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Topical salicylic acid treatment of digital dermatitis in dairy cows: Drug resides in milk and clinical efficacy

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Abstract

Salicylic acid (SA) provides similar treatment efficacy to tetracycline-class antibiotic drugs for the treatment of digital dermatitis (DD) in dairy cows. No milk withholding time following SA treatment has been established in the United States. The objective of this study was to generate data about drug residues in milk following topical treatment of DD lesions with SA. Cows with active (M2 stage) DD lesions were assigned to 1 of 3 treatments: salicylic acid paste, salicylic acid powder, or tetracycline powder. Lesions were photographed digitally and thermographically and scored using the M-stage scoring system before treatment and at 7 and 28 d post-treatment. Milk samples were collected before treatment and at 4 h, 8 h, 24 h, 36 h, and 48 h afterward. Most cows did not have detectable salicylic acid in their milk more than 24 hours after treatment; however, 3 cows had detectable levels of SA at 36 hours post treatment. Treatments did not differ in their effects on DD lesions. These data suggest that milk from treated cows should not be used or sold for at least 48 hours following the topical treatment of DD lesions with salicylic acid.

Key words: digital dermatitis, dairy cow, salicylic acid, tetracycline, milk withdrawal

Introduction

Digital dermatitis (DD) is a contagious disease characterized by painful and ulcerative skin lesions that commonly lead to lameness in cattle, diminishing animal welfare and productivity. Among the infectious causes of lameness, DD has the highest prevalence on dairy farms across North America.[5,23] It has been estimated that for every DD lesion, there is a financial loss of approximately US$133 to the producer.[4] Digital dermatitis results from a multifaceted disease process. *Treponema* spp of spirochete bacteria can be isolated from DD lesions, but induction of DD has been more successful using processed biopsies of active DD lesions than using pure bacterial cultures.[9,13,17]

In North America, DD has commonly been treated with antibiotic drugs. Tetracyclines have been a treatment of choice using a 1-time topical application of 2 to 5 g per affected lesion in the form of a powder or paste, with or without a bandage.[7] The use of tetracyclines to treat DD is an extra-label drug use (ELDU) in the United States. This type of therapeutic drug use requires a valid veterinarian-client-patient relationship and a written prescription. Currently, the Food Animal Residue Avoidance Databank recommends a 24 h milk withholding time after the topical use of tetracycline, provided there is no oral ingestion.[10] Water-soluble powdered dosage forms of tetracyclines are available only with a prescription from a veterinarian since 2017, due to concerns about the development of antimicrobial drug resistance.[22]

Salicylic acid (SA) is a candidate for a non-antibiotic form of treatment for DD due to its keratolytic and anti-inflammatory effects.[24] Salicylic acid was reported to be more effective than chlortetracycline at improving lesions and decreasing pain in cows with DD, with a 71% lesion improvement rate 30 d after treatment.[3,19] Salicylic acid is typically applied directly onto the lesion in the form of a powder or as a paste with a bandage. No tolerance has been established for SA in animals and their products in the United States, and no published data about residues of SA in milk after using it to treat DD in cattle could be found in the literature.

The objective of this study was to describe the presence and persistence of drug residues in milk after topical treatment of DD lesions with SA. In addition, we descriptively compared the efficacy of SA to tetracycline when treating DD, and evaluated use of thermographic imaging as a method for categorizing the severity of DD lesions.

Materials and Methods

All procedures involving animals were approved by the North Dakota State University Institutional Animal Care and Use Committee, protocol #A19088.

Animals

This study was performed at the North Dakota State University Dairy Research and Teaching Unit in Fargo, ND. All cows were housed indoors in free-stall housing with ad libitum access to water and a ration formulated to meet the nutritional demands of lactating cows, which was delivered once daily at 6:00 am and pushed up throughout the day. Cows were milked twice daily in a tandem milking parlor at 4:00 am and 3:00 pm. Cows were screened for enrollment based upon observation of an active DD lesion in the milking parlor by study personnel and/or observation of a DD lesion by management on the farm. Cows flagged for screening were examined by a veterinarian in a standing hydraulic chute, and those with active lesions (M2, described below) were enrolled in the study and treated immediately following examination. Cows were enrolled in August and October 2019 and March 2020 in 3 cohorts. Cows with illnesses other than lameness (e.g. mastitis, metabolic disease) or a history of medical treatment that had not completed the drug withholding period(s) + 7 d were excluded from enrollment.

Scoring of Digital Dermatitis Lesions

All lesion scoring was performed by the same veterinarian, a specialist in bovine lameness (author G. Cramer). Lesions were scored using a system described by Zincola et al:[25] M0 - healthy tissue; M1 - early ulcerative lesion, diameter up to 2 cm; M2 - painful ulcerative lesion > 2 cm diameter; M3 - lesion is covered by a scab; M4 - chronic proliferative lesions; M4.1 - chronic lesions with a small area of ulceration.

Treatment Groups

Cows were blocked by day of enrollment then randomly assigned to 1 of 3 treatment groups:

- TPO: 2g tetracycline hydrochloride powder[a] mixed with 6 mL propylene glycol[b], applied topically and left unbandaged.
- SPO: 5g of SA powder[c] applied topically and covered with a coflex bandage.
- SPA: 6 mL of 38% SA paste[d] (2.28 g SA) applied topically then covered with a coflex bandage.

Because DD is painful condition, there was no untreated control group. Randomization was achieved by using the Randomizr package version 0.20.0 in the R program version 3.60.[e] If there was more than 1 lesion on a cow/hoof, the largest and most active lesion was used for the study. Milk was not marketed for 24 hours after treatment in all groups.

The study herd suspended the use of footbaths to manage DD in the herd during study periods to avoid confounding treatment results.

Study Design

The sample size for this study was based on the US regulatory agency standards for establishing withdrawal times for veterinary drugs, FDA guidance for industry #207, which uses the Veterinary International Conference on Harmonization guideline 48.[21] This guideline recommends 20 cows per treatment group for the determination of residues and was the goal for the study.

Study procedures were as follows:

Day -1: Baseline milk sample was collected from study candidates.

Day 0: The affected foot was cleaned and a digital photo and thermographic image were obtained with the cow restrained in a standing position in a hydraulic chute. The following were recorded: affected limb, stage of lesion, treatment type, and treatment time. Milk samples were collected at approximately 4 and 8 h post treatment.

Day 1: Milk samples were collected at approximately 24 h and 36 h post treatment.

Day 2: Milk samples were collected at 48 h post treatment. Bandages were removed after the 48 h milk sample was collected.

Days 7 and 28: Each lesion was scored by a veterinarian (G. Cramer), and the lesion was photographed with digital and thermographic cameras.

Digital and Thermographic Photography

Digital photos were taken using an iPad Air 2[f] on day 0 for groups 2 and 3, on d 7 for all groups, and on d 28 for groups 1 and 2. A thermographic image using a FLIR E8 Wifi camera[g] was taken of all lesions on d 0, d 7, and d 28. The lesion on each thermographic image was outlined, and the interdigital cleft was indicated using the FLIR software (Figure 1). The software then established a minimum, maximum, and average temperature of the skin surface within the outlined lesion.

Milk Collection and Drug Assays

Milk samples were collected in the parlor at milking times via hand-stripping of all quarters immediately after milking was completed. At the 12 h sampling time, which was not during a normal milking time, animals were hand-stripped to collect the sample. Each sample collected ranged from 5 to 10 mL in volume per the laboratory requirements. All milk samples were stored in a -112° F (-80° C) freezer from shortly after collection until they were shipped overnight on dry ice to the Analytical Chemistry Services lab at the Iowa State University Veterinary Diagnostic Laboratory.

Milk Tetracycline Assay

Bovine milk concentrations of tetracycline were determined using UHPLC mass spectrometry. Oxytetracycline was used as the internal standard. A Q Exactive Focus orbitrap was coupled to a Dionex Ultimate 3000.[h] The mobile phases consisted of A: 0.1% formic acid in water and B: 0.1% formic acid in methanol. A Thermo Accucore C18 column was used[h] (100 mm x 2.1 mm, 2.6 μ*m* particles) with column temperature set to 95° F (35° C). The injection volume was 5 μL. The following ions were used for identification and quantification: tetracycline (m/z 445.161) 154.05 and 410.13 and oxytetracycline (m/z 461.155) 381.06 and 426.12. The retention

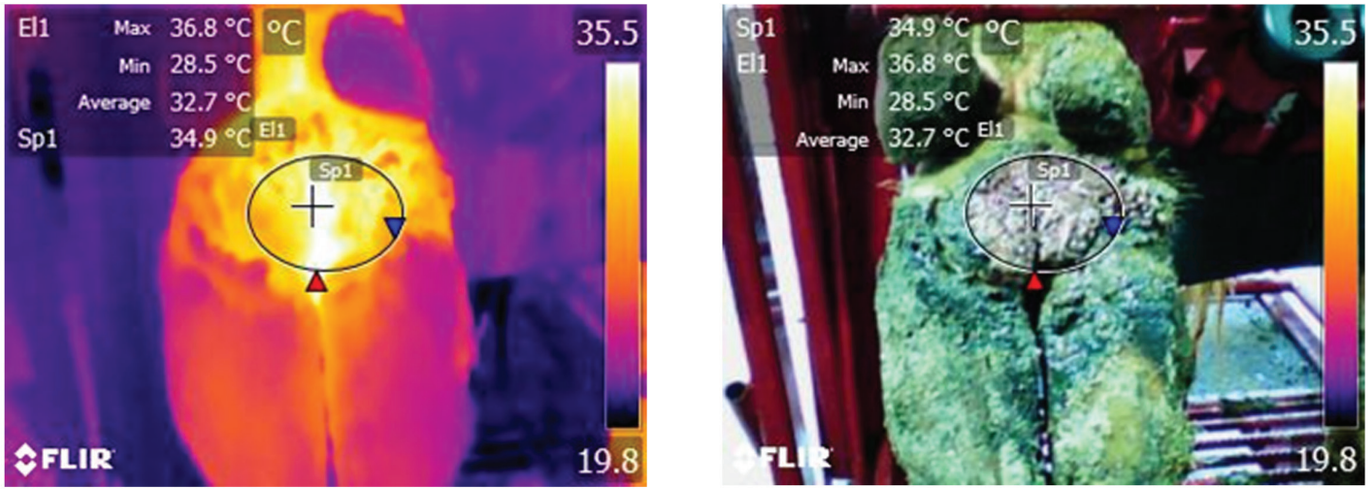

Figure 1. Example of thermographic and digital images of an untreated M2 lesion generated by the thermographic camera.

times were 2.21 and 2.25 for tetracycline and oxytetracycline, respectively.

Calibration curves were calculated using Quan Browser portion of the Xcalibur software[l] and a linear fit. Calibration curves were 5 to 300 ng/mL. The correlation coefficient (r^2) exceeded that of 0.99. The QCs were within a tolerance of ±15% of the nominal value. The limit of detection (LOD) was 3 ng/mL and the limit of quantitation (LOQ), which was based on the calibration curve, was 5 ng/mL.

Milk Salicylic Acid Assay

Salicylic acid concentration was determined using ultra high-pressure liquid chromatography (UHPLC) with fluorescence detection. The UHPLC used was a Thermo Vanquish Flex system.[h] Calibrators were spiked into blank matrix at 7 concentrations ranging from 20 to 5000 ng/mL. Three quality controls were spiked into blank matrix at 150, 1500, and 3500 ng/mL.

The mobile phases consisted of: A) 3.5mM phosphate solution with 0.1% formic acid in water and B) HPLC grade acetonitrile. Salicylic acid was detected at an Excitation wavelength of 295nm, an Emission wavelength of 410nm, and a retention time of 1.92 (± 0.014) min.

Thermo Chromeleon software[m] was used to process quantitative results. All calibrations consisting of 7 points between 20ng/mL and 5000ng/mL and a blank resulted in linear curves with $r^2 \geq 0.997$. The lower limit of quantification (LOQ) was 20ng/mL (the concentration of the lowest calibrant). All QC samples were calculated within 10% of their nominal value.

Statistical Analyses

Drug residue data were analyzed using PROC MIXED in SAS version 9.4.[i] Lesion score data were analyzed using PROC GLIMMIX in SAS. Fixed effects included treatment, day, cohort, and the interactions of treatment with day and cohort. The 2-way interaction of day with cohort and the 3-way interaction of treatment, day, and cohort were tested and removed from the model because $P > 0.1$. DIM was included as a covariate. Repeated measures were included using the random statement in GLIMMIX with cow as the subject. Different covariance structures were tested and the covariance structure with the best fit based on AIC was chosen.

Temperature data were analyzed using PROC MIXED in SAS. Score, treatment, day, and their 2- and 3-way interactions were fit as fixed effects. If interactions had a P-value > 0.1, they were removed from the model. Group was fit as a random effect. A repeated measures statement with cow as the subject was utilized. Different covariance structures were tested and covariance structure with the best fit based on AIC was used. Both contrast and estimate statements were used to compare score M2 with M3, and with M4 and M4.1. Statistical significance was defined a priori as $P \leq 0.05$ for all measures.

Results

Fifty-four cows (18 per treatment group) completed all study procedures, except for 9 cows (3 per treatment group) in cohort 3 that did not have 28 d observations due to travel restrictions related to the SARS-COV-2 pandemic. Twenty-eight day lesion data were not collected for 1 cow in cohort 2 due to being culled for reasons unrelated to the study.

Drug Residues in Milk

Thirty-six cows did not have detectable drug residues at any time point (SA paste = 11, SA powder = 12, tetracycline = 13). Of the 18 cows with drug concentrations above the LOQ in their milk, 13 had drug levels ranging from 20.4 ng/mL to 87.4 ng/mL for SA and 6 ng/mL to 26 ng/mL for tetracycline

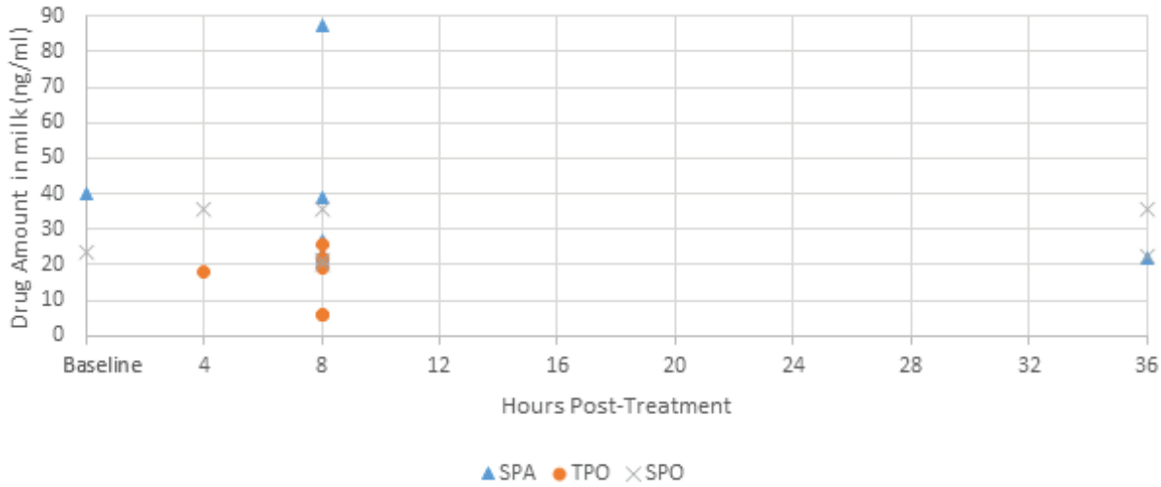

Figure 2. Drug amounts found above the 5 ng/mL (LOQ) for tetracycline and 20 ng/mL (LOQ) for salicylic acid in milk samples by time after treatment. TPO: 2 g tetracycline hydrochloride powder mixed with 6 mL propylene glycol, applied topically and left unbandaged; SPO: 5 g of salicylic acid powder, applied topically and covered with a coflex bandage; SPA: 6 mL of 38% salicyclic acid paste (2.28 g salicylic acid) applied topically then covered with a coflex bandage.

starting 4 to 8 h post-treatment, with no detectable amounts by 24 h (Figure 2). Two cows had concentrations of SA above the LOQ on the baseline sample (1 cow each in the paste group and in the powder-treated group). Two cows treated with SA powder did not have a detectable amount of drug in milk samples until 36 h after treatment. One cow treated with SA paste had 87.4 ng/mL of SA in milk at 8 h, a non-detectable amount at 24 h, and then 22.1 ng/mL at 36 h. There were no detectable drug levels in milk from any cows 48 h after treatment, in any treatment group.

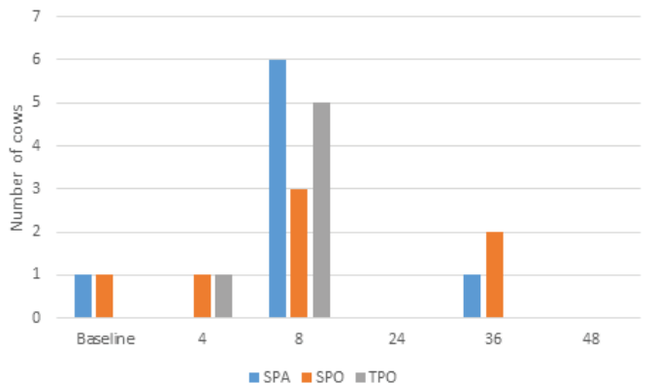

Figure 3. The number of cows that showed positive (detectable) results of drug by time. N = 18 cows per treatment group. SPA: 6 mL of 38% salicyclic acid paste (2.28 g salicylic acid) applied topically then covered with a coflex bandage; SPO: 5 g of salicylic acid powder, applied topically and covered with a coflex bandage; TPO: 2 g tetracycline hydrochloride powder mixed with 6 mL propylene glycol, applied topically and left unbandaged.

Clinical Treatment Outcomes

All DD lesions were scored M2 on d 0 as a requirement for enrollment in the study. A transition from M2 to stages M3, M4, or M4.1 was considered "improvement". There were no lesions that resolved completely to M0. Lesion scores at each observation and the number of cows with lesions that improved, stayed the same, or worsened compared to the previous evaluation are shown in Figures 4a and 4b. There was no evidence of a treatment effect on DD lesions (P=0.54). There was a treatment by day effect on mean lesion scores, with improvement on d 7 across all treatments analyzed together (P<0.0001).

Lesion temperature

Based on thermographic imaging, acute M2 lesions had higher mean temperatures (84 ± 2.9° F [28.9 ± 1.6° C]) compared to M3 lesions (80.2 ± 3.4°F [26.8 ± 1.9°C], P = 0.03). Mean M2 lesion temperature did not differ from mean M4 or M4.1 temperature (82.9 ± 3.1°F [28.3 ± 1.7° C] and 82.8 ± 3.1°F [28.2 ± 1.7°C], respectively; P = 0.27 and P = 0.34, respectively).

Discussion

Salicylic acid is non-antibiotic DD treatment option for producers, hoof trimmers, and veterinarians. Our primary objective was to establish parameters to suggest a milk withholding time after the topical treatment DD with SA; the authors could find no published data of this kind. Drug residues, especially from antimicrobial drugs, continue to grow as a public health concern.[20]

We found that tetracycline levels in milk of treated cows fell below the LOQ (5 ng/mL) by 24 h in all cows. This

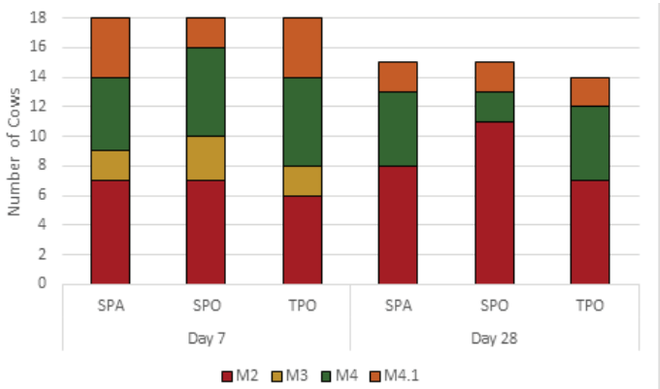

Figure 4a. Lesion scores at d 7 and d 28 for each treatment. SPA: 6 mL of 38% salicyclic acid paste (2.28 g salicylic acid) applied topically then covered with a coflex bandage; SPO: 5 g of salicylic acid powder, applied topically and covered with a coflex bandage; TPO: 2 g tetracycline hydrochloride powder mixed with 6 mL propylene glycol, applied topically and left unbandaged.

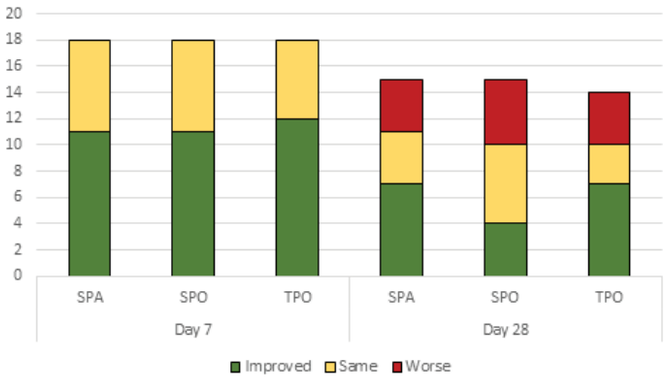

Figure 4b. Number of cows with lesions that improved, stayed the same, or worsened compared to the previous evaluation (d 7 compared to d 0 and d 28 compared to d 7). SPA: 6 mL of 38% salicyclic acid paste (2.28 g salicylic acid) applied topically then covered with a coflex bandage; SPO: 5 g of salicylic acid powder, applied topically and covered with a coflex bandage; TPO: 2 g tetracycline hydrochloride powder mixed with 6 mL propylene glycol, applied topically and left unbandaged.

agrees with previous research; Cramer et al recommended that cows treated with 5g tetracycline paste topically for DD should follow a milk withholding time of 24 h.[6]

Our results also indicated that the majority of cows (67%) never had quantifiable levels of SA in their milk at any time after treatment, but most cows that did had levels below the LOQ of SA by 24 h post treatment. However, there were 3 cows that still had detectable SA in their milk for up to 36 h after treatment. There were also 2 cows that had detectable levels of SA (23.5 ng/mL and 39.8 ng/mL) during the baseline milk sample collections. The farm confirmed that there was no risk of treatment with SA prior to our sampling. In humans, small amounts of SA are found to be naturally occurring in the blood serum when no salicylate treatment or ingestion has taken place.[1] SA is a phenolic compound found naturally in plants that plays a central role in disease resistance to pathogen infection, and people with a vegetarian diet were found to have a higher level of salicylates in their serum compared to non-vegetarians.[1,2] It is possible that some dairy cows may have low levels of naturally occurring SA in their blood (and milk) due to being an herbivorous species. It is also possible that those samples were mis-labelled during research procedures or mis-coded at the analytical laboratory.

The detection of SA for up to 36 h after topical treatment of DD with SA paste or powder is likely due to prolonged and possibly intermittent absorption from the site of application rather than slow elimination of the drug. Salicylates have been found to have a short elimination half-life of less than an hour after oral administration in cattle.[12] Because there is no current tolerance for SA in milk, based on our findings a milk withholding time of at least 48 hours should be observed after topical treatment of DD with SA to ensure that there is no residual drug present in the milk of treated cows.

Salicylic acid has been shown to be an effective tool in treating DD when compared to tetracycline, and it has been used for many years in Denmark as a non-antibiotic option for treatment of DD.[3,19] It is thought to have keratolytic and anti-inflammatory characteristics, which may aid in eliminating the bacteria in the deeper layers of the epidermis as well as promoting epidermal repair.[24]

Although not a primary objective of this study, we descriptively compared clinical outcomes for the 3 treatments. We found that SA paste, SA powder, and tetracycline had similar effects. All treatment groups showed improvement by d 7 with no evidence of treatment differences in lesion score on d 7. Lesions worsened between d 7 and 28 after treatment, regardless of treatment group. Our results are similar to those of previous studies comparing SA and tetracyclines. A study done by Jacobs et al compared the effects of 3 different treatments: tetracycline, "HealMax"[j], and "HoofSol"[k]. One of the ingredients in "HealMax" is methyl salicylate, which is a methyl ester of SA. They found that "HealMax" was similar in effectiveness to tetracycline when evaluated 1 week after a single treatment.[16] In 2013, Schultz and Capion found that topical treatment with SA proved to be 1.75-fold better than chlortetracycline in terms of clinical improvements, and 2.5 times greater at reducing lesion size by d 14 and 34 after treatment.[19]

It is important to note that we did not observe complete healing, and many of the lesions regressed back to an M2 lesion by d 28 among all treatments. Transitions between lesion stages have been observed to occur over 1 to 2 weeks.[15,18] Our results agree with the shorter transition period, as we had lesions transition between stages from 0 to 7 d and from 7 to 28 d. It is difficult to isolate the reason for the frequent reoccurrence of this disease; a contributor is likely the process of *Treponome* spp spirochete bacteria encysting deep into the epidermal layers.[3,8]

Thermography can be a useful tool in identifying inflammation in the hooves of dairy cows.[11] We chose to evaluate the

thermographic images because there is a significant increase in temperature of lame feet caused by DD lesions or hoof horn lesions compared to healthy feet, and the heel area is the most accurate for detecting this temperature difference.[14] Our primary goal for using this tool was to see if temperature was associated with lesion score. We did find that M2 lesions had higher mean temperatures than M3 lesions, but the difference is too small to be diagnostically useful. One thing to note is that our study did not have any observed M0 or M1 lesions. Studies with all stages of DD included might be more likely to detect a correlation between temperature and lesion score, as would studies with more lesions enrolled; the current study was powered for the drug residue objective, not for clinical objectives.

Conclusion

In conclusion, we recommend withholding milk from treated cows from sale or consumption for at least 48 h following topical use of SA to treat DD. Our results also confirm the recommended 24 h milk withholding time after topical use of tetracycline to treat DD.

Endnotes

[a] TC Vet 324, VetOne, MWI, Meridian, ID
[b] Propylene Glycol, VetOne, MWI, Meridian, ID
[c] Salicylic Acid Powder USP, JorVet, Jorgensen Labs, Loveland, CO
[d] Hoof Gel with Salicylic Acid 38%, JorVet, Jorgensen Labs, Loveland, CO
[e] R Core Team, 2020, www.r-project.org
[f] Apple Inc., Cupertino, CA
[g] FLIR Systems OÜ, Estonia
[h] Thermo Scientific, San Jose, CA, USA
[i] SAS Institute Inc., Cary, NC
[j] AgroChem Inc., Saratoga Springs, NY
[k] Diamond Hoof Care Ltd., Intracare BV, Veghel, the Netherlands
[l] Xcalibur Software, Thermo Fisher Scientific, Waltham, MA
[m] Chromeleon™ Data System, Thermo Fisher Scientific, Waltham, MA

Acknowledgements

This project was funded by the Hoof Trimmers Association and the American Association of Bovine Practitioners Foundation. Thank you to the management and staff of the North Dakota State University Dairy Teaching and Research Center for technical assistance. Thank you to the Analytical Chemistry Services Lab at the Iowa State University Veterinary Diagnostic Laboratory for the analytic chemistry work on this project. The authors declare no conflicts of interest.

References

1. Battezzati A, Fiorillo G, Spadafranca A, Bertoli S, Testolin G. Measurement of salicylic acid in human serum using stable isotope dilution and gas chromatography–mass spectrometry. *Analytical Biochemistry* 2006;354:274-278.
2. Blacklock CJ, Lawrence JR, Wiles D, Malcolm EA, Gibson IH, Kelly CJ, Paterson JR. Salicylic acid in the serum of subjects not taking aspirin. Comparison of salicylic acid concentrations in the serum of vegetarians, non-vegetarians, and patients taking low dose aspirin. *J Clin Pathol* 2001;54:553-555.
3. Capion N, Larsson EK, Nielsen OL. A clinical and histopathological comparison of the effectiveness of salicylic acid to a compound of inorganic acids for the treatment of digital dermatitis in cattle. *J Dairy Sci* 2018;101:1325-1333.
4. Cha E, Hertl JA, Bar D, Gröhn YT. The cost of different types of lameness in dairy cows calculated by dynamic programming. *Prev Vet Med* 2010;97:1-8.
5. Cramer G, Lissemore KD, Guard CL, Leslie KE, Kelton DF. Herd- and cow-level prevalence of foot lesions in Ontario dairy cattle. *J Dairy Sci* 2008;91:3888-3895.
6. Cramer G, Solano L, Johnson R. Evaluation of tetracycline in milk following extra-label administration of topical tetracycline for digital dermatitis in dairy cattle. *J Dairy Sci* 2019;102:883-895.
7. Cutler JH, Cramer G, Walter JJ, Millman ST, Kelton DF. Randomized clinical trial of tetracycline hydrochloride bandage and paste treatments for resolution of lesions and pain associated with digital dermatitis in dairy cattle. *J Dairy Sci* 2013;96:7550-7557.
8. Döpfer D, Ter Huurne AAHM, Cornelisse JL, Van Asten AJAM, Koopmans A, Meijer FA, Bosma RB. Histological and bacteriological evaluation of digital dermatitis in cattle, with special reference to spirochaetes and *Campylobacter faecalis*. *Vet Rec* 1997;140:620-623.
9. Evans NJ, Brown JM, Demirkan I, Murray RD, Vink WD, Blowey RW, Carter SD. Three unique groups of spirochetes isolated from digital dermatitis lesions in UK cattle. *Vet Microbiol* 2008;130:141-150.
10. Food Animal Residue Avoidance Databank, Available at: http://www.farad.org/wdilookup/wdi_cattle.html. Accessed March 16, 2021.
11. Gianesella M, Arfuso F, Fiore E, Giambelluca S, Giudice E, Armato L, Piccione G. Infrared thermography as a rapid and non-invasive diagnostic tool to detect inflammatory foot diseases in dairy cows. *Polish J Vet Sci* 2018;21:299-305.
12. Gingerich DA, Baggot JD, Yeary RA. Pharmacokinetics and dosage of aspirin in cattle. *J Am Vet Med Assoc* 1975;167:945-948.
13. Gomez A, Cook NB, Bernardoni ND, Rieman J, Dusick AF, Hartshorn R, Socha MT, Read DH, Döpfer D. An experimental infection model to induce digital dermatitis infection in cattle. *J Dairy Sci* 2012;95:1821-1830.
14. Harris-Bridge G, Young L, Handel I, Farish M, Mason C, Mitchell MA, Haskell MJ. The use of infrared thermography for detecting digital dermatitis in dairy cattle: What is the best measure of temperature and foot location to use? *Vet J* 2018;237:26-33.
15. Holzhauer M, Bartels CJ, Döpfer D, van Schaik G. Clinical course of digital dermatitis lesions in an endemically infected herd without preventive herd strategies. *Vet J* 2008;177:222-230.
16. Jacobs C, Orsel K, Mason S, Barkema HW. Comparison of effects of routine topical treatments in the milking parlor on digital dermatitis lesions. *J Dairy Sci* 2018;101:5255-5266.
17. Krull AC, Cooper VL, Coatney JW, Shearer JK, Gorden PJ, Plummer PJ. A highly effective protocol for the rapid and consistent induction of digital dermatitis in Holstein calves. *PloS One*, 2016;11:e0154481.
18. Nielsen BH, Thomsen PT, Green LE, Kaler J. A study of the dynamics of digital dermatitis in 742 lactating dairy cows. *Prev Vet Med* 2012;104:44-52.
19. Schultz N, Capion N. Efficacy of salicylic acid in the treatment of digital dermatitis in dairy cattle. *Vet J* 2013;198:518-523.
20. Silbergeld EK, Graham J, Price LB. Industrial food animal production, antimicrobial resistance, and human health. *Annu Rev Public Health* 2008;29:151-169.
21. US Food and Drug Administration, Guidance for Industry #207, Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs In Food-Producing Animals: Marker Residue Depletion Studies to Establish Product Withdrawal Periods VICH GL48(R), March 2015 Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cvm-gfi-207-vich-gl48-studies-evaluate-metabolism-and-residue-kinetics-veterinary-drugs-food Accessed June 16, 2021.

22. US Food and Drug Administration. Veterinary Feed Directive. US Federal Register, pp 31707-31735, document # 80 FR 31707, Available at: https://www.federalregister.gov/d/2015-13393
23. USDA. Dairy, 2007 Part IV: Reference of dairy cattle health and management practices in the United States. USDA:APHIS:VS, CEAH. Fort Collins, CO. Available at: https://www.aphis.usda.gov/animal_health/nahms/dairy/downloads/dairy07/Dairy07_dr_PartIV.pdf Accessed April 4, 2020.
24. Weber J, Richter S, Freick M. Comparison of the therapeutic efficacy of salicylic acid paste with a polyurethane wound dressing for the treatment of digital dermatitis lesions in dairy cows. *Res Vet Sci* 2019;125:7-13.
25. Zinicola M, Lima F, Lima S, Machado V, Gomez M, Döpfer D, Guard C, Bicalho R. Altered microbiomes in bovine digital dermatitis lesions, and the gut as a pathogen reservoir. *PLoS One* 2015;10:e0120504.