Comparative efficacy of stannous fluoride and copper sulfate footbath solutions for the treatment and prevention of digital dermatitis in lactating dairy cows

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ABSTRACT

The objective of this study was to determine whether a novel footbath solution containing stannous fluoride (SnF2) was superior to 5% copper sulfate solution for the treatment and prevention of digital dermatitis (DD) in dairy cattle. Study 1 was conducted over 4 weeks in Missouri and involved 34 lactating Holstein-Friesian cows with hind feet DD lesions. Cows in group SF walked through a footbath containing a proprietary formulation of SnF2 once weekly, whereas cows in group CS walked through a 5% CuSO4 footbath once daily for 5 d each week. Study 2 was conducted over 8 weeks in California and involved 40 lactating Holstein-Friesian cows with hind feet DD lesions. Cows in group SF walked through a SnF2 footbath for 3 consecutive days then once a week for the following 7 weeks. Cows in group CS walked through a 5% CuSO4 footbath 3 times each week for 8 weeks. Data collection included lesion type, lesion area, locomotion score, and pain score. Digital dermatitis was actively transmitted in study 1, and lesion area and locomotion scores were lower in group SF than group CS. In contrast, DD was not actively transmitted in study 2, and lesion area and locomotion scores were similar in groups SF and CS. Stannous fluoride delayed the development of active DD lesions in study 1 compared with copper sulfate, with a lower relative risk (0.57, P < 0.001) of a hind foot developing an active DD lesion over 28 d. However, SnF2 decreased the rate that active DD lesions transitioned to M3, M4, or M0 lesions compared with 5% copper sulfate in both studies, with the relative risk of a hind foot with an active DD lesion transitioning to M3, M4, or M0 in group SF being slightly lower in study 1 (0.83, P = 0.042) and study 2 (0.90, P = 0.020) than group CS. Our findings demonstrated that walking cows through a stannous fluoride footbath once per week in a herd undergoing active transmission of infection was more effective in preventing active DD lesions, but less effective in treating active DD lesions, than walking cows through a copper sulfate footbath 4 times per week. The novel SnF2 footbath solution shows promise for controlling DD in dairy herds that want an alternative footbath solution to CuSO4 and are interested in limiting the environmental accumulation of copper.

Key words: Treponema spp., lameness, hoof, digital dermatitis, footbath

INTRODUCTION

Digital dermatitis (DD) is a contagious disease that is a common cause of lameness in dairy cattle housed in intensive production systems (Rodriguez-Lainz, 1996; Read et al., 1998). Over 90% of DD lesions affect the posterior aspect of the foot in an area between the bulbs of the heel below the dew claws (Britt, 1998). Besides being a significant animal welfare problem, DD has been associated with economic losses due to reduced milk production and weight loss (Losinger, 2006). Digital dermatitis is currently viewed as a polymicrobial multifactorial disease (Beninger et al., 2018), with active DD lesions having a distinct microbiome dominated by Treponema spp., a Gram-negative anaerobic spirochete (Zinicola et al., 2015).

Treatment and prevention of DD has involved topical applications of antimicrobials or antiseptics via bandages or sprays, intramuscular injections of antimicrobials, walking cattle through footbaths containing antiseptic or antimicrobial solutions, or vaccination (Laven and Logue, 2006; Nuss, 2006). The benefits of footbaths include mass treatment and a potential decrease in the transmission of infection from infected cows to non-infected animals (Laven and Proven, 2000). Use of
antimicrobial footbath solutions such as tetracycline, chlortetracycline, lincomycin, or erythromycin has decreased due to concerns over antimicrobial residues in the environment and the potential for increased antimicrobial resistance in bacteria. Use of antimicrobial footbath solutions in the United States constitutes extra-label use and requires a veterinary prescription. Copper sulfate is a commonly used antiseptic footbath solution to treat and control DD (Solano et al., 2017) because of its widespread availability, low cost, and ease of use. The authors of a 2019 meta-analysis of footbath studies concluded that a 5% CuSO4 solution used at least 4 times/week was effective in treating DD (Jacobs et al., 2019). Copper sulfate is also effective in preventing active DD lesions when 5% CuSO4 solutions are applied for 4 consecutive milkings every 2 weeks compared with monthly footbath treatments (Speijers et al., 2012). After 150 to 300 cows have passed through the footbath, the CuSO4 solution is believed to become ineffective and is disposed of by land application. Government agencies have expressed concern that the frequent application of CuSO4 solutions in footbaths will result in unacceptably high concentrations of copper in the soil; consequently, the use of CuSO4 solutions in footbaths has been restricted to low concentrations (0.5% CuSO4) in the Netherlands (Holzhauer et al., 2008a). There is therefore widespread interest in developing alternative antiseptic solutions for the treatment and prevention of DD in dairy cattle.

Stannous fluoride (SnF2) has been used to treat and prevent gingivitis in humans for over 50 years (Andres et al., 1974; Klukowska et al., 2017), and topical 0.2% and 0.4% SnF2 preparations have been used to treat superficial pyoderma in dogs (Seltzer et al., 2010) and bacterial skin infections in horses (Marsella and Akucewich, 2007), respectively. In vitro studies have demonstrated that SnF2 has antibacterial effects on the oral microflora (Attramadal and Svatun, 1984) and Treponema spp., including Treponema denticola (Hughes and Yotis, 1986) that is associated with bacterial uptake of tin (Camosci and Tinanoff, 1984), enhanced oxygen-dependent antibacterial activity of neutrophils (Shapira et al., 1997), and inhibition of selenoprotein synthesis (Jackson-Rosario and Self, 2009). Stannous fluoride also reduces inflammation by decreasing Toll-like receptor activation associated with lipopolysaccharides (Klukowska et al., 2017). Neither tin(II) or fluoride is believed to provide a significant risk to the development of resistant bacterial organisms (Andres et al., 1974; Attramadal and Svatun, 1984). We therefore hypothesized that a SnF2 solution would be superior to 5% CuSO4 solution for the treatment and prevention of DD in lactating dairy cattle walking through a footbath. The primary aims of this study were therefore to compare the effectiveness of the 2 footbath solutions in the treatment and prevention of DD, as assessed by lesion type, lesion area, locomotion score, and pain score.

MATERIALS AND METHODS

Study 1

Animals and housing. Study 1 was conducted during February and March at the University of Missouri Foremost Dairy Research farm that maintained a rolling herd average (305 d) of 22,500 lb of milk per cow and average daily milk production of 71 lb. The herd was composed of 280 Holstein and Guernsey cows and the annual turnover of lactating cows was 29%. Mean bulk tank somatic cell count was 309,000 cells/mL. Cows were housed in a free stall barn with mattresses covered by a mixture of limestone, sand, and shavings. Barn aisles were flushed twice daily when the ambient temperature was above 25°C and scraped daily when the temperature was below 25°C. Cows were fed a total mixed ration twice daily and milked twice daily.

Standard hoof care on the farm included cows having their feet trimmed every 6 mo and walked through a 5% copper sulfate footbath 3 times per week. A survey of 270 cows examined by the foot trimmer over a 12-mo period revealed a total of 741 hoof diagnoses; 28% of feet examined were normal. Of the hoof diagnoses, 53% were DD. Of the cows diagnosed with DD, 76% had one episode, 19% had 2 episodes, and 4% had 3 episodes on the same foot within the 12-mo survey period. Lame cows with DD lesions were treated with topical oxytetracyline or chlortetracycline in a liquid formulation and wrapped with a bandage (3M Vetwrap bandaging tape; 3M Animal Care, St. Paul MN).

Experimental method. In accordance with Institutional Animal Care and Use approval, lactating Holstein-Friesian cows in the herd were examined and 34 cows with visible DD lesions in one or both hind limbs and a lameness score of ≥2/5 (Sprecher et al., 1997) were enrolled in the study. We elected to examine only the hind feet of lactating dairy cattle because the prevalence of DD is much lower in the front feet (Murray et al., 1996; Solano et al., 2016). Cows were randomly assigned to one of 2 groups (group SF; group CS) using a random number generator (Excel, Microsoft Corp.).

The stannous fluoride footbath was designed to provide a continuous flow of water over a porous rubber mat in the base (Figure 1). The active ingredient in the footbath solution (SnF2) was formulated as a semi-liquid slow-release stannous fluoride emulsion with a specific gravity >1; as such, the emulsion sank into the porous rubber mat and contacted and adhered to the sole and heel area of the foot through hydraulic action when the cow walked through the footbath. Adherence was facilitated...
using a sustained release technology based on encapsulation of SnF₂ inside a sticky cell wall and movement of SnF₂ through the cell wall over time. A pilot study was conducted before starting the field studies to monitor the adherence and subsequent rate of release of SnF₂ from the foot over time by attaching an UV dye to the SnF₂ emulsion. The results of the pilot study indicated that the UV color started to visibly fade after one week, suggesting that weekly treatment intervals would provide sufficiently prolonged exposure to SnF₂ at the site of infection.

Both footbaths were filled to a depth of at least 10 cm to ensure coverage of the typical lesion site for DD and were set up with a ‘pre-rinse’ footbath to remove debris and reduce contamination of the treatment footbaths. On d 0, cows in group SF walked through a modified footbath with interior dimensions of 7 feet 6 inches long and 2 feet 8 inches wide containing the proprietary formulated SnF₂ solution, whereas cows in group CS walked through a footbath with interior dimensions 2 footbaths joined lengthwise each with interior dimensions of 5 feet 5 inches long and 3 feet 5 inches wide containing 5% CuSO₄ solution. Thereafter, cows in group SF were treated once each week (on d 7, 14, and 21) for a total of 4 treatments over the 28-d study period, while cows in group CS walked through a 5% CuSO₄ footbath 5 times a week (Monday through Friday) for a total of 20 treatments during the 28-d study period.

Locomotion score and lesion pain score were determined before cattle walked through the footbath because the examination protocol had the potential to remove residual footbath solution and therefore negatively impact possible treatment efficacy. A 5-point locomotion score (Sprecher et al., 1997) was assigned to each cow on d 0 (immediately before the first treatment), 7, 21, and 28 by visual examination before the cow entered the foot trimming chute. Hind feet were examined on d 0, 7, 14, 21 and 28 by restraining each cow in a vertical hydraulic foot-trimming chute. A weekly time interval for examination was selected based on typical treatment responses (Laven and Logue, 2006; Holzhauer et al., 2008b). A pain score was assigned to each hind foot on d 0 and 7 to assess if there was a reduction in pain associated with treatment. The pain score was assigned by the same investigator (TSM) using a method adapted from a standardized DD pain assessment method using a pressure sprayer (Britt et al., 1999). Specifically, a cold stream of water from a parlor hose turned fully on was applied sequentially to the plantar aspect of the left hind and right hind feet with the cow standing. The pain score was recorded as: P0, no movement of the foot after application of the water stream; P1, cow picked up the foot and returned it to the floor within 2 s of application of the water stream; P2, cow picked up the foot and held the foot above the floor for more than 2 s.

The presence and nature of any DD lesions were then scored by the same investigator (TSM) using the 5-point scoring system developed by Döpfer et al., (1997) when the cow was in the foot trimming chute based on visual inspection of the plantar aspect of each hind foot between the heel bulbs. The heel area of each hind foot was cleaned with water and wiped dry with an individual paper towel. A lesion score was then recorded, based on the clinical appearance and size of the lesion, as well as pain reaction to touch, and consisted of 5 categories: M0,
normal skin, no signs of disease; M1, small (0.5 to 2 cm diameter) DD lesion that was usually not painful; M2, erosive hyperemic DD lesion > 2 cm diameter that was usually painful on palpation; M3, healing stage of M2 manifest as the presence of a scab with minimal pain; and M4, hyperkeratotic cutaneous DD lesion that was usually not painful on palpation (Zinicola et al., 2015). The score M4.1 that has been assigned in some other studies (Berry et al., 2012) was scored as M1 in this study because M1 is considered an active infection (Jacobs et al., 2017). In addition, classes M1, M2, and M4.1 all contain a circumscribed lesion that is red-gray in color, painful, and prone to bleed; many DD studies focus on these 3 lesion types because they can cause lameness (Biemans et al., 2018).

An objective measure of DD lesion evolution has been recommended for evaluating treatment and prevention efficacy (Ariza et al., 2017; Jacobs et al., 2019). We hypothesized that changes in the area of active and inactive DD lesions provided this desired measurement. A digital image of the plantar aspect of each hind foot was therefore obtained of the foot at a distance of approximately 30 cm while the foot was lifted and restrained in the foot trimming chute. Standardized information was provided in each digital image including date, cow number, left or right hind-limb, a 1 cm length bar for calibration purposes, and a RGB color code chart.

A 28-d study duration was selected based on the findings of a 2008 study (Holzhauer et al., 2008b) that topical treatment of M2 lesions with chlortetracycline resulted in a change in lesion type from M2 of 79% per week. In that study, an effective topical treatment was estimated to decrease M2 lesions to 21% after 7 d, 4.4% after 14 d, 0.9% after 21 d, and 0.2% after 28 d. In addition, it is reasonable to assume that within the M2 lesion category, a decrease in lesion area would indicate some resolution of the infection, whereas an increase in lesion area would indicate expansion of the infection. Cows that did not demonstrate sufficient healing of the DD lesion at the end of the 28 d study period were treated with the standard farm protocol that entailed cleaning the hoof, corrective foot shaping as described (Ando et al., 2009), and application of 10 mL of oxytetracycline (200 mg/mL) on a single gauze 4 inch square and wrapped with a bandage (1/3rd of a roll of Vetwrap™, 10 cm width).

**Study 2**

**Animals and housing.** Study 2 was conducted over the summer at a commercial dairy in southern San Joaquin Valley of California. The dairy milked 984 cows twice daily with an annual turnover of 27.4%. The rolling herd average (365 d) was 25,528 lb milk per cow with individual cows averaging 64 lb milk per day. Average bulk tank somatic cell count was 156,000 cells/mL. Cows were fed a TMR twice daily and housed in pens of approximately 100 cows in free stalls with sand used for bedding. The barn was flushed twice daily; however, during the study the flush system broke for a period of about 3 weeks during which one half of each study pen was not flushed but was scraped when needed. The standard footbath protocol implemented on this farm was for cows to walk through a 5% copper sulfate footbath 5 times per week.

**Experimental method.** Two pens with a minimum of 20 Holstein-Friesian cows with DD lesions on one or both hind feet (pen prevalence approximately 20%) and a lameness score of ≥2/5 were randomly assigned to one of 2 treatment groups. Assignment based on pen was undertaken to minimize disruption to the herd structure on the farm. Pens were assigned to either SF or CS based on coin toss and cows within the pen with lesions were identified with a colored ear tag to make drafting easier at subsequent data collection periods. Cows in group SF (n = 20) were walked through a proprietary formulated footbath containing SnF₂ on d 0, 1, and 2 (i.e., initially treated for 3 consecutive days) then once a week for 7 more weeks for a total of 10 treatments over the 8-week study period. Cows in group CS (n = 20) walked through a 5% CuSO₄ footbath 3 times a week (Monday, Wednesday, and Friday) for a total of 24 treatments during the 8-week study period. Footbaths were filled as described in study 1.

The hind feet of all cattle in the study were evaluated on d 0 (immediately before walking through the footbath) and on d 28 and 56. The 4-week time interval for examination was selected based on typical treatment responses (Laven and Logue, 2006) and the results of study 1. An 8-week study duration was selected based on the results of study 1.

Hind feet were examined before cattle walked through the footbath using the following standardized protocol. Locomotion scores were not obtained. A pain score was assigned to each cow on d 0, 28 and 56 based on the response to application of a water stream to the hind feet as described in study 1. The hind feet were then examined as for Study 1. The presence and nature of any lesions were recorded using the scoring system described in study 1 by the same investigator (AK). Both hind feet of enrolled cattle were examined at each examination, including those feet without a DD lesion at the start of the study. A digital image of the plantar aspect of each foot was taken from a standard distance and analyzed as described in study 1 by an investigator (TSM) who was masked to treatment assignment.

Cows identified by the farm staff that developed lameness during the course of the study (a lameness score of 3 or greater using Sprecher et al., 1997) were examined by trained farm staff or a veterinarian. If a diagnosis of DD was made the cow’s foot was treated according to the
standard farm protocol, which involved topical application of tetracycline powder to the lesion and application of a bandage. If a cow had a lameness score of 4 or 5 out of 5, the lameness was deemed severe and the cow was removed from the study.

**Analysis**

Digital images were analyzed by an investigator (Study 1, PDC; Study 2, TSM) who was masked to treatment assignment. A freely available software program (ImageJ 1.42i; www.rsb.info.nih.gov/ij) was used to quantify lesion area. The mm scale on the photograph was used to calibrate distance in mm/pixel and the lesion perimeter freely drawn by hand on 3 separate occasions and the mean, SD and coefficient of variation (CV) values for each lesion calculated.

**Statistical analysis**

Statistical analysis was performed by an investigator (PDC) who was masked to treatment assignment. The hind foot was considered the statistical unit for analysis. Digital dermatitis lesion scores were categorized as active (score M1, M2), inactive (score M3, M4), or absent (score M0) (Zinicola et al., 2015). This categorization approach was based on studies showing the presence of a higher number of *Treponema* organisms in active lesions (Mumba et al., 1999), particularly *T. phagedenis, T. medium,* and *T. pedis* (Beningier et al., 2018) compared with inactive lesions, belief that stage M4 is associated with chronic disease compared with stages M1, M2, and M4.1 that are associated with acute disease (Watts et al., 2018; Pirkkalainen et al., 2024), and our assumption that the presence of epithelial erosion would increase transmission of infection from the foot to the environment and subsequently to an uninfected foot.

For hind feet with active DD lesions on d 0, 4 (study 1) or 3 (study 2) measures were used to compare the treatment efficacy of the 2 foot-bath solutions: 1) geometric mean lesion area on d 0, 7, 14, 21, and 28 (study 1) or d 0, 28 and 56 (study 2); 2) pain score on d 0 and 7 (study 1) or d 0, 28, and 56 (study 2); 3) the percentage of hind feet that developed a new DD lesion (score M1, M2, M3 or M4) during the study.

Statistical power was estimated for differences in active lesion area as we hypothesized this would provide the most sensitive indicator of treatment and prevention efficacy. Based on an active lesion area of 6.0 cm², SD = 1.5 cm², α = 0.05, and β = 0.20, we estimated that a study would need to enroll at least 17 feet with an active DD lesion (M1 or M2) to detect a 25% difference in lesion area between groups (MedCalc® Statistical Software Ltd., Ostend, Belgium). We therefore planned to enroll a minimum of 15 cows in each group on the assumption that at least 15% of cows with an active DD lesion on one hind foot would have an active DD lesion on the other hind foot.

Continuous variables were expressed as mean ± SD or least squares means and 95% confidence interval for the group and *P* < 0.05 was considered significant. Age for the 2 groups was compared using the Mann-Whitney U test. A statistical software program (SAS 9.4, SAS Inc., Cary, NC) was used for analyses. Lesion area was log-transformed before analysis to stabilize variance and was summarized at each measurement day as geometric mean and 95% confidence interval. Changes in lesion area over time were evaluated using mixed models ANOVA for repeated measures (PROC MIXED) and an autoregressive (1) matrix, with foot nested within cow and the main factors of interest being treatment (2 levels), time (5 or 3 levels), and the interaction between treatment and time. This analysis investigated whether implementation of the treatment protocols for 28 d (study 1) or 56 d (study 2) was associated with a change in lesion area, and whether the response to treatment was different for the 2 groups. The number of existing DD lesions that were clinically improved were compared for the 2 footbath solutions using Fishers exact test (PROC FREQ). This analysis investigated whether the lesion type changed from active to chronic following implementation of the treatment protocols. The incidence of new DD lesions in feet without DD lesions or M0, and the number of new DD infections over time were evaluated using the Cochran-Mantel-Haenszel adjustment.
RESULTS

Study 1

Thirty-four cows were enrolled with 17 cows (34 hind feet) in each group. All cows completed the study. The lactation number of enrolled cows was similar in both groups \((P = 0.096)\); group SF: median, 2.0 and interquartile range, 1.0 to 2.0; group CS: median, 2.0 and interquartile range, 2.0 to 2.25. The median DIM for cows in group SF (122 d; interquartile range, 69 to 167 d) was similar \((P = 0.49)\) to that in group CS (132 d; interquartile range, 105 to 254 d). The study was conducted during February and March with mean maximal temperatures ranging from 8 to 65°F. The free stall barn was too cold to scrape on several days during the study. The median CV for measuring lesion area was 5.3%.

There was no difference \((P = 0.80)\) in the number of hind feet with DD in group SF (65%, 22/34) or group CS (71%, 24/34) at the start of the study (Day 0). The number of hind feet with DD lesions during the 28-d study period increased \((P < 0.001)\) from 68% (46/68) on d 0 to 91% (62/68). There was no difference \((P = 0.20)\) in the number of hind feet with DD in group SF (85%, 29/34) or group CS (97%, 33/34) at the end of the study (d 28). Lesion type changed during the study, with a decrease in the percentage of hind feet without DD, indicating transmission of infection (Figure 2, top panel).

Repeated measures ANOVA on DD lesion area on all hind feet (68 feet from 34 cows) indicated that there was a significant effect of time \((P < 0.001)\) on geometric mean lesion area, with lesion area being increased by d 7 or d 14 in groups CS and SF, respectively (Figure 2, top panel). The effect of treatment was not significant \((P = 0.13)\), and interaction effect between treatment and time on lesion area was not significant \((P = 0.76)\).

Treatment of active DD lesions. One cow in group SF had a M4 lesion on the right rear foot and a M3 lesion on the left rear foot on d 0 and was therefore categorized as not having an active infection on study enrollment. Data analysis of treatment efficacy of hind feet with active DD (scores M1 or M2) on d 0 was therefore confined to 39 feet (group SF, 18; group CS, 21) from 33 cows.

The percentage of active DD lesions decreased in both groups during the study (Figure 2). There was no difference in the number of hind feet with active DD lesions at the start of the study that were clinically improved by d 7 (SF, 3; CS, 5; \(P = 0.70)\), d 14 (SF, 4; CS, 9; \(P = 0.31)\), d 21 (SF, 8; CS, 13; \(P = 0.34)\), or d 28 (SF, 9; CS, 14; \(P = 0.34)\). However, there was an overall effect of group \((P = 0.042)\) on the percentage of hind feet with an active DD lesion that transitioned to a chronic lesion or M0 during the 28 d of the study based on Cochran-Mantel-Haenszel adjustment for the effect of time. The relative risk of a hind foot with an active DD lesion transitioning to a M3, M4, or M0 lesion in group SF over the 28 d was 0.83 (95% CI, 0.70 to 0.99) compared with group CS.

Repeated measures ANOVA indicated that there was a significant effect of time \((P < 0.001)\), but not treatment \((P = 0.32)\), or the interaction between treatment and time \((P = 0.0059)\), on locomotion score (Figure 4). Locomotion score was increased in group CS at all 3 examination times after treatment was started (day = 0). In contrast, locomotion score in group SF was only increased on d 28 and was lower than that of group CS.

![Figure 2. Change in DD lesion type throughout study 1 conducted in Missouri (top panel; 34 dairy cows) and study 2 conducted in California (bottom panel; 40 dairy cows) for the hind feet of dairy cows walking through a footbath containing stannous fluoride (SF2) or copper sulfate (CuSO4). Feet without DD lesions were score M0. Active DD lesions were score M1 and M2. Chronic DD lesions were score M3 and M4.](image-url)
There was no difference ($P = 0.67$) in the percentage of hind feet that were painful in group SF (89%, 16/18) or group CS (81%, 17/21) at the start of the study (Day 0). Similarly, there was no difference ($P = 1.00$) in the percentage of hind feet that were painful in group SF (85%, 15/18) or group CS (86%, 18/21) at the end of the study (Day 28).

Prevention of active DD lesions. Twelve hind feet in group SF and 10 hind feet in group CS did not have a DD lesion (score M1, M2, M3, and M4) on the hind feet of treated cattle. The center panel is the area of all active DD lesions (score M1, M2) on the hind feet. The bottom panel is the area of all new active DD lesions (score M1, M2) on hind feet that did not have DD lesions on d 0. The error bars are the 95% confidence interval. *$P < 0.05$ compared with time = 0 d. †$P < 0.05$ compared with copper sulfate at the same time.

Forty Holstein-Friesian cows were enrolled with 20 cows (40 hind feet) in each group. Thirty-eight cows completed the study; 2 cows (both in group CS) were removed from the study between d 28 and 56 due to acute lameness attributed to DD. On d 28, one of these animals had a M2 DD lesion in one hind foot (lesion area, 2.9 cm²) and the second cow had a M2 DD lesion in one hind foot (lesion area, 10.8 cm²). The number of cows removed from the study and provided a rescue treatment in group SF (0/20) was not statistically significant ($P = 0.49$) to that in group CS (2/20). The median lactation number of enrolled cows in group SF (1.0, interquartile range, 1.0 to 1.0) was lower ($P < 0.001$) than that in group CS, median, 2.0, interquartile range, 1.0 to 3.0). The median DIM at the start of the study for cows in group SF on d 14 (42%, 5/12), d 21 (50%, 6/12), and d 28 (58%, 7/12) was not statistically different ($P = 0.099$, $P = 0.20$, and $P = 0.16$, respectively) to that in group CS on d 14 (80%, 8/10), 21 (80%, 8/10), and d 28 (90%, 9/10). There was an overall effect of group ($P < 0.001$) on the percentage of hind feet with a new DD lesion during the 28 d of the study based on Cochran-Mantel-Haenszel adjustment for the effect of time. The relative risk of a hind foot developing a DD lesion in group SF over the 28 d was 0.57 (95% CI, 0.41 to 0.79) compared with group CS.

Repeated measures ANOVA confined to 22 hind feet without DD on d 0 (SF, 12 feet; CS, 10 feet) indicated there were significant effects of treatment ($P = 0.036$), time ($P < 0.001$), and the interaction between treatment and time ($P = 0.037$) on new lesion area. Geometric mean lesion area was increased by d 7 or d 14 in groups CS and SF, respectively, and was lower in group SF than group CS on d 7 (Figure 2, bottom panel).
group SF (122 d; interquartile range, 69 to 167 d) was similar \( (P = 0.49) \) to that for cows in group CS (median, 132 d; interquartile range, 105 to 254 d). The study was conducted during summer with mean high temperatures of 87°F (range, 84–94°F) and mean low temperatures of 60°F (range, 56–65°F). Calculated heat indices (>105°F) on some days were in the extreme range which limited our ability to handle cows for long periods of time. The median CV for measuring lesion area was 6.1%.

The percentage of active DD lesions remained constant in group SF and decreased slightly in group CS during the study (Figure 2). The percentage of hind feet with DD in group SF (90%, 36/40) or group CS (73%, 29/40) at the start of the study (Day 0) were not statistically different \( (P = 0.083) \); however, on d 28 the percentage of hind feet with DD was higher \( (P = 0.048) \) in group SF (90%, 36/40) than group CS (70%, 28/40). The percentage of hind feet with DD for the 2 groups was unchanged at the end of the study (d 56) from d 28 when feet lesions for the 2 cows at the time of rescue were included.

Lesion type changed very little during the study, indicating minimal transmission of infection (Figure 2, bottom panel). Repeated measures ANOVA on DD lesion area on all hind feet (80 feet from 40 cows) were compatible with no effect of treatment \( (P = 0.056) \), time \( (P = 0.69) \), or the interaction between treatment and time \( (P = 0.71) \) on geometric mean lesion area (Figure 5, top panel).

**Treatment of active DD lesions.** Analysis of treatment efficacy of active DD lesions (scores M1 or M2) on d 0 was conducted on 49 hind feet (group SF, 27; group CS, 22) from 40 cows. There was no difference \( (P = 0.62, P = 0.39, P = 1.00, \) respectively) in the percentage of hind feet that were painful in group SF on d 0 (89%, 24/27), d 28 (93%, 25/27), or d 56 (85%, 23/27) compared with group CS on d 0 (95%, 21/22), d 28 (82%, 18/22), or d 56 (85%, 17/20), respectively.

There was no difference between the 2 groups in the percentage of hind feet that were clinically improved on d 28 (SF, 4%, 1/27; CS, 14%, 3/22; \( P = 0.31 \)) or d 56 (SF, 4%, 1/27; CS, 23%, 5/22; \( P = 0.077 \)). However, there was an overall effect of group \( (P = 0.020) \) on the percentage of hind feet with an active DD lesion that transitioned to a M3, M4, or M0 lesion during the 56 d of the study based on Cochran-Mantel-Haenszel adjustment for the effect of time. The relative risk of a hind foot develop-

**Prevention of active DD lesions.** Four hind feet in group SF and 11 hind feet in group CS did not have a DD lesion (score M1, M2, M3 or M4) on d 0. The percentage of hind feet with a DD lesion in group SF on d 28 (0%, 0/4) and d 56 (25%, 1/4) were not statistically different \( (P = 1.00, P = 0.48, \) respectively) to that for group CS on d 28 (0%, 0/11) and d 56 (9%, 1/11). There was no overall effect of treatment \( (P = 0.44) \) on the percentage of hind feet with a new DD lesion during the 56 d of the study based on Cochran-Mantel-Haenszel adjustment for the effect of time. The relative risk of a hind foot develop-

Figure 5. Least squares geometric mean lesion area for the hind feet of dairy cows walking through a footbath containing stannous fluoride \( (n = 20 \) cows) or copper sulfate \( (n = 20 \) cows) in study 2 conducted in California. The top panel is the area of all DD lesions (M1, M2, M3, and M4) on the hind feet of treated cattle. The center panel is the area of all active DD lesions (M1, M2) on the hind feet. The bottom panel is the area of all new active DD lesions (M1, M2) on hind feet that did not have DD lesions on d 0. The error bars are the 95% confidence interval. *P < 0.05 compared with time = 0 d.
oping a DD lesion in group SF over the 28 d was 1.06 (95% CI, 0.89 to 1.26) compared with group CS.

Repeated measures ANOVA confined to 15 hind feet without DD on d 0 (SF, 4 feet; CS, 11 feet) indicated that there was no effect of treatment ($P = 0.11$), time ($P = 0.070$), or the interaction between treatment and time ($P = 0.074$) on geometric mean lesion area (Figure 5, bottom panel).

**DISCUSSION**

Our 2 studies were designed to compare the efficacy of a novel footbath solution in treating dairy cows with DD against that of the most used footbath solution (5% copper sulfate). As such, the 2 studies enrolled cows with at least one hind foot affected with DD and treated the cows for 28 or 56 d. The study design and duration limited our ability to look at the incidence of new infections because only 22 hind feet (from 34 cows) in study 1 and 14 hind feet (from 40 cows) in study 2 did not have DD at the start of the study. Moreover, study 2 was conducted in the heat of the California summer, which may account for the difference in results compared with study 1 that was carried out during the coldest part of the Missouri winter. This difference is consistent with findings elsewhere that unhygienic, moist environments promote the spread of DD (Rodriguez-Lainz et al., 1996; Somers et al., 2003). The flush system breakdown in California did not appear to have an impact on incidence of lesions in either treatment group. This could have been due to effective pen scraping during this period to maintain pen hygiene. The prewash may have compensated for change in hygiene. It would be helpful to include hygiene scores as part of the screening process to provide an estimate of pen hygiene in future projects. Despite these limitations, stannous fluoride delayed the development of active DD lesions and the associated increase in lameness scores in study 1 compared with copper sulfate, with the relative risk (0.57) of a hind foot developing an active DD lesion over the 28 d in group SF being lower ($P < 0.001$) than group CS. Taken together, our findings demonstrated that walking cows through a stannous fluoride footbath once per week in a herd undergoing active transmission of infection (study 1) was more effective in preventing active DD lesions than walking cows through a copper sulfate footbath 4 times per week. The preventive effect of a stannous fluoride footbath could not be evaluated in the California trial (study 2) because transmission of infection was not evident.

There are very few randomized clinical trials that document the efficacy of footbath solutions or topical antiseptic solutions in treating or preventing DD in lactating dairy cows (Thomsen, 2015; Ariza et al., 2017; Jacob et al., 2019). A 2010 study (Speijers et al., 2010) appears to be the only trial using a negative control to demonstrate that a 5% copper sulfate footbath solution applied at 4 consecutive milkings each week for 5 weeks was effective in curing active DD lesions (score M1 and M2), based on a lower percentage of active DD lesions in the CuSO4 group of 21% vs. 70% in the negative control group at the end of the study. This finding was consistent with that of a large study conducted on 19 farms demonstrating superiority of a CuSO4 footbath to a quaternary ammonium-based footbath in decreasing the prevalence of active DD lesions (Jacobs et al., 2017). This finding was also consistent with a large study on 9 farms that demonstrated a 5% copper sulfate footbath solution applied at 4 consecutive milkings each week for 12 weeks decreased the prevalence of active DD lesions and increased the prevalence of hind feet without DD lesions compared with the farms previous CuSO4 footbath protocol (Solano et al., 2017). The Missouri and California studies reported here can therefore be considered as positive control studies. We used a 5% copper sulfate footbath instead of a negative control (water bath) in our studies because of animal welfare concerns regarding withholding an effective treatment from animals that may lead to unnecessary pain and discomfort, and potentially contribute to the spread of the disease within the group (Britt et al., 1996; Laven and Logue, 2006). In addition, we wanted to test the effect of the novel stannous fluoride formulation against the most commonly used footbath solution (5% copper sulfate) to determine whether the new stannous fluoride footbath solution could replace copper sulfate footbaths as a treatment or preventive measure for DD. We found that the stannous fluoride footbath solution slightly decreased the transition rate of active DD lesions to M3, M4, or M0 compared with 5% copper sulfate footbath solution, with the relative risk of a hind foot with an active DD lesion transitioning to a M3, M4, or M0 lesion in group SF being slightly lower in study 1 (0.83, $P = 0.042$) and study 2 (0.90, $P = 0.020$) than group CS. Whether this treatment outcome was due to decreased efficacy or decreased frequency of footbathing in group SF will require additional studies.

Clinical inspection of the foot, as done in the 2 studies reported here, provides the opportunity to take digital pictures of the lesion to verify assignment of lesion score and measure lesion area. Mean area of active DD lesions at the start of each study approximated 5 cm$^2$ in study 1 and 7 cm$^2$ in study 2. Other studies have reported active DD lesion mean areas of 7 cm$^2$ (Nishikawa and Taguchi, 2008) and 4 cm$^2$ (Sellera et al., 2021). Larger DD lesions take longer to heal than smaller lesions (Nishikawa and Taguchi 2008) and healing of DD lesions is slower when the lesion area is greater than 2.5 cm$^2$, with the odds of incomplete healing increasing by 23% for each extra 1 cm$^2$ of lesion size (Nishikawa and Taguchi, 2008). Pri-
miparous cows also have a greater likelihood of incomplete healing (Nishikawa and Taguchi 2008). The failure to identify healing of active DD lesions (as assessed by lesion area) in group SF in study 2 may have been due to a lack of efficacy in promoting healing or because a higher proportion of animals in group SF were heifers or had large DD lesions at the start of the study. Alternatively, there may have been insufficient time to observe healing since the study periods were 28 to 56 d for study 1 and 2, respectively.

Both footbaths were set up with a ‘pre-rinse’ water bath to reduce contamination of the active ingredients by gross fecal matter on the feet of the cows. It is possible that the act of cleaning the cow’s feet with the flowing water in the stannous fluoride footbath played a role in the treatment response. However, results of other studies have demonstrated that washing the plantar aspect of cows’ hooves in the milking parlor with water from a hose does not reduce lameness due to DD (Shearer et al., 1995; Britt et al., 1996). Cows in study 1 and most of study 2 were treated with the stannous fluoride footbath once per week, and we considered this frequency of cleaning the cow’s feet unlikely to impact the overall effect of treatment.

Locomotion scores did not decrease in group CS in this study during 28 d of treatment. This finding raises the question as to whether 5% copper sulfate footbaths are appropriate for treating DD in cattle with painful active lesions, manifest as lameness. Because DD is a localized polymicrobial disease (Beninger et al., 2018), many studies have found the optimal treatment of affected animals should be application of a topical antimicrobial such as oxytetracycline in a manner that ensures sustained contact with the lesion (Hernandez et al., 1999; Cutler et al., 2013; Krull et al., 2016; Klawitter et al., 2019), or the use of non-antibiotic alternatives in the same manner (Holzhauer et al., 2011). Moreover, disinfectants would likely be more efficacious in preventing new DD lesions than treating existing DD lesions, because most of the associated bacterial infection is located below the epithelial surface and therefore not readily accessible to disinfectants (Döpfer et al., 1997). A recent economic analysis (Robcis et al., 2023) investigated the optimal time allocation between lameness detection and footbath application in herds with lame cows due to DD but did not specifically evaluate the time involved and consumable costs of individual treatment of lesions. Future studies appear indicated to compare the treatment efficacy of 5% copper sulfate footbaths in lame cattle with DD to that provided by topical treatment with oxytetracycline.

**CONCLUSION**

We used a randomized controlled study that had practical treatment and evaluation schedules and protocols, and clinically relevant end-points. Change in geometric lesion area (as measured by digital photography) provided a sensitive measure of treatment and prevention efficacy. Our findings demonstrated that walking cows through a stannous fluoride footbath once per week in a herd undergoing active transmission of infection was more effective in preventing active DD lesions, but less effective in treating active DD lesions, than walking cows through a 5% copper sulfate footbath 4 times per week. With further testing in commercial dairy herds, application of a stannous fluoride footbath solution may provide a suitable alternative to a copper sulfate footbath solution for farmers interested in using a less labor-intensive footbath regimen and limiting environmental accumulation of copper.

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