 5 min. Then isopropyl myristate (75% w/w, Namsiang Co., Ltd., Thailand) was added and mixed by vortex mixer until a homogeneous texture was observed. All ingredients were sterilized by autoclaving at 121ºC for 15 min except clove essential oil and absolute ethyl alcohol. All steps were performed in a biological safety cabinet (Biosafe 1.2, Heto-Holten, Denmark) using aseptic technique.

2.2 Animals

This study was approved by the Institutional Animal Care and Use Committee of Khon Kaen University, based on the Ethics of Animal Experimentation of National Research Council of Thailand (number IACUC-KKU-87/63). Client-owned dogs were examined at the dermatology clinic of the Animal Hospital, Faculty of Veterinary Medicine, Khon Kaen University. Animals were diagnosed with superficial pyoderma based on clinical signs of pustules, alopecia, and peripheral epidermal collarettes with crusts. All diagnoses were confirmed by positive bacterial culture for coagulase-positive staphylococci. Dogs with other infections, deep pyoderma, a history of food allergies, atopic dermatitis, external parasites, hormonal disorders, and other systemic diseases were excluded from the experiment. The dogs used for skin irritation tests were in good skin health based on history taking and physical examination. None of the animals had been treated with systemic or topical antibiotics or anti-inflammatory drugs in the previous 14 days.

2.3 Sample size calculation

2.3.1 Therapeutic efficacy test

Sample size was calculated for a non-inferiority trial with the data for a continuous variable by using the program N4STUDIES version 1.4.1 according to the following equation (Chow, Shao, & Wang, 2003):

$$n = \frac{\left(Z_{1-\alpha}+Z_{1-\beta}\right)^2 \sigma^2 \left(1+\frac{1}{\kappa}\right)}{(\varepsilon-\delta)^2}$$

Here σ = standard deviation, ε = mean difference between groups (clove essential oil spray and chlorhexidine gluconate spray groups), δ = non-inferiority margin, κ = ratio between groups, α = significance level (0.05), and β = type II error probability (0.2). As there are limited studies on the efficacy of clove essential oil formulations in treating canine dermatitis, the variable values in the computational formula were predicted based on two efficacy studies of essential oil in the treatment of skin diseases in animal models (Lee *et al.* (2007) and Asawapattanakul (2013)). Based on these studies, the standard deviation of %clinical index score (CIS) was 5% when α=0.05 and β=0.20, the mean difference between groups was 10%, the non-inferiority margin was defined as a %CIS reduction equal to 20%, and the proportion between groups was 1:1. Therefore, the sample size was four dogs in each group.

2.3.2 Skin irritation tests

Sample size was calculated for a randomized controlled trial with the data for a continuous variable by using the program N4STUDIES version 1.4.1 according to the following equation (Rosner, 2000):

$$n_{trt} = \frac{\left(Z_{1-\frac{\alpha}{2}}+Z_{1-\beta}\right)^2 \left(\sigma^2_{trt}+\frac{\sigma^2_{con}}{r}\right)}{\Delta^2}$$

$$r = \frac{n_{con}}{n_{trt}},\ \Delta = \mu_{trt}-\mu_{con}$$

Here μ_{trt} = mean in treatment group (clove essential oil spray), μ_{con} = mean in control group (normal saline

solution), σ_{trt} = standard deviation in treatment group, σ_{con} = standard deviation in control group, α = significance level (0.05), and β = type II error probability (0.2). The variable values in the computational formula were predicted based on Asawapattanakul (2013). The irritation score means of the treatment and control groups were 1 and 0.25, respectively, the standard deviation was 0.25 in each group, and the proportion between groups was 1:1. Therefore, the sample size was 4 tested areas in each group. As the treatment and control tested areas were on the same animal, 4 dogs were used in this experiment.

2.4 Experimental design

This was a non-inferiority randomized controlled trial comparing the efficacy of 5% w/w clove essential oil spray with 2% w/v chlorhexidine gluconate spray (OLIC (Thailand) Ltd., Thailand). Dogs with confirmed superficial pyodermas were randomly assigned to two groups; Group A received 5% w/w clove essential oil spray and Group B received 2% w/v chlorhexidine gluconate spray. Medications were applied topically to the full lesion area (0.01 g/cm^2), 2 times a day, 8 h apart for 15 days. The primary investigator was blinded to the treatment group throughout the study.

2.5 Clinical evaluation

The clinical lesion scores were assessed from 3 criteria including pustules, crusts and scaling, and erythema by the primary investigator on days 0 (before treatment), 5, 10, and 15 after the 1st treatment. A clinical lesion score for each criterion was assigned from 0 to 3 with 0 = no lesion, 1 = mild, 2 = moderate, and 3 = severe. The CIS was obtained by summation of the scores for each criterion for a possible CIS range of 0 to 9. A 50% reduction in CIS was considered a clinical difference. Clinical resolution was defined by negative coagulase-positive staphylococci culture (Miller *et al.*, 2013; Rosales *et al.*, 2005).

The number of viable coagulase-positive staphylococci (CoPS) that were recovered from the treatment areas of the affected animals was investigated along with clinical lesion evaluation (days 0, 5, 10, and 15). Bacterial samples were collected by sampling the treatment area with a sterile cotton swab dipped into sterile pH 7.4 phosphate-buffered saline solution (PBS). The samples were mixed using a vortex mixer for 5 min and then 10-fold diluted with PBS to 10^{-1} to 10^{-5}. Then 100 µl aliquots of dilutions 10^{0}-10^{-5} were inoculated onto blood agar plates (Becton Dickinson, France) and incubated at 37ºC for 24 h. The white and creamy white pinpoint colonies of beta-hemolytic gram-positive cocci, which were positive to catalase and coagulase tests and negative to an oxidase test, were enumerated and are expressed as colony-forming units per square centimeter (CFU/cm^2) (Markey, Leonard, Archambault, Cullinane, & Maguire, 2013).

The %CIS reduction and %CoPS reduction were determined from the percent reduction of CIS and CoPS cell counts, respectively, of each follow-up compared to the initial score (before treatment) and calculated as mean±SD for each treatment group.

2.6 Skin irritation test

The acute dermal irritation/corrosion test was performed according to the Organization for Economic Co-operation and Development (OECD) guidelines (Organization for Economic Co-operation and Development, 2015) with some modifications. Briefly, healthy dogs had 4 x 4 cm areas on the left and right sides of the midline of the abdomen trimmed of hair using scissors at least 24 hours before testing. The exposed skin areas were randomly assigned to be treated with either 5% w/w clove essential oil spray or normal saline solution (control) applied topically (0.01 g/cm^2) twice a day, 8 h apart for 4 days. The testing sites were covered with sterile gauze and non-irritating tape. The irritation criteria included any erythema or edema in the inoculated areas and were assessed before testing and 15 min, and 1, 6, and 24 h after the first application each day. Erythematous skin was assigned a score from 0 to 3 with 0 = no lesion, 1 = the skin has mild redness, 2 = the skin has moderate redness, and 3 = the skin has severe redness or the skin is burnt. Edematous skin was assigned a score from 0 to 3 with 0 = no lesion, 1 = the skin has mild edema (with a clear margin of swelling), 2 = the skin has moderate edema (raised 1 mm above the surrounding skin level), and 3 = the skin has severe edema (raised more than 1 mm above the surrounding skin level and extending beyond the applied area). The irritation score was obtained by summation the scores for each criterion for a possible score range of 0 to 6 (Asawapattanakul, 2013).

2.7 Statistical analysis

Statistical analysis was performed with SPSS (KKU license, Windows version 19.0, SPSS Inc., USA) using $P < 0.05$ as criterion for significance. The normality of the data was assessed by the Shapiro-Wilk test. Differences in CIS, %CIS reduction, CoPS cell count, and %CoPS cell count reduction between the two groups were analyzed using independent sample T-test. The non-inferiority margin (δ) was a 20% difference between the 5% w/w clove essential oil spray and 2% w/v chlorhexidine gluconate spray in %CIS reduction. The 5% w/w clove essential oil spray was not inferior to the 2% w/v chlorhexidine gluconate spray if the lower margin of the 95% confidence interval (CI) of the %CIS reduction difference was not greater than the non-inferiority margin (Sickafoose, Hosgood, Snook, Westermeyer, & Merchant, 2010).

3. Results

3.1 Animals used in the study

A total of 12 mixed-breed dogs were used. The animals were aged 5 months to 13 years old (average of 3.8±3.7 years) and weighed between 6 and 22 kg (average of 15.5±5.4 kg) at the start of the experiment. Eight dogs with localized superficial pyoderma were used to study the therapeutic efficacy of the two spray formulations and four dogs with healthy skin were used to test for the skin irritation potential of the 5% w/w clove essential oil spray.

There were no significant differences in the average ages and weights of the dogs in the 5% w/w clove essential oil spray and 2% w/v chlorhexidine gluconate spray treatment

groups (mean±SD; 4.1±6.0 years and 12.5±7.2 kg vs 4.9±3.1 years and 19.0±3.2 kg, respectively. All P-values > 0.05). Skin lesions consisting of erythema, pustules, alopecia, epidermal collarettes, crusts, and skin scales were found at several sites on the dogs, including the back, abdomen, inguinal and axillary regions. The causative pathogen was found to be *S. aureus* in 3 animals (2 in the 5% w/w clove essential oil group and 1 in the 2% w/v chlorhexidine group) and *S. pseudintermedius* in 5 animals (2 in the 5% w/w clove essential oil group and 3 in the 2% w/v chlorhexidine group). Table 1 shows that there were no significant differences between the 2 treatment groups in initial CIS and CoPS cell counts.

Table 1. Initial clinical index score (CIS) and coagulase-positive staphylococci (CoPS) cell count of tested dogs with localized superficial pyoderma

Treatment	CIS	CoPS cell count (CFU/cm^2)
Clove oil spray	7.25±1.25	0.95±1.31 x10^5
Chlorhexidine spray	8.25±0.96	2.90±2.63 x10^5
P-value	0.780	0.174

Clove oil spray = 5% w/w clove essential oil spray (n=4). Chlorhexidine spray = 2% w/v chlorhexidine gluconate spray (n=4). The values represent the mean±SD.

3.2 Efficacy of clove essential oil spray on canine superficial pyoderma

The CIS and CoPS cell count follow-up results on days 5, 10 and 15 after the first treatment showed that the 5% w/w clove essential oil and 2% w/v chlorhexidine gluconate sprays were both able to improve the canine superficial pyoderma skin lesions with no adverse effects (Figures 1 and 2). There were no significant differences between the two groups in the percent reduction from the initial CIS and coagulase-positive staphylococcal cell counts at each follow-up. Both the clove essential oil spray and the chlorhexidine gluconate spray reduced the number of CoPS recovered from the skin lesions by more than 2-log$_{10}$ CFU/ml (99%) at the first follow-up, 5 days after initiating treatment, and negative CoPS cultures were collected from the lesions at the second and third follow-ups, 10 and 15 days after initiating treatment (Table 2). There were small but non-significant differences in the %CIS reductions between the two groups at each follow-up. Treatment of lesions with the chlorhexidine gluconate spray resulted in a clinical difference (CIS reduction of at least 50%) from the first follow-up (%CIS reduction of 50.99±10.90%) while clove essential oil spray showed a clinical difference at the second follow-up (%CIS reduction of 66.87±13.09%, Table 2). Nevertheless, the data analysis indicated that the therapeutic effect of 5% w/w clove essential oil spray on canine superficial pyoderma was non-inferior to 2% w/v chlorhexidine gluconate spray at each follow-up. At the first follow-up 5 days after initial treatment, the noninferiority margin of the mean %CIS reduction in the chlorhexidine treated group (50.99±10.89%) was -10.20%, with a mean difference between groups of 9.53% and a 95% CI of -7.86 to 26.91%. In the second follow-up 10 days after starting treatment, chlorhexidine spray had a mean %CIS reduction of 74.70±13.16% with a -14.94% noninferiority margin and a 7.83% difference between treatment groups, which was within the 95% CI of -14.87 to 30.55%. In the third follow-up 15 days after the first treatment, chlorhexidine spray showed a mean %CIS reduction of 90.87±11.03% with a noninferiority margin of -18.17% and a mean difference between groups of 7.14%, which was within the 95% CI of -13.09 to 27.37%.

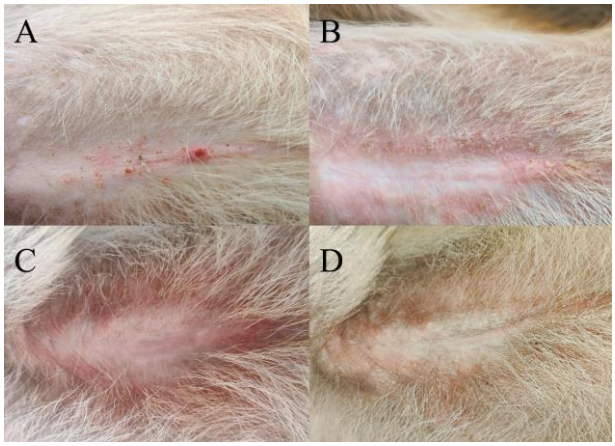

Figure 1. Clinical lesion of 5% w/w clove oil spray treated dog. (A) The initial lesion shows severe erythematous papules with pustules and moderate crusts and scales on the ventral abdomen (umbilical region) of a dog (CIS = 7). (B) In the 1st follow-up, the pustules were resolved but moderate erythematous papules and mild scaling remain (CIS = 3). (C) The 2nd follow-up shows mild erythematous skin (CIS = 1). (D) The 3rd follow-up shows normal skin appearance (CIS = 0).

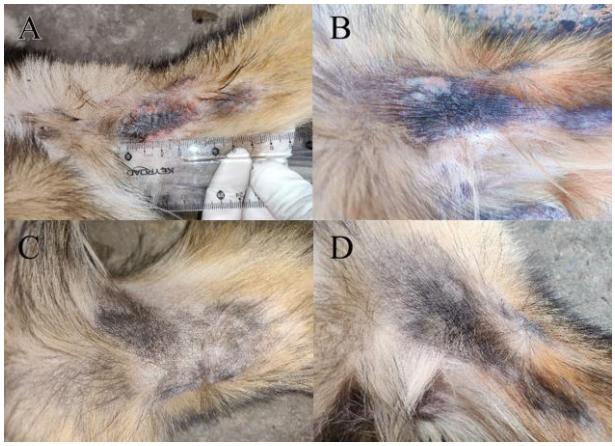

Figure 2. Clinical lesion of 2% w/v chlorhexidine spray treated dog. (A) The initial lesion shows severe erythematous papules with pustules and crusts and scales on the medial axillary region of a dog (CIS = 9). (B) In the 1st follow-up, the pustules were resolved but moderate erythema with mild scaling remains (CIS = 3). (C) The 2nd follow-up shows mild scaling with no erythema (CIS = 1). (D) The 3rd follow-up shows normal skin appearance (CIS = 0).

3.3 Skin irritation test of clove essential oil spray

The results of the irritation test of clove essential oil spray on dog skin are shown in Figure 3. None of the dogs

Table 2. The percent reductions from initial for clinical index score (CIS) and coagulase-positive staphylococci (CoPS) cell count in experimental animals treated with 5% w/w clove essential oil spray (n=4) and 2% w/v chlorhexidine gluconate spray (n=4)

Treatment	Follow up					
	1st		2nd		3rd	
	%CIS reduction	%CoPS reduction	%CIS reduction	%CoPS reduction	%CIS reduction	%CoPS reduction
Clove oil	41.47±9.13	99.76±0.49	66.87±13.09	100.00±0.00	83.73±12.32	100.00±0.00
Chlorhexidine	50.99±10.89	99.67±0.17	74.70±13.16	100.00±0.00	90.87±11.03	100.00±0.00
p-value	0.741	0.119	0.733	N/A	0.999	N/A

Clove oil = 5% w/w clove essential oil spray (n=4). Chlorhexidine = 2% w/v chlorhexidine gluconate spray (n=4). The values represent the mean±SD. N/A = not applicable; the statistic cannot be computed.

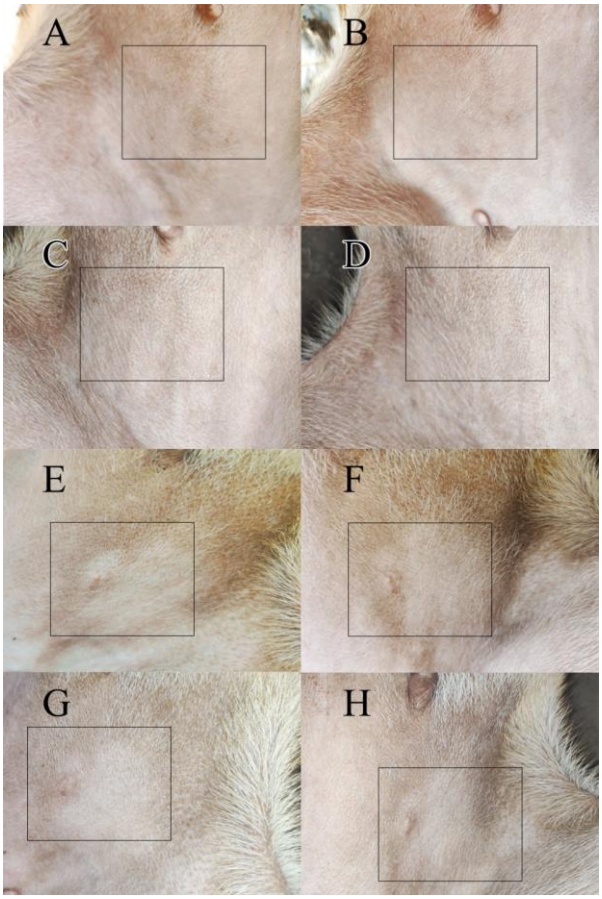

Figure 3. The characteristic of exposed skin areas (in squares) of a dog at day 4 of skin irritation test (irritation score = 0). (A-D) At 15 min, 1, 6, and 24 h after applied with 5% w/w clove essential oil spray, respectively. (E-F) At 15 min, 1, 6, and 24 h after applied with normal saline solution (control), respectively

showed irritation signs (irritation score = 0) on the tested skin areas, with 5% w/w clove essential oil spray or normal saline solution applied, at any follow-up.

4. Discussion

A previous investigation of superficial pyoderma in dogs aged 5 months to 13 years (mean = 5 years) found thatthe major lesion characteristics were pustules, erythematous papules, and crusting with scaling that appeared on the thorax, abdomen, and inguinal and axillary areas of the dogs (Ravens, Vogelnest, Ewen, Bosward, & Norris, 2014). In the current study, superficial pyoderma lesions were observed on the back, abdomen, and inguinal and axillary regions of dogs and clinical lesion scores were evaluated from the severity of three inflammatory lesion characteristics: papules and pustules, crusting and scaling, and erythema. The degree of pruritus was not evaluated because the dogs used in the study did not present with this clinical sign. While this is the first report examining the *in vivo* efficacy of a topical 5%w/w clove essential oil lipid-based spray formulation on canine superficial pyoderma, there are a few previous reports showing that clove has the potential to treat skin infections in animals. Al-Ameedi and Nahi (2019) reported that an ointment containing clove bud extract reduced dermal scar tissue in infectious wounds caused by *S. aureus* in dogs by more than penicillin ointment. Their histopathological study showed that clove ointment reduced the accumulation of inflammatory cells in the wound site and restored tissue structures more quickly. Reports of Eman-abdeen and El-Diasty (2015) and Mousa and Eman (2018) demonstrated that pure clove essential oil reduced lesion size and hair loss from dermatophytosis in cattle and buffalo. Similarly, Lee *et al.* (2007) reported that an ointment containing eugenol, a major constituent of clove essential oil, improved skin lesions caused by *Microsporum gypseum* in a guinea pig model.

A topical spray formulation has advantages over cream, ointment, lotion, and shampoo preparations. Sprays are more convenient to use as the user does not have to touch the animal or the drug directly as it can be applied from a small distance. In addition, sprays are suitable for application over small and large areas in cases of localized and generalized superficial pyoderma. The frequency and duration of topical application depends on the clinical experience of the primary veterinarian. Generally, topical therapy for treating canine superficial pyoderma is applied 1-2 times daily until 7 days after the lesions resolve (Hillier *et al.*, 2014; Koch *et al.*, 2012). In the current study, application of 5% w/w clove essential oil spray to lesions twice a day for 15 days at a dose of 0.01 g/1 cm^2 of lesion area resolved superficial pyoderma and did not cause any skin irritation.

Previous *in vitro* studies from our research group have revealed that clove essential oil has high antibacterial activity against *S. pseudintermedius* isolated from dogs with superficial pyoderma, with minimum planktonic inhibitory

concentration and minimum planktonic bactericidal concentrations of 0.078% v/v and 0.156% v/v, respectively (Aiemsaard *et al.*, 2020b). Furthermore, a 5% w/w clove essential oil lipid-based spray was able to reduce the number of viable *S. pseudintermedius* bacteria *in vitro* by more than 6-$\log_{10}$ CFU/ml (99.9999%) within 15 minutes (Aiemsaard *et al.*, 2020a). Thus, our developed lipid-based spray formulation promotes the effects of clove essential oil both *in vitro* and *in vivo*. Clove essential oil is very compatible with this lipid-based spray formulation since its major active constituents are terpenes (eugenol and caryophyllene), which are lipophilic. In addition, the lipid-based spray formula helps to moisturize the skin and increases the time that the drug adheres to the skin, which promotes the time-dependent antimicrobial effects of clove essential oil (Kaur & Kaushal, 2019).

Clove essential oil has some advantages over conventional antibiotics as a topical antimicrobial agent. Clove essential oil has shown good antibacterial effects against many multidrug-resistant bacterial strains such as *S. aureus*, *Streptococcus suis*, *Acinetobacter baumanni*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis* (Abdullah, Hatem, & Jumaa, 2015; Wongsawan, Chaisri, Tangtrongsup, & Mektrirat, 2020) and is also effective against biofilm-forming *S. pseudintermedius* and *S. aureus* (Aiemsaard *et al.*, 2020b; Chamdit & Siripermpool, 2012). In addition, clove essential oil has low toxicity showing no cytotoxic effects against human keratinocyte (HaCaT) and epithelial (CoN–CRL-1790) cell lines at concentrations of 32 and 125 µg/ml, respectively (Oliveira Ribeiro *et al.*, 2020). Additionally, Mektrirat, Janngeon, Pikulkaew, and Okonogi (2016) showed that the mild cytotoxic effects of a microemulsion containing clove essential oil against murine peritoneal macrophages was due to the vehicle rather than the clove essential oil. Furthermore, an *in vivo* irritation test in guinea pig models demonstrated that clove essential oil at concentrations of 5, 10, and 15% w/w in a hydrocarbon base ointment did not cause any skin irritation (Mukhlishah, Sugihartini, & Yuwono, 2017).

Eugenol is the major chemical constituent of clove essential oil making up 97.76-98.87% of the content (Aiemsaard *et al.*, 2020a; Wongsawan *et al.*, 2020). It has shown antimicrobial activities against several microbial species and acts by disrupting the cell membrane, which results in the leakage of intracellular substances from the cells and eventually cell death (Guimarães *et al.*, 2019). Eugenol has also been shown to reduce the *in vitro* production and expression of several proinflammatory biomarkers such astumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-8, matrix metalloproteinase-9, interferon γ-induced protein (IP)-10, interferon-inducible T-cell α chemoattractant (I-TAC), and monokine induced by γ interferon (MIG). These anti-inflammatory effects of eugenol are likely to help in treating skin inflammation, which can be seen via its ability to reduce edematous skin in a *Propionibacterium acnes*-induced inflammation mouse model (Han & Parker, 2017; Tsai *et al.*, 2017).

5. Conclusions

A spray of 5% w/w clove essential oil was effective for the treatment of canine superficial pyoderma with non-inferiority to 2% w/v chlorhexidine gluconate spray. Topical application of clove oil spray 2 times a day reduced the CIS and CoPS cell counts by 83.73% and 100%, respectively, at 15 days after the first treatment. No skin irritation was observed in dogs during the treatment or in an independent skin irritation test. This study demonstrates that this novel clove essential oil spray has the potential to treat superficial pyoderma in dogs. Further studies should investigate the efficacy of the clove essential oil spray in combination with systemic antibiotics and other topical drugs commonly used to treat canine superficial pyoderma.

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