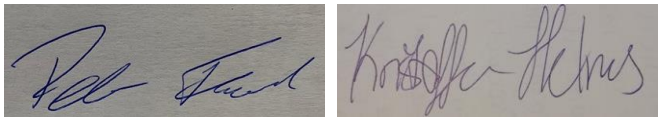
UNIVERSITY OF COPENHAGEN
FACULTY OR DEPARTMENT

Master Thesis

Peter Faudel -gjk226 & Kristoffer Sig Helms -fsj448



A comparison of clinical effect of two non-antibiotic products to chlortetracycline for the treatment of digital dermatitis in dairy cattle

Supervisors: Nynne Capion & Jörg Matthias Dehn Enemark

Submitted on: 21 January 2019

Name of department:	Department of Veterinary Clinical Science – Section for Medicine and Surgery
Authors:	Peter Faudel & Kristoffer Sig Helms
Title and subtitle:	A clinical comparison of two non-antibiotic products to chlortetracycline for treatment of digital dermatitis in dairy cattle
Topic description:	Clinical trial comparing the curative effect of salicylic acid and a solution of copper and zinc minerals to chlortetracycline when treating digital dermatitis in 3 Danish dairy herds during a 28-day study period.
Supervisors:	Nynne Capion & Jörg Matthias Dehn Enemark
Submitted on:	21 January 2019

ABSTRACT

Digital dermatitis (DD) is an infectious bacterial disease possibly caused by *Treponema* spp. The disease is a dermatitis and epidermitis characterized by ulcerative or papillomatous lesions on the feet of cattle. It is a major production and welfare problem in the dairy industry commonly treated with topical antibiotics, but with an increasing focus on limiting antibiotic use and avoiding antibiotic resistance non-antibiotic alternatives are needed.

The aim of this study was to compare two non-antibiotic alternatives to an antibiotic spray commonly used in the treatment of DD. Three Danish farms were visited at routine hoof-trimming over a three-month period in the fall of 2018 and 62 lesions were enrolled in the study. Eligible lesions were photographed and clinically scored before randomly enrolled to one of three treatments. Initial treatment was carried out on lifted legs in the trimming chute and all follow up treatments and evaluations were done on standing cows in the milking parlor. The cows were followed for 28 days with treatments in the first seven days after hoof-trimming and observations at d10 and d28 after hoof-trimming. The cows were treated with one of the three products: chlortetracycline spray (Cyclo spray, Dechra Veterinary Products A/S, 7171 Uldum, Denmark), salicylic acid (Salicylsyre Jørgen Kruuse A/S, 5550 Langeskov, Denmark) or a spray containing a solution of chelated copper and zinc minerals (Repiderma, Intracare BV, Netherlands). Photographs and registrations on all lesions were done on d0, d10 and d28. Products were compared regarding their ability to complete heal and clinically improve lesions appearance and reduce lesion length. All three products showed ability to improve lesions on d10 and d28 with at least 76% of lesions improved d10 and at least 57% on d28. The ability to complete heal lesions were considerably lower for all products with a maximum of 39% on d28 for salicylic acid. In regard to lesion length reduction salicylic acid maintained a continuous reduction beyond d10 with 60% reduced lesion length d28 whereas the two other products did not reduce lesion length beyond d10 never exceeding 39%.

This indicates that both non-antibiotic products are valuable alternatives to chlortetracycline. Eight lesions were biopsied to evaluate any difference in location and amount of *Treponema* spp. at the start and end of the study. Unfortunately, none of these lesions changed clinical or histological appearance during the study.

TABLE OF CONTENTS

1. **ABSTRACT……...……3**

2. **INTRODUCTION……...…..5**

3. **MATERIALS AND METHODS……..9**
   a. **Population……9**
   b. **Cow and Lesion selection……..…10**
   c. **Randomization……..10**
   d. **Experimental procedures and Treatments...……..……10**
      i. **Products and treatments protocol…….11**
      ii. **Evaluation of DD lesions……14**
      iii. **Histological evaluation of Tissue samples…….15**
   e. **Baseline comparison…….16**
   f. **Statistical Analyses……16**
      i. **Statistical analyses on DD CS-scores……16**
      ii. **Statistical analyses on DD lesion length……17**

4. **RESULTS……17**
   a. **Study Population……...17**
   b. **Baseline comparison…….19**
   c. **Interobserver agreement……..20**
   d. **Products Effect on Clinical Appearance…….……20**
      i. **Complete Healing……20**
      ii. **Clinical Improvement…….21**
      iii. **Development of lesions between d10 and d28……..22**
   e. **Characteristics of different Progressions……23**
   f. **Effect of Products on Lesions Size...……24**
   g. **Progression of the Mature lesions……27**
   h. **Histological Evaluation…….29**

5. **DISCUSSION……..29**

6. **CONCLUSION……36**

7. **ACKNOWLEDGEMENTS……36**

8. **REFERENCES……37**

INTRODUCTION

Digital Dermatitis (**DD**) is an infectious disease of cattle occurring worldwide throughout the intensive dairy industry (Laven and Logue, 2006; Orsel et al., 2018). The disease affects the feet of cattle with up to 88% located on the plantar and palmar surface in the area from the dewclaws to the heel bulb and the interdigital space (Read and Walker, 1998). DD is a dermatitis and epidermitis with an initial active state that progresses into a mature state (Döpfer et al., 1997; Krull et al., 2016). The initial active lesion is characterized by an oval shape with reddish to grey color, a raw or moist, flat or concave surface with granulomatous or erosive ulcers. As the active lesion progresses into a mature state, it's appearance changes to a grey color and a protruding surface covered with small filiform papillae (Read and Walker, 1998; Krull et al., 2016). The active stage of the condition causes pain that can lead to lameness (Döpfer et al., 1997; Read and Walker, 1998), ultimately resulting in decreased animal welfare and production losses due to reduced milk yield (0.5% pr. lactation), reduced conception rate (0.89 Risk Ratio), and higher risk of culling (0.14%) (Cha et al., 2010; Ettema et al., 2010). The mature stage is considered non-painful but as the DD lesion is often in its mature stage for a long period of time before transitioning back to an active or healing stage, the mature stage is assumed to be the most contagious stage with a reproduction ratio >2 compared to the active stage with a reproduction ratio <0.06 (Biemans et al., 2018).

The exact pathogenesis remains uncertain but is considered a multifactorial disease with an interaction between environmental skin factors, such as moist conditions, poor hygiene (Wells et al., 1999; Relun et al., 2013) and an oxygen poor environment causing a slight maceration of the skin, in combination with pathogens (Gomez et al., 2012; Krull et al., 2014). The predominant infectious agents that are considered to cause DD are spirochetes, especially *Treponema* spp., as they are commonly identified in large numbers from DD lesions (Read et al., 1992; Demirkan et al., 1999) and by histological examination of tissue samples from DD lesions (Blowey et al., 1994; Döpfer et al., 1997).

It is assumed that the healing process of DD lesions initiates with reepithelialization from the stratum basale in the epidermis. If the lesion advances to a mature state, the epidermis thickens by hyperkeratosis and the papillated epidermal hyperplasia covers the dermal papilla. In this process, the stratum basale and basement membrane is damaged which prolongs the reepithelialization to normal skin tissue (McGavin and Zachary, 2007).

In order to control DD on herd level, identification and prompt treatment of lesions are key elements. Identification of DD lesions in cattle restrained in the trimming chute remains the gold-standard method for DD lesion scoring. Scoring in the milking parlor has shown levels of sensitivity (**Se**) of 0.90-1 and specificity (**Sp**) of 0.80-0.99 (Relun et al., 2011; Solano et al., 2017) when investigating for the presence vs. absence of lesion. Se and Sp were both increased when placing a mirror under the heel to visualize lesions not visible from the observer's point of view.

Different scoring systems have been developed to describe stages and progress of DD lesions including features of macroscopic appearance, size and shape, and histological evaluation of dermis and epidermis (Döpfer et al., 1997; Read and Walker, 1998; Manske et al., 2002; Krull et al., 2016).

The 4 point M-stage scale developed by Döpfer et al. (1997) and later modified into a 6 point M-stage scale by Berry et al. (2012) is commonly used to describe DD lesion progression. In the early M-1 stage the DD lesion is red, granulomatous and <2 cm across, the lesion can heal to normal skin, M-5 (M-0 in some studies), but often progresses into a painful, red and ulcerative M-2 stage >2 cm across. A non-painful M-3 stage is seen when the lesion surface is covered by a dark rubbery scab as a sign of healing. If an M-2 lesion is left untreated, it will often develop into a chronic M-4 lesion characterized by a raised surface, a grey color and papillomatous projections (Berry et al., 2012). The chronic M-4 lesion can become an M4.1 lesion if it develops a small M-1 active foci, which is prone to redevelop into a new M-2 lesion. A recent study by Krull et al. (2014) monitored 53 cows' natural DD lesions progression every three to four weeks for a period of three years. This study compared the clinical appearance of DD lesions with microbiota isolated from those lesions using DNA sequencing. They found that the bacterial composition of DD lesions change significantly as the lesion develops through its stages, and they further subdivided the early stages of DD lesions into two different groups developing the 7-point IOWA-scoring system. Overall conclusions from both studies were that advanced DD lesions are preceded by early DD lesion and that DD lesion progresses systematically (Berry et al., 2012) even after topical oxytetracycline treatment, with lesion scores highly correlating (either one stage greater or one stage less) to previous lesion scores ($r=0.7913$; $P < 0.0001$) (Krull et al., 2014).

A detailed scoring system is preferred by authors describing the gross pathology, development and progress of DD lesions, but to evaluate the effect of treatment on DD lesions with a specific product, authors often choose to simplify their scoring system. One can either dichotomize the M-stage scale in different ways, for instance categorize M-2 lesions and M-4.1 lesions as active and M-5, M-1, M3 and M-4 as in-active (Holzhauer et al., 2011; Jacobs et al., 2018). Others quantify treatment effect as the amount of exact numeric reduction in lesion size (Nishikawa and Taguchi, 2008; Kofler et al., 2015; Christensen and Larsen, 2016) or divide lesion size into scores ex. score 1: lesion size <2.5 cm and score 2: lesion size >2.5 cm (Shearer and Hernandez, 2000). It is the assumed that a reduction in lesion size after treatment is a sign of healing and a treatment success. Finally, some studies divide the clinical appearance of DD lesions into active or early, mature, healing and healed as done by Shearer and Hernandez (2000) and Cutler et al. (2013). These terms are based on observations from Read and Walker (1998) on 183 DD lesions from cows in California. The term "healed" describes lesions with normal skin features with no moist or scab surface, the term "healing" describes a dry DD lesion covered by or developing a scab with assumed normalizing skin features underneath, an "active" DD lesion is characterized by a raw, pink-red granulomatous or ulcerative appearance and "mature" DD lesions are grey in color with a raised surface covered by small filiform papillae (Read and Walker, 1998; Hernandez and Shearer, 2000; Cutler et al., 2013a).

The curative effect of different treatment products varies among studies. One reason is the different criteria definition of *treatment success*, *treatment effect* and *cure rate* (Krull et al., 2016). In some studies, the term *cure rate* describes lesions that appear "completely healed" and cannot be distinguished from the surrounding normal skin. Others use *cure rate* to describe a shift from an active lesion stage to any other lesion stage and interpret this change as an improvement.

Another reason for the difference in curative effect among studies is due to the difference in treatment protocol, ex. length of study period, the concentration of the treatment product or how often treatment should be carried out (Laven and Logue, 2006). A study period of thirty days is common as it leaves enough time for the treatment to show effect (Hernandez et al., 1999; Nishikawa and Taguchi, 2008; Berry et al., 2010; Capion et al., 2018), and therefore a 28-day study period with observations on d0, d10 and d28 was chosen in our study to allow for comparison with similar studies.

Macroscopic clinical appearance of DD lesions can differ from the microscopic appearance of DD lesions (Demirkan et al., 1998; Berry et al., 2010). To assess complete healing and lesion

progress after treatment there is a need for a combination of clinical and histological evaluation (Berry et al., 2010; Capion et al., 2018).

Individual treatment is often topical applying either paste, sprays or bandages containing antibiotics, acids or metallic ions on the DD lesions (Laven and Logue, 2006). The curative effect of topical tetracycline on DD lesions has previously been shown to be as high as 72-73% (Van Amstel et al., 1995; Britt and McClure, 1998), but recent studies show a lower curative effect of topical tetracycline around 9-50% (Shearer and Hernandez, 2000; Cutler et al., 2013a; Krull et al., 2016). Authors often observed a good initial response the first 10 days after topical tetracycline treatment, but antibiotic resistance by *Treponema* spp. due to long-term exposure of low doses of tetracycline were suggested by authors (Shearer and Hernandez, 2000; Nishikawa and Taguchi, 2008) as an explanation of recent years' minimal curative effect of tetracycline when following lesions for more than 10 days.

With an increased focus on antibiotic resistance and prudent use of antibiotics in food production, a demand for alternatives in the treatment and prevention of DD is currently becoming a hot topic. Topical salicylic acid and topical mineral formulations of zinc and copper have previously shown treatment effect on DD lesions comparable to that of topical tetracycline (Holzhauer et al., 2011; Fiedler et al., 2015; Kofler et al., 2015; Jacobs et al., 2018).

The aim of the recent study was to compare the effect of three commercially available topical skin products on DD lesions under Danish conditions. Two non-antibiotic products, Salicylic Acid (**SA**) (Salicylsyre Jørgen Kruuse A/S, 5550 Langeskov, Denmark) and a spray containing chelated copper and zinc minerals (**M**) (Repiderma, Intracare BV, Netherlands) were compared to chlortetracycline 2.45% (**CTC**) (Cyclo spray, Dechra Veterinary Products A/S, 7171 Uldum, Denmark). Comparison of treatment effect was based on a 28-day trial period with clinical evaluation of all lesions on enrolled cows d0, d10 and d28. To support clinical findings, histological evaluation of lesions was done based on tissue samples taken on d0 and d28 from a random 1/3 of cows from each treatment group.

MATERIAL AND METHODS

The study was designed as a randomized multicentric, parallel group, controlled field study. All procedures were carried out in agreement with The Animal Experiments Inspectorate (Fødevarestyrelsen, Glostrup, Denmark).

Population

The Sample size was calculated using OpenEpi sample size calculator (http://www.openepi.com/SampleSize/SSCohort.htm visited January 6, 2018) which uses Fleiss, Statistical Methods for Rates and Proportions 2nd Ed., formulas 3.18 & 3.19 using a 95% confidence interval and a power of 80. The expected difference in effect (ability to improve a DD lesion) between the products was estimated to be 30% (Holzhauer et al., 2011; Kofler et al., 2015) resulting in a minimum sample size of 39 lesions for each product group. This would require a total sample size of 117 lesions.

Herds were selected by contacting hoof trimmers from Jutland, Funen and Zealand in Denmark. Based on willingness to cooperate, three farmers and two hoof trimmers were selected to participate in the study. The three herds selected, which were located on Zealand, had a known problem with DD and had not used preventive measures against DD on herd level for three weeks prior to study enrollment. The farms all had a herringbone milking parlor and the cows were milked twice a day with approximately 12 hours interval.

Farm A consisted of 111 Holstein cows and approximately 60 cows were trimmed at d0. All cows were housed in a barn with mattresses in cubicles, a rubber floor and manure scraper cleaning the floor in two-hour intervals. The first two weeks of Farm A's study period, the cows were on pasture during the day between morning and evening milking.

Farm B consisted of 166 Red Danish (RDM) cows, approximately 60 cows were trimmed at d0. All cows were housed in a barn with mattresses in cubicles and had a bedding material consisting of a mixture between straw and chalk. The floor type was slatted concrete. Half the barn had a manure scraper cleaning the floor in two-hour intervals and the other half a manure scraping robot to clean the floor.

Farm C consisted of 222 cross-breeds cows from a Holstein line, approximately 200 cows were trimmed on d0. These cows were also housed in a barn with mattresses in cubicles with sawdust as a bedding material. The floor consisted of slatted concrete with a manure scraper cleaning it in two-hour intervals.

All three farmers had previously used CTC treatment for DD with good results but reported a reduced effect in recent years. All three farmers currently used SA for DD treatment during regular hoof trimming and all had an on-farm trimming chute for individual treatment of severe lame cows.

Cow and Lesion selection

Cows with active or mature DD lesions were included in the study, if the lesions were located on their hind feet, within an area where it was assumed that the lesion would be visible on the standing cow in the milking parlor. Lesions located to low on the heel bulb, close to, or inside the interdigital space were not included in the study. Lesions were classified by the authors as either active or mature based on observations from Read and Walker (1998) and later modified into a scoring system by Hernandez and Shearer (2000). Active lesions were characterized by shape (round, oval, flat or concave) and appearance (raw, moist, red-yellow to gray and granular to strawberry-like surfaces). The mature lesions were greyish in color with a round or oval shape and a protruding surface covered with raised small filiform papillae or hair (Hernandez and Shearer, 2000).

Randomization

Cows were randomized between treatment groups using random.org's list randomizer (https://www.random.org/lists/ visited September 18, 2018) in clusters of three. Cows with DD lesions on both hind legs were allocated the same treatment group and both lesions were included in the further analysis to maximize our sample size. One third randomly assigned cows from each farm and treatment group were biopsied from one DD lesion on d0 and d28 for later histological evaluation.

Experimental procedure and Treatments

Cows came to the trimming chute for routine hoof trimming. The hind legs were thoroughly washed using the hoof trimmers washing equipment (pressure hose) and if any excess dirt remained, it was wiped off using a paper towel. Lesions that met the enrollment requirements were scored and photographed while the cow was restrained in the trimming chute. Photographs were taken with an iPhone 8 (Apple Inc Cupertino, CA, USA). A ruler applied with a study no. was included in the photo of the lesion. It was attempted to line the ruler as parallel and close to the lesion as possible to avoid any measurement errors. The ruler allowed for measuring the lesion length later and the study no. allowed for a blinded score of the clinical appearance from

the photograph. The study no. was stored together with the cow's identification tag for later analysis. Lesions were scored based on clinical appearances as shown in the scoring scheme (Table 1) and their highest lesion lengths were measured using Microsoft Paint (Microsoft Corp., Redmond, WA, USA).

A central lesion biopsy was taken from every third cow enrolled in each treatment group from every herd using a 6 mm punch biopsy (Jørgen Kruuse A/S, 5550 Langeskov, Denmark). Prior to the biopsy, 3-5 mL procaine (Procamidor Vet. - Salfarm Danmark A/S, 6000 Kolding, Denmark) were administered subcutaneously proximal to the lesion. Biopsies were stored in biopsy containers that were randomly numbered in advance to ensure a blinded histological evaluation. The biopsy No. was stored together with the study no. and the cow's identification tag for later analysis. Biopsies were finally fixated in 10% neutral buffered formalin until further processing. Assigned cows were biopsied a second time at the end of the trial on d28 using the same procedure as d0.

Treatments on d0 were applied while the cows were restrained in the trimming chute and with the relevant leg fixated. Follow up treatments were undertaken while the cows were standing in the milking parlor. Lesions were washed with water from a pressure hose located in the milking parlor and wiped off using paper towels before every treatment, thereby preventing excess dirt from potentially hampering the treatment effect of the applied products or compromising the evaluation of the lesion.

Products and Treatment protocol. In the recent study, CTC treatment followed the manufacturer's recommendations for DD treatment and sprayed the lesions for three seconds twice with a 30 second interval, from a distance of 15-20 cm from the lesion. All applications of product CTC were carried out by the authors.

Lesions treated with product SA were fully covered on the lesion area with a layer of approximately 0.5 cm in thickness. This corresponds roughly to 5-10 grams for each lesion. After application of SA the lesion was wrapped with a bandage. The bandage consisted of a thin layer of cotton wool in the interdigital space and a flexible bandage (Vet-flex®, Jørgen Kruuse A/S, 5550 Langeskov, Denmark) on top. Every bandage was applied by the hoof trimmer. Bandages were removed at d2, while cows were standing in the milking parlor, using a knife or a

long-shaft bandage knife (Viking Forbindingskniv, VIKINGDANMARK, 8200 Århus, Denmark).

Treatment with product M was performed by spraying the lesion twice for three seconds with a 30 sec. interval from a distance of 15-20 cm from the lesion. Following this, the lesions were wrapped with a bandage as described for product SA, bandages were removed d3. All applications of product M were carried out by the authors.
Treatment schedule for all products can be seen in fig. 1

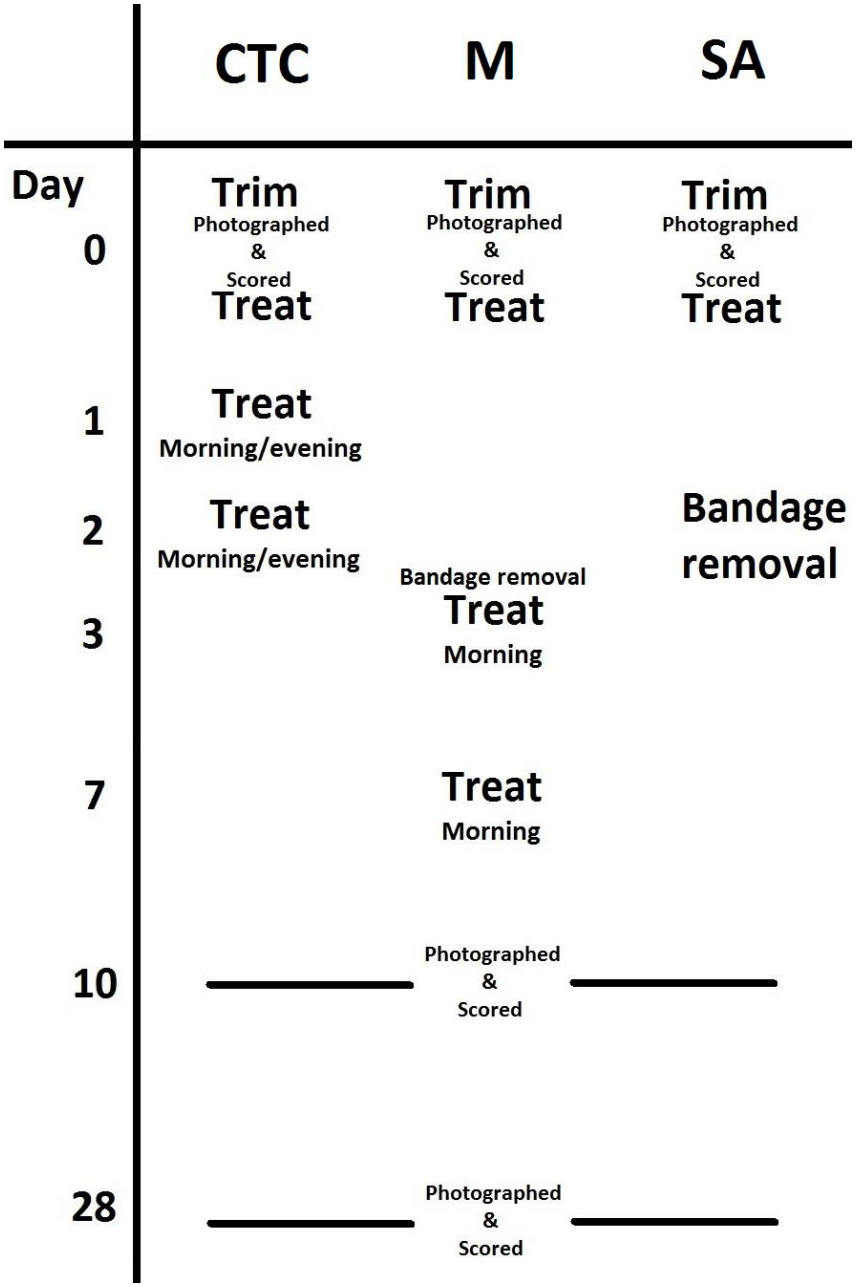


Fig. 1. Treatment (Treat) and scoring schedule for digital dermatitis in the 28 day study period conducted in the recent study. Using chlortetracycline spray (CTC) treating two times daily, twice with 30 sec. interval, for three days. Using a spray with Minerals (M) treating twice with 30 sec. interval once on d0 before applying a bandage, treating twice with 30 sec. interval again on d3 after bandage removal and twice with 30 sec. interval at d7. Using Salicylic Acid (SA) in powder form covering the DD lesion and applying a bandage at d0 and removing the bandage at d2. Scoring using Clinical scores: CS-0=Healed, CS-1=Healing, CS-2= Active lesion and CS-3= Mature lesion Trim=hoof trimming. D0 observations and treatment were done on fixated cows in the trimming chute, the remainder of observations and treatments were done on standing cows in the milking parlor.

Evaluation of DD lesions. Lesions were evaluated before each treatment or after removal of bandage and all lesions were photographed d0, d10 and d28. If the lesion appeared completely healed, the previously affected area was photographed.

Evaluation of treatment effects was based both on combined observations from previously described scoring systems (Table 1) (Read and Walker, 1998; Hernandez and Shearer, 2000; Manske et al., 2002; Cutler et al., 2013a) and the approximated reduction in highest length of the lesion after treatment. Analyzing the products effect on both the DD lesions clinical features and the change in their lesion length would be more adequate compared to analyzing lesion length or clinical appearance alone.

Table 1. Clinical Scoring (CS) system with four levels CS-0,-1,-2,-3 for Digital Dermatitis lesions used in the recent study based on the predominant clinical appearance of the Digital Dermatitis lesion with description of the characteristics for each score modified from Read & Walker (1998), Hernandez & Shearer (2000) Manske et al., (2002), Cutler et al., (2013) and Krull et al., (2014).

CS[1]	Predominant clinical appearance
0	Lesion is either no longer visible, or visible but covered by dry pink skin
1	Healing: Lesion area is visible but dry and displaying normalizing skin features or is covered by a dry necrotic scab with assumed normalizing skin features underneath.
2	Active: Oval moist, raw, reddish to gray, flat or concave with an erosive, granulomatous or ulcerative surface
3	Mature: Grayish in color with a protruding surface covered with small filiform papillae

[1]CS: Clinical score

The change in lesion size was calculated as the percentage of the change in lesion length between two measurements of the same lesion from the three observations (d0, d10 and d28). Examples of CS-score 1, 2 and 3 are illustrated in fig. 2.

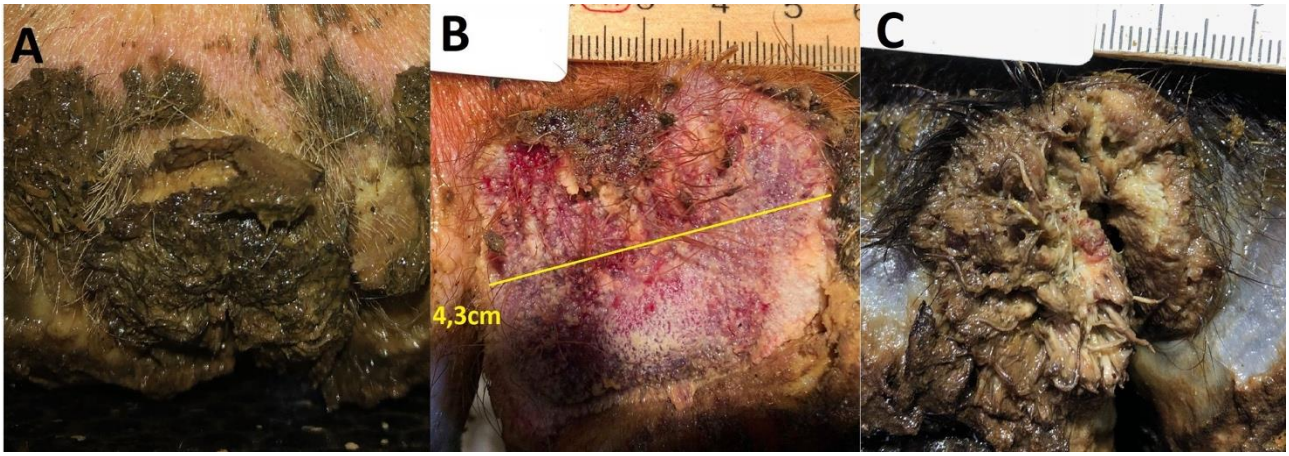


Fig. 2. Examples of different digital dermatitis lesions stages using the CS-score (Clinical scores: CS-0=Healed, CS-1=Healing, CS-2= Active lesion and CS-3= Mature lesion). (A): CS-1 with a raised necrotic scab, showing signs of loosening at the edges. (B): CS-2 with a characteristic red erosive surface, slightly under the level of the skin, Yellow line=measuring line, measured to be 4.3 cm. (C): CS-3 with a characteristic gray color and raised filiform papillae, protruding well above the surface of the skin.

Evaluation of the lesions in the milking parlor was done looking at the lesion from behind as the cows stood in a herringbone system. In the cases where the lesion was located too low to allow for direct visualization, a cosmetic mirror glued to a kitchen spatula that allowed for visual inspection and photographing of the lesion, when placing the cosmetic mirror on the floor behind the hind leg so the lesion was reflected in the mirror. A powerful headlamp (Led Lenser H7.2, Ledlenser GmbH & Co. KG, Solingen, Germany) with 250 lumen and an intense focus option was used to improve visibility in the milking parlor.

Histological evaluation of Tissue samples. The tissue samples from the biopsy containers were prepared and embedded in paraffin wax. The tissue samples were then sliced perpendicularly to the skin surface. From each tissue sample two slices were stained. One slice was stained with hematoxylin and eosin (H&E) and used as a reference for the orientation in the tissue sample. The second slice was stained using Levaditi's method (Campbell and Rosahn, 1950), a silver nitrate stain where the morphology of spirochetes can be seen as black stained long twisted bacteria. The tissue samples were histologically examined to support the macroscopic findings. The presence and rough quantity estimates of *Treponema* spp. were assessed, and it was evaluated whether *Treponema* spp. was located on the surface or how deep they were located in the epidermis and dermis. After blinded evaluation of the silver stained slices, the d0 tissue samples were
compared to the corresponding d28 tissue sample to assess any change after treatment.

Baseline comparison

Baseline comparison of all cows and DD lesions included in the study on d0 were compared in between groups. Variables of interest included lactation stage (Days in Milk, DIM), parity, daily milk yield from the latest yield control and the distribution of lesion length. Means, standard deviation and range were calculated using PROC MEANS in SAS Studio (SAS Institute).

Statistical analyses

All data was entered into Microsoft Excel (Microsoft Corp., Redmond, WA, USA), the statistical analyses were performed using SAS, and P-values <0.05 were considered statistically significant in all analyses. The foot within a cow was considered the statistical unit.

Before descriptive statistical analyses, interobserver agreement was calculated using a Cohens Kappa κ (Cohen, 1960). The authors scored lesions from photographs independently using the CS-score 0-3 (table 1), off farm site. The Kappa analysis was calculated in SAS using PROC FREQ with the agree option for agreement coefficients. Results were interpreted as suggested by Landis and Koch (1997) where interpretations of Kappa values are divided into five categories: $\kappa \leq 0.2$ = Poor, $0.2 < \kappa \leq 0.4$ = Fair, $0.4 < \kappa \leq 0.6$ = Moderate, 0.6 $\kappa \leq 0.8$ = Good and $0.8 < \kappa$ = Very good.

After the Kappa calculation, disagreement between the authors were discussed and in the event of disagreement between authors, the CS-score was discussed until agreement was reached.

The overall objective with the statistical analyses was to compare the effect of product CTC to that of product SA and M, in their ability to completely heal a DD lesion, to improve (reduce) a DD lesions CS-score, considering a score of CS-0 the best possible outcome, and the ability to reduce a DD lesions length during the 28-day study period. All comparisons were made between corresponding observations from d0 and d10, d10 and d28 or d0 and d28. Comparison of treatment effect was assessed on the active lesions and the mature lesions separately.

Statistical analyses on DD CS-score. Using Fisher's exact test in SAS, the three products were compared in their ability to completely heal a lesion and improve the lesions clinical appearance. Complete healing was defined as lesions transitioning from CS-1, -2 & -3 to CS-0. Improvement of the lesions' clinical appearance was defined as a transition from CS-scores 2 & 3, "No Improvement", to CS-scores 0 & 1, "Clinical Improvement".

Statistical analyses on DD lesion length. Comparison of the 95 % confidence interval for Mean and Standard deviation of length after each observation day by treatment group was performed to display the development in average lesion length in between treatment groups. A paired T-test was performed using PROC TTEST with the paired option in SAS, to analyze if the products could statistical significantly reduce the lesions length through the 28-day study period.

RESULTS

Study Population

The herds collectively contributed with 55 cows, seven cows from Farm A, 11 from Farm B and 37 from Farm C. Ten cows from Farm C contributed with both hind legs resulting in a total of 65 lesions from the 55 cows. After blinded CS-scoring of lesions on photographs from d0, 55 lesions scored CS-2. Ten lesions from Farm C scored CS-3 and three lesions from Farm C scored CS-1 and were excluded from further analyses.

During the course of the 28-day study period, 15 cows with a total of 17 DD lesions were excluded from the study, these were distributed as follows:

From group CTC, five cows with five lesions were excluded, all on d28. Two cows were excluded as their DD lesions were positioned too low to register and photograph, two were excluded because they were dried off and moved to a dry cow barn that did not allow for proper lesion evaluation. Finally, one cow was treated for DD with SA, as farm personnel observed what they described as severe limping from the legs previously treated.
From group M, six cows with a total of seven lesion were excluded during the trial period. Two cows were excluded at d10, as their DD lesions were positioned too low to register and photograph, one of those cows was registered again at d28 as the cow was now standing in a position to allow for proper evaluation of the DD lesion. Two cows were excluded on d28, one because it was dried off and moved to a dry cow barn that did not allow for proper lesion evaluation and one because of culling. The cow was not culled because of limping, lameness or leg issues. Finally, two cows were treated for DD, one with SA and one with CTC, as farm personal observed what they described as severe limping from the legs previously treated with M. One of those cows had a history of repeatedly recurrence of DD lesions and its DD lesion was also biopsied on d0.
From group SA, four cows with a total of five lesion were excluded from the study at d28. One

was dried off and moved to a dry cow barn that did not allow for proper lesion evaluation, one was culled, but not because of limping, lameness or leg issues. Two were re-treated with SA as farm personnel observed what they described as severe limping from the legs previously.

In ⅕ of group CTC's, ⅓ of group M's and ½ of group SA's excluded cows, exclusion happened as a consequence of necessary re-treatment of the DD lesion before the end of the study period.

After the exclusion of the lesions above, we were left with 62 lesions from d0, 59 lesions from d10 and 46 lesions from d28 (Table 2).

This yielded 167 registrations for further analyses. Cows excluded at d28 were included on d10 analyses if they were not already excluded at that time.
Eight cows were biopsied on d0 (two SA, three CTC and three M) but only six out of the eight (one SA, two CTC and three M) were available for biopsying on d28 - one was culled and one was dried off, resulting in a total of 14 biopsies, where one from the M product group was re-treated with CTC. The reason we do not have biopsies from every 3rd cow at Farm C was due to the compromise with the hoof trimmer at Farm C, to only biopsy three cows to avoid slowing him down.

Table 2: Distribution of Digital dermatitis lesions by farm (A,B and C) and product (Chlortetracycline=CTC, Salicylic Acid=SA and Minerals=M), on d0, d10 and d28. Distribution of biopsies by farm and product at d0/d28

Location	Product	No. of lesions d0	No. of lesions d10	No. of lesions d28	No of biopsies d0/d28
Farm A	CTC	2	2	2	1/1
	SA	2	2	1	0/0
	M	3	3	2	1/1
Farm B	CTC	4	4	3	1/1
	SA	4	4	4	1/1
	M	3	3	3	1/1
Farm C	CTC	12	11	8	1/0
	SA	10	10	8	1/0
	M	12	11	9	1/1
Farm C Mature lesions	CTC	1	1	1	0/0
	SA	6	6	4	0/0
	M	3	2	1	0/0

Baseline comparisons

No statistically significant difference between cows or lesions were detected in the three product groups, in regard to parity, DIM, daily milk yield from the latest yield control or lesions length at d0, as illustrated by Means and Standard deviation (Table 3).

Table 3: Product group characteristics *(Chlortetracycline=CTC, Salicylic Acid=SA and Minerals=M)* with Mean ± SD for Parity, Days in Milk (DIM), Daily Milk yield from the latest yield control (Daily yield) and Lesions length in cm at d0

Product	No. of lesions	Parity	DIM	Daily yield	Lesion Length d0
CTC	18	2.06 ± 1.11	184.67 ± 102.87	35.95 ± 6.31	2.39 ± 0.99
SA	16	1.38 ± 0.62	176.00 ± 86.61	28.89 ± 9.82	2.39 ± 0.93
M	18	1.66 ± 0.91	185.72 ± 96.87	31.79 ± 5.06	2.24 ± 0.79

Interobserver agreement

Agreement between authors scoring DD lesions using the scoring system from Table 1 on photographs were "Very good", according to Landis and Kocks(1977) definition $\boldsymbol{\kappa} \geq 0.8$, with an unweighted $\boldsymbol{\kappa}$ =0.87 (95% CI: 0.81 - 0.93) and a weighted $\boldsymbol{\kappa}_w$ = 0.89 (95% CI: 0.83 - 0.95).

Products Effect on Clinical Appearance

A total of 52 CS-2 DD lesions from 49 cows were included in the analyses of the products effect on changes in clinical appearance of the DD lesions.

Complete Healing. The distribution of lesions CS-scores at d10 and d28 can be seen in fig. 3. At d10, four lesions appeared completely healed (CS-0), two out of 16 (13%) from the SA product group, one out of 17 (6%) from product group M and one out of 17 (6%) from product group CTC. At d28, nine lesions appeared completely healed (CS-0), five out of 13 (39%) were from the SA product group, two out of 13 (15%) from the CTC product group and two out of 14 (14%) from the M product group.

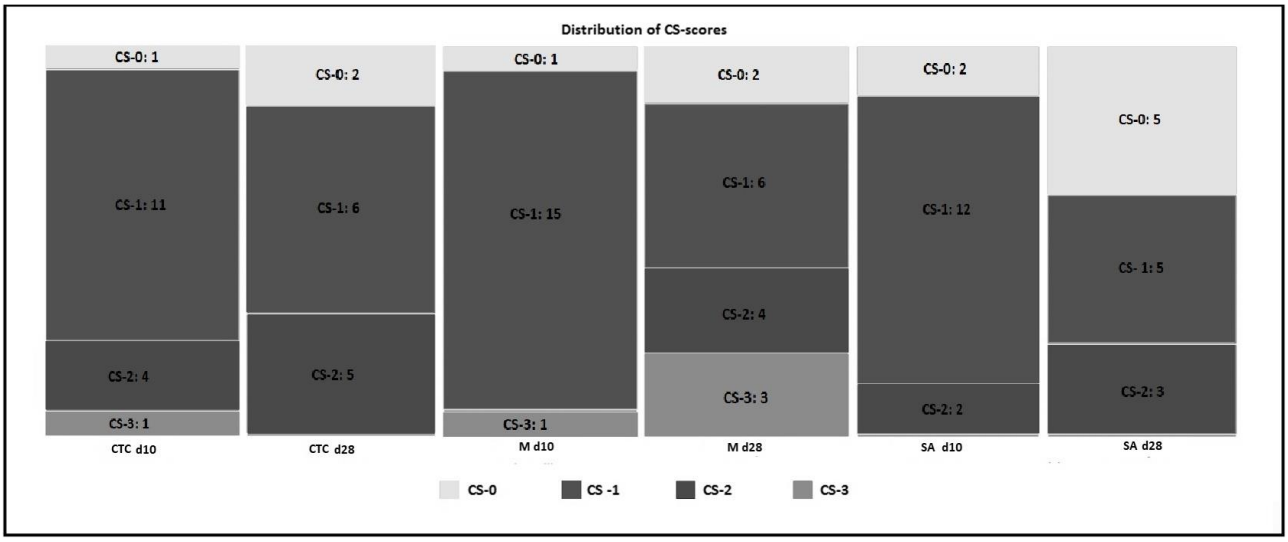


Fig 3: Distribution of digital dermatitis CS-scores (Clinical scores: CS-0=Healed, CS-1=Healing, CS-2= Active lesion and CS-3= Mature lesion) by product *(Chlortetracycline=CTC, Salicylic Acid=SA and Minerals=M)* and day, shown as proportions, the number of Digital Dermatitis lesions is not the same in treatment groups and on different days due to exclusion of cows.

When subjected to three independent Fisher's-exact tests comparing the groups two by two, none were found to be different ($P<0.05$) from one another in the ability to completely heal the DD lesions at either d10 (SA vs M P=0.60, CTC vs SA P=1, CTC vs M P=1) or d28 (SA vs M 0.19, CTC vs SA P=0.39, CTC vs M P=1).

Clinical Improvement. The ability of the products to "Clinical Improvement" - change the CS-score from CS-2 to CS-0 or CS-1 - was examined on d10, 86% of the lesions included at d10 (50 lesions) were improved, 14 out of 16 (88%) from the SA product group, 16 out 17 (94%) from product group M and 13 out of 17 (76%) from product group CTC (Table 4).

Table 4: Digital Dermatitis lesions response to product *(Chlortetracycline=CTC, Salicylic Acid=SA and Minerals=M)* shown as "Responds/Number of observations in product group (proportion responding in percent)" at d10 and d28 for products CTC, SA and M. Clinical improvement (CS-0 or CS-1) Complete Healing(CS-0) using Clinical scores: CS-0=Healed, CS-1=Healing, CS-2= Active lesion and CS-3= Mature lesion

Product	Response	d10	d28
CTC	Clinical Improvement	13/17(76%)	9/13(69%)
CTC	Complete Healing	1/17(6%)	2/13(15%)
SA	Clinical Improvement	14/16(88%)	10/13(77%)
SA	Complete Healing	2/16(13%)	5/13(39%)
M	Clinical Improvement	16/17(94%)	8/14(57%)
M	Complete Healing	1/17(6%)	2/14(14%)

On d28, 68% of the lesions included (40 lesions) were improved compared to their d0 CS-score, 10 out of 13 (77%) were from the SA product group, nine out of 13 (69%) from the CTC product group and eight out of 14 (57%) from M product group.

Of the 39 DD lesions with observation on both d28 and d10, 23 DD lesions had remained "Improved", three remained "Not Improved", three became "Improved" and 10 changed from "Improved" to "Not Improved" between d10 and d28.

Development of lesions between d10 and d28. Of the 13 DD lesions in product group SA on d28, nine lesions had remained "Improved". One remained "Not Improved". One became "Improved" and two changed from "Improved" to "Not Improved" between d10 and d28.

Of the 14 DD lesions in product group M on d28, eight lesions had remained "Improved". One remained "Not Improved". Zero became "Improved" and four changed from "Improved" to "Not Improved" between d10 and d28. One did not have a d10 observation.

Of the 13 DD lesions in product group CTC on d28, seven DD lesions had remained "Improved". One remained "Not Improved". Two became "Improved" and three changed from "Improved" to "Not Improved" between d10 and d28.

When subjected to three independent Fisher's-exact tests comparing the groups two by two none were found to be different ($P<0.05$) from one another in the ability to "Improve" the DD lesions at either d10 (SA vs M P=0.60, CTC vs SA P=0.65, CTC vs M P=0.17) or d28 (SA vs M P=0.25, CTC vs SA P=0.66, CTC vs M P=0.70).

Characteristics of different Progressions

Average length of the healed lesions in each group were 2.39 cm, 1.03 cm and 2.50 cm for product group CTC, M and SA respectively. In general, the clinical score was reduced from CS-2 to healing CS-1 or CS-0 at d10, except from one lesion in the SA group with a CS- 2 and one lesion in the CTC group with a CS-3 at d10.

All healing lesions transitioned from active to healing within d10, except for one lesion in the CTC product group which transitioned from active to healing between d10 and d28. Mean percentage reduction in healing lesions length was 7%, 48% and 24% after d10 for product group CTC, M and SA respectively. Mean percentage reduction in healing lesions length between d0 and 28 was -5%, 52% and 61% for product group CTC, M and SA respectively. This indicates that the lesion length in product group CTC on average increased even though its clinical appearance seemed healed. As the healing scab would sometimes cover a larger area than the active lesion underneath, this could explain the increase in length of healing CTC lesions.

All lesions active at d28 transitioned from an active stage at d0, to a healing stage at d10 and back to active stage at d28, except from one lesion from the CTC and one from the SA product group that remained active throughout the study period.

Only three lesions went from an active stage at d0 to a mature stage at d28. All three lesions were in the product M group and all three were over 3 cm in lesion length.

Effect of Products on Lesions Size

A total of 52 CS-2 DD lesions in 49 cows were included in the analyses of the products' effect of the products on changing lesions size

For each product group, a mean, 95% confidence limits for mean and standard deviation were calculated for both the numeric lesion length and percentage change in lesion length from d0 to d10 and from d0 to d28 (Table 5). All products were able to reduce the average length of the lesion the first 10 days after treatment. The largest reduction on lesion length in product CTC and M groups were seen after d10 with an initial reduction on lesion length of 21% and 39% for CTC and M, respectively. The percentage reduction in lesion length between d0 and d28 did not differ from that of d0 to d10 in product groups CTC and M. Product SA's average reduction of lesion length was 30% from d0 to d10, and 60% between d0 and d28 (fig 4). There was no statistically significant difference in the numeric lesion length or percentage reduction in lesion length between the product groups on d0, d10 or d28. However, product group CTC was the only group where the lower 95% confidence limit for percentage reduction in lesion length, included negative values (-14.97-52.30)

Table 5: Mean, 95% confidence limits for mean and standard deviation for both Lesion length in cm and the percent reduction in lesion length between d0 and d10 and d0 and d28. No statistically significant difference (p<0.05) were detected between any treatment groups *(Chlortetracycline=CTC, Salicylic Acid=SA and Minerals=M)*.

Product		**d0**	**d10**	**d28**
CTC	N	18	16	12
	Mean length in cm.± SD	2.4 ±1.0	1.9±1.0	1.8 ±0.9
	(CI-95%)	(1.9-2.9)	(1.4-2.4)	(1.2-2.4)
	Mean length reduction in % from d0 ± SD		21.1 ±32.2	18.7 ±55.7
	(CI-95%)		(4.0-38.3)	(-15.0-52.3)
SA	N	16	15	12
	Mean length in cm. ±SD	2.4 ±0.9	1.5 ± 0.9	0.9 ±1.1
	(CI-95%)	(1.9-2.9)	(1.0-1.9)	(0.2-1.6)
	Mean length reduction in % from d0 ± SD		30.2 ±37.1	66.7 ±46.0
	(CI-95%)		(9.7-50.8)	(31.4-89.9)
M	N	18	16	14
	Mean length in cm. ±SD	2.2 ±0.8	1.6 ±0.8	1.6 ±1.1
	(CI-95%)	(1.8-2.6)	(1.2-2.1)	(1.0-2.2)
	Mean length reduction in % from d0 ± SD		38.7 ±33.9	39.2±41.4
	(CI-95%)		(21.8-55.5)	(15.3-63.1)

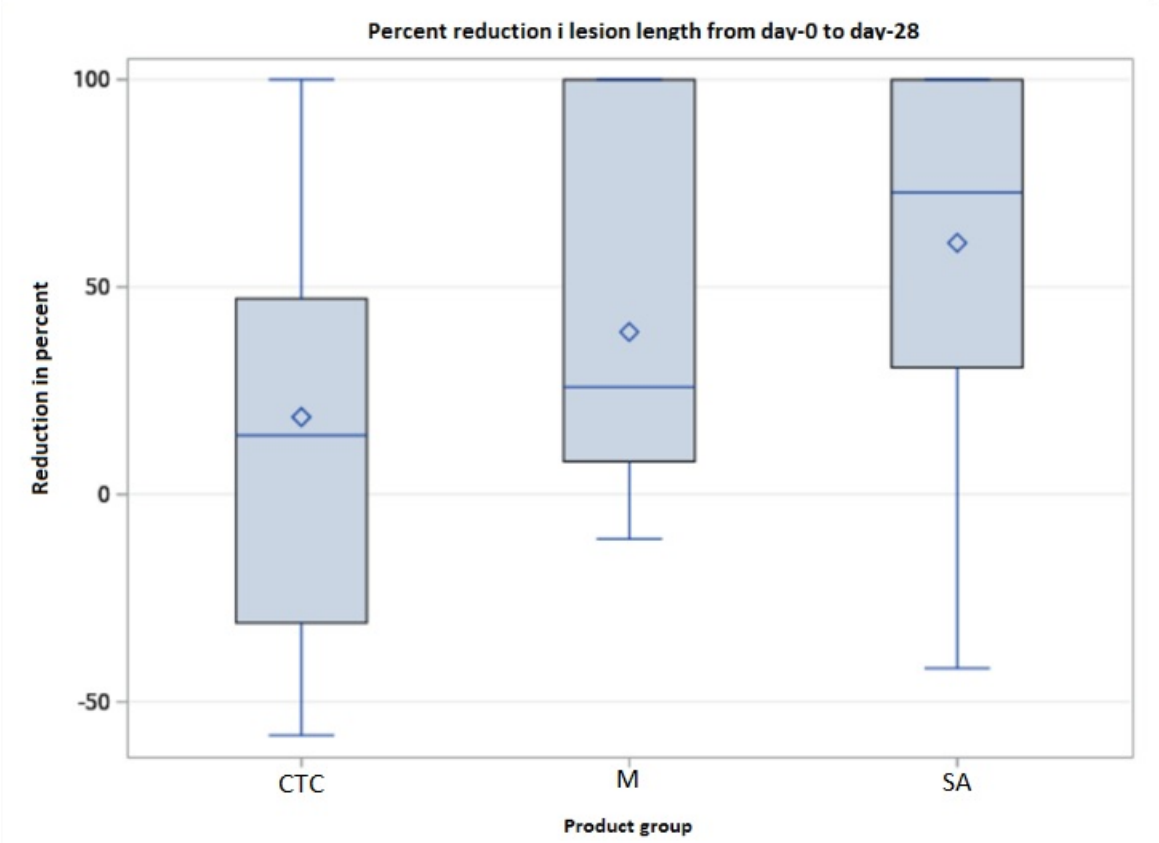


Fig 4: Boxplot generated in SAS-studio showing boxplots of the lesion length reduction in percent for treatment groups *(Chlortetracycline=CTC, Salicylic Acid=SA and Minerals=M)* between d0 and d28 where a negative reduction in percent means an increase in lesion length and 100% reduction (the maximum possible reduction) in lesion length means complete healing. The boxplot shows minimum observation, lower quartile, mean, median, upper quartile and maximum observation.

Using a paired T-test in SAS, the products' own ability to reduce the numeric lesion length between d0 and d10, d0 and d28 and d10 to d28 was calculated (table 6). A statistically significant difference in average numeric lesion length was detected in all product groups between d0 and d10, but only the SA and M product group had a statistically significant mean difference between d0 and d28. The mean difference in numeric lesion length between d10 and d28 were negative for the CTC and M product group, but all three product groups had negative lower confidence limits for the mean difference in numeric lesion length between d10 and d28.

Table 6: Results of using a paired T-test in SAS-studio to detect statistically significant differences (p<0.05) between means of reduction in lesion length within product groups *(Chlortetracycline=CTC, Salicylic Acid=SA and Minerals=M)* between d0 and d10, d0 and d28 and d10 to d28. with Mean difference (95% confidence limits) and Standard deviation (SD)

Product		d0-d10	d0-d28	d10-d28
CTC	Mean difference (CI-95%)	0.58 (0.23-0.93)	0.58 (-0.22-1.38)	-0.12 (-0.83-0.59)
	SD	0.64	1.26	1.12
	t-test (p-value)	3.61 (0.0026)	1.61 (0.13)	-0.37 (0.72)
SA	Mean difference (CI-95%)	0.95 (0.34-1.56)	1.50 (0.64-2.35)	0.55 (-0.07-1.18)
	SD	1.1	1.35	0.94
	t-test (p-value)	3.33 (0.0049)	3.85 (0.0027)	1.95 (0.079)
M	Mean difference (CI-95%)	0.64 (0.34-0.93)	0.60 (0.35-0.85)	-0.19 (-0.46-0.07)
	SD	0.55	0.43	0.42
	t-test (p-value)	4.60 (0.0003)	5.21 (0.0002)	-1.60 (0.13)

Progression of the Mature DD lesions

At d0 10 DD lesions classified as mature were treated. These were not used for descriptive analyses but underwent subjective analyses. Of the 10 mature DD lesions, six of the lesions were from the SA product group, three were from the M product group and one was from the CTC product group. One lesion from the M product group was excluded at d10 because of inaccessible placement.

By d10, four lesions were still CS-3, one from the CTC product group and three from the SA product group. One from the M product group was reduced to CS-2. Four lesions were reduced to CS-1, three from the SA product group and one from the M product group.

By d28, two DD lesions from the SA product group and one DD lesion from the M product group were excluded due to treatment by the farm personnel as a consequence of lameness.

From the remaining six, two from the SA product group remained CS-3 between d10 and d28- one from the CTC product group and one from the SA product group had changed from CS-3 to CS-1. The last two remained CS-1 between d10 and d28, one from product groups M and SA respectively.

All three products succeeded in changing at least one CS-3 to a CS-1 over the course of the 28-day study period.

Table 7. Clinical score of 10 mature lesions on d0, d10 and d28 treated with Clinical scores: CS-0=Healed, CS-1=Healing, CS-2= Active lesion and CS-3= Mature lesion *Chlortetracycline=CTC, Salicylic Acid=SA and Minerals=M*

Lesion	d0	d10	d28
M-1	CS-3	Excluded	
M-2	CS-3	CS-1	CS-1
M-3	CS-3	CS-2	Re-treated
CTC-1	CS-3	CS-3	CS-1
SA-1	CS-3	CS-1	CS-1
SA-2	CS-3	CS-3	CS-3
SA-3	CS-3	CS-3	CS-1
SA-4	CS-3	CS-1	Re-treated
SA-5	CS-3	CS-1	Re-treated
SA-6	CS-3	CS-3	CS-3

Histological Evaluations

Due to treatment with SA bandage of one cow by farm personnel as a consequence of lameness and one cow drying off during the study period, two out of eight DD lesions were not biopsied on d28. This left us with six complete sets of biopsies distributed as three sets, two sets and one set from product groups M, CTC and SA respectively, where one cow from the M group had been re-treated with CTC by the farmer before d28.

None of the biopsied DD lesions had an improved CS-score on d28, and one progressed into a CS-3. The histopathological evaluation of the tissue samples showed numbers of *Treponema* spp. so high that they were indistinguishable from one another at the top of the epidermis. Bacteria were found down through the epidermal layers until the dermal papillae. No apparent difference was found in samples between d0 and d28 in all but one sample. From that sample, it was not possible to confirm the presence of *Treponema* spp. at d0, however the corresponding d28 sample showed large quantities of *Treponema* spp.

Initially, all eight biopsied DD lesions were CS-2. Of the remaining biopsied DD lesions, five out of six healed to CS-1 by d10. Four out of those five returned to CS-2 and one had increased to CS-3 by d28. The last DD lesion remained CS-2 throughout the study period.

DISCUSSION

The 28-day study period with observation days on d10 and d28 were chosen to allow a comparison to studies with a similar time frame. The d10 observation allowed us to evaluate any rapid changes to the lesions post treatment and the d28 observation allowed us to evaluate the lesions progression after the initial effect of treatment should have worn of. Another reason for choosing the 28-day study period were observations from Krull et al (2016) where DD natural lesion progression was followed for three years. These observations suggested that lesions typically did not change morphological appearance in less than 14 days, even after oxytetracycline hydrochloride treatment (Krull et al., 2016). This observation suggested that an active lesion could progress to a healing stage after 14 days and a healed state after 28 days. Any lesions not regressing at d28 could be considered a failed treatment. However, our study did not evaluate the long-term effects of treatment which could be of interest to the user of the products. The long-term success of treatment with topical antibiotics is reported low, with Berry et al. (2012) re-treating 54% of all lesion treated with topical 10 g lincomycin on a cotton ball left in a bandage for four days, during a 341-day study period and Read and Walker (1998) using topical

tetracycline reported recurrence of 48% of all lesions 7-12 weeks after complete therapeutic response was observed. These observations, indicating that the initial response to treatment is often better than long-term response, is confirmed in our own results (table 5). It its however difficult to determine if the lesion's long-term response is dependent on treatment only or affected by reinfection as well.

It is difficult to distinguish between lesions that don't respond to treatment and lesions with a successful treatment that suffer reinfection between two observations. This could potentially lead to underestimated cure rates. The difference between a non-successful treatment and reinfection can't be distinguished when evaluating the appearance of the lesions only, unless it were possible to follow the amount of bacteria in the lesions and determine when a microbiological cure was complete. It was our interest to evaluate if the location, in the epidermis and dermis, and the amount of *Treponema* spp. would change during the study period, depending on the treatment product. Any reduction in bacteria amount could have been associated with the products ability to cure the lesion. Unfortunately, all lesions that were biopsied remained active at the end of the study period. This could lead to the assumption that biopsying active DD lesion could have a negative effect on the healing after treatment, but this observation has not been observed in other studies.

We did not employ any negative control in our study. This was done from an ethical point of view as no animal should go untreated. This means that it can be argued that we can't distinguish what is treatment effect and what is natural lesions progression. In a study where a negative control group were included no natural healing was observed after 12 days (Cutler et al., 2013a). Jacobs et al. (2018) however found the probability of clinical cure of saline to be 33% after one week and 10% after 10 weeks when lesion were treated weekly. Even though tetracycline and mineral solutions were significantly better at curing lesion during the first study weeks, no difference among saline and other treatments were reported after week 7. This might highlight the potential effect of good foot hygiene as all feet where washed once a week before scoring and re-treatment (Jacobs et al., 2018).

We should be able to compare the treatment groups considering that cows were equally distributed between treatment groups independent of farm and the fact that any possible small effect of natural healing would affect the treatment groups equally.

The influence of the different housing and management conditions on the three farms could be a point of interest in terms of the three product groups comparability, however treatment and lesion scoring were easily reproducible between farms as all farms had a herringbone milking parlor and similar housing and management conditions.

Cows with DD lesion on both hind legs were enrolled into the same treatment group. On d0 four cows from product group SA and M contributed with both hind legs and two cows from the CTC product group contributed with both hind legs. If any individual cow effect would affect the result of treatment, cows contributing with both hind legs would influence the results two-fold. Due to exclusion of some lesions from cows contributing with both legs in the study, only two M and one CTC cow contributed with both legs in the statistical analysis. All six of these lesions progressed to a healing state at d10 and one lesion from the M product group progressed back to an active state. If any individual cow effect would occur, it is assumed to have a minimal impact on our analysis.

The use of some of the more elaborate scoring systems is a good way to describe the details of the natural lesion progression and pathogenesis of DD lesions, but the sensitivity and specificity of detecting and accurate scoring of DD lesions decreases when a detailed scoring system is used in the milking parlor. A Danish study from 2008 showed Se of 0.84 and Sp of 0.51 when scoring DD lesions on a 6-point scale in a herring bone milking parlor (Thomsen et al., 2008). However, they didn't use a mirror to visualize smaller lower placed lesions and they only allowed approximately 15 sec. pr. evaluation. Compared to evaluating DD lesions in a trimming chute, the Se and Sp of evaluating DD lesions in the milking parlor is lower, but can increase to 0.90-1 and 0.80-0.99 for Se and Sp respectively when simplifying the scoring systems into fewer categories, increasing the time spend on each evaluation and utilizing a mirror to visualize lower placed small DD lesions (Relun et al., 2011; Solano et al., 2017). As we also photographed each lesion for later evaluation, we deemed scoring in the milking parlor a good alternative to the time and work consuming scoring in the trimming chute.

As we expected active lesions to heal or initiate a healing transformation after treatment, our scoring system was therefore simplified to focus on changes that implied only clear signs of improvement or deterioration.

In contrast to what some authors have done, we did not interpret every lesion change from active to mature as signs of improvement, but only considered lesions that changed from an active stage

706 to a healing stage as signs of actual improvement. This could explain the differences between our
707 results and Holzhauer et al. (2011) who interpreted a change from M2 stage to anything else as
708 cured. He found a cure rate 92% for a similar M product and 58% cure rate for a similar CTC
709 product when following a treatment protocol similar to ours. Our reason for not interpreting a
710 change from active to mature as improvement is because a mature lesion is thought to be the
711 most contagious lesion (Biemans et al., 2018) and it is likely to develop a new active area within
712 the mature lesion and thereafter redevelop into new an active lesion if not responding to
713 treatment (Berry et al., 2012). The reason for other studies to interpret the transition from active
714 to mature lesion as a sign of improvement is that it is often considered a non-painful state
715 (Döpfer et al., 1997; Read and Walker, 1998).

716 As our clinical scoring didn't account for lesion length, we decided to analyze length as an
717 individual factor. To account for the inaccuracy associated with deciding lesions margins, we
718 only included measurements from lesions on photographs of good quality where sharp lesion
719 margins were clearly distinguishable from surrounding normal tissue. Lesion length were
720 included to evaluate any correlation between healing and reduction in size. We did see a
721 reduction in size in many lesions which could lead to the theory of a somewhat circular healing.
722 It is theorized that the lesion could be more superficial at the borders of the lesions than at the
723 center and thus easing the healing at the lesion borders resulting in a circular healing. Further
724 research on DD lesions initial lesion size influence on healing would be welcomed. If any
725 negatively correlated relation between and lesion size and healing is evident as suggested by
726 Nishikawa and Taguchi (2008), lesion size reduction after treatment could be used as an
727 expression of treatment success.

728 Pain score is another way to evaluate treatment effect on DD lesions. In general, pain response of
729 DD lesions seems to decrease after treatment with products similar to those used in this study
730 (Read and Walker, 1998; Shearer and Hernandez, 2000; Capion et al., 2018). Pain score has been
731 based on cows response to palpation of lesions, spraying water on lesion and estimating how
732 long the cow raises its leg or touching lesion with a pressure algometer and recording at what
733 pressure the cow raises its leg (Cutler et al., 2013b; Schultz and Capion, 2013; Capion et al.,
734 2018). Even though pain score seems to be an expression of treatment success, it is a very
735 subjective assessment and we deemed it too unreliable to use as cows could react very different
736 to pain stimuli, and it would be difficult to replicate the same amount of pain stimuli between
737 cows and farms. One reason being that the water pressure hose available in the milking parlor
738 had very different pressure between the farms. We did, however, observe that cows with active

lesions raised their legs quicker and for a longer period of time than those with healing lesions when being washed with a pressure hose.

This study was not able to detect any difference ($P<0.05$) between products SA, M and CTC, in their ability to completely heal, reduce lesion length or clinically improve DD lesions. However, tendencies indicated a higher healing rate and lesion length reduction in the SA product group compared to both the M and the CTC product group.

However, our small sample size makes it hard to compare the proportions of healed and healing lesions between product groups, as a small number of successful cases would affect the proportion greatly. Therefore, it could be more reliable to compare the products effect by their ability to reduce the size of DD lesions.

The tendency towards SA being more efficacious on d28 is illustrated by the findings in lesion length reduction. Lesions treated with SA were the only lesions displaying a continuous steady lesion length reduction of over the 28-day study period - 30% at d10 and 61% at d28 in contrast to lesions treated with M and CTC which mainly saw reduction in the first 10 days of the period 39% and 21% for M and CTC respectively (table 5). The findings of SA being consistently effective over time is supported by the findings by Capion et al. (2018) and Fiedler et al. (2016).

This could indicate a good short-term effect of products M and CTC but limited long term improvement as the lesion size reduction on d28 was almost identical to those of d10 for M and CTC. Similar results of a 9-20% cure rate of topical CTC treatment have been reported in recent years (Hernandez and Shearer, 2000; Krull et al., 2016).

The superior reduction for M after ten days (table 5) could be attributed to the fact that M were the only group with a treatment at d7. Our results stand in contrast to those found by Holzhauer et al. (2011) who used a product with a similar mineral composition and a similar protocol and found a more consistent ability of 92% cure rate after 28-day. However, he defined cure rate as course where any change from M-2 to another M-stage occurred.

Product CTC's confidence interval for percent reduction in lesion length on d28 holds negative values (table 5), meaning the lesions would grow and not reduce their size, indicating none to adverse effects of treatment. The previous use of CTC on the three farms could result in development of antibiotic resistance by *Treponema* spp., which could explain the reduced healing of CTC treated DD lesions as suggested by Shearer and Hernandez (2000). But when

769 comparing the effect of product CTC to SA and M it should be considered that CTC was the
770 only product group that was not bandaged at d0. Any positive effect of a bandage would
771 therefore not influence the CTC treated lesion.

772 Before the study was conducted, we expected to find SA and M to be more efficacious than CTC
773 with approximately 30% difference. The 117 lesions needed to detect this 30% difference
774 ($P<0.05$) would also have allowed for a more thorough statistical analysis. The effect of any
775 explanatory variables such as lesion size at d0 and individual cow effects and their possible
776 interactions could have been analyzed using multivariable analysis. As we suspected, an
777 interaction between treatment and lesion size on d0 would influence the lesions ability to heal or
778 reduce in size, it would have been relevant to make an ANCOVA or Binary logistic regression in
779 SAS to analyze if lesion size or any other cow or lesion factors would influence our result.
780 However, this would require a larger sample size than the 52 lesions included in our study. One
781 study analyzed "the risk of incomplete healing" using binary logistic regression. This study
782 included 89 cows with DD lesions treated with 5ml 100mg/ml oxytetracycline on a cotton pad
783 held in place using an elastic band and followed for 29 days. "Primiparous cows" (OR 4.1, 95%
784 CI 1.375-12.313) and "Lesion size on day 0 (cm^2)" (OR 1.21 95% CI 1.045-1.450) turned out to
785 be significantly ($P<0.05$) related to the risk of incomplete healing (Nishikawa and Taguchi,
786 2008). Another study with 98 cows treated with either 10 g lincomycin paste or an non-antibiotic
787 paste, used multivariate logistic regression and found DD lesions on 3+ lactation cows to be 4-5
788 times more likely to change from an active stage to non-active stage after 29 days (Moore et al.,
789 2001).

790 We hoped it was easy to obtain photographs of the DD lesions of consistently good quality in the
791 milking parlor, but the process of obtaining viable photographs turned out to be hard and at times
792 dangerous for both equipment and authors. Evaluation in the trimming chute is a more reliable
793 method and it is safe for the observer to clean and palpate lesions. Separating cows and fixating
794 them in the trimming chute is a time-consuming process so to save time and for convenience for
795 farm personnel scoring at d10 and d28 was done in the milking parlor during milking. But
796 scoring DD lesions in the milking parlor can be affected by the awkward position cows can be
797 standing in and if several enrolled cows enter in the milking parlor at the same time, scoring and
798 photographing has to be done before the cows finish milking. Reflecting back on the study, we
799 would have liked to evaluate all the DD lesions in a trimming chute as in hindsight the downside
800 of scoring DD lesions on fixated cows in the trimming chute outweighs the benefits of scoring
801 DD lesions on standing cows in the milking parlor. As the sample size were a lot larger on Farm

C, more cows could enter the milking parlor at the same time and therefore less time could be spend evaluating each lesion on Farm C compared to Farm A and B.

When advising farmers to use a specific product in their treatment for DD, one must consider the curative effect of the products, the labor intensity of the treatment and the environmental impact of the product i.e. the risk of antibiotic resistance. When comparing the products in terms of labor intensity, product CTC and M are sprays and can be applied in the milking parlor. In this study, lesions treated with product M needed re-treatment for a longer period of time and were also bandaged and therefore we do not know its efficacy when used as spray without bandage. In this study product SA and M are more labor intensive than product CTC and can only be performed in a trimming chute. The environmental consideration regarding the three products is mainly the risk of the development of antibiotic resistance when using CTC, since none of the two other products are known to have any environmental effects.

In future research it could be beneficial to agree on a single system for scoring lesions to evaluate effect of different treatments. This would make comparison between studies transparent and would increase the overall understanding of what different products' effect is. Bearing this in mind, it would also be beneficial to agree in what time frame effect should be evaluated and when recurrence is relevant to account for. A system for scoring and evaluating lesions after treatment should be simple and easily applicable to different treatment regimes, but detailed enough to accurately describe changes expected. As the pain associated with active DD lesions is a concern of animal welfare, it would have been ideal to include pain scores in our analyses. In a previous study SA were superior to CTC in lowering pain of treated lesions after 14 days (Schultz and Capion, 2013). SA ability to lower pain score would have been interesting to compare to that of M, but we deemed pain scores to unreliable as the study was carried out on three different farms and their different management practices could have affected their response to pain stimuli.

Based on the difficulties we found in this study in relation to obtaining data of good quality in the milking parlor we would argue that future studies should consider obtaining all data on legs fixated in the trimming chute. This is a time-consuming process that could influence farmers willingness to participate, but for acquiring data of good quality the difference between these two methods is considerable. Inclusion criteria for lesions in our study were that they would be located in an area that allowed us to evaluate and photograph them consistently. Unfortunately, the hoof trimmer at Farm C was working with at a very high pace and we were forced to make

quick decisions on whether to include lesion or not, only to exclude some lesions later as we discovered that they were located too low for proper evaluation in the milking parlor. As the biopsy procedure was time consuming compared to the hoof trimmers pace at Farm C, it was decided to limit the biopsy sample size to three cows. This was unfortunate for the histological aspect of the study, as the small sample size limited the comparisons between macroscopic and microscopic appearance of the lesions. If lesions were evaluated in the trimming chute, interdigital lesions could have been enrolled as well and have increased our sample size.

To avoid finding insignificant results in future research, the number of products tested should be held in reference to the expected sample size available and the expected difference between products.

Even though it might be appealing to be able to compare several products commonly used to new products, it might be better to compare new product to common product with similar treatment regime or to common products with a high cure rate to evaluate if new products should be implemented in the treatment strategies against DD.

CONCLUSION

We found the effect of SA and M to be equal or tending to superior to that of CTC in their ability to clinical heal, improve and reduce length of DD lesions, when using the treatment protocol described in this study. The results could indicate that product SA or M should be used when hoof trimming to reduce the use of antibiotics, but because CTC and M followed two different treatment protocols, it can't be concluded that M has a tendency to be is superior to CTC if they followed the same treatment regime

ACKNOWLEDGEMENTS

The authors would like to extend the most grateful thanks to both the farmers and hoof-trimmers for participating. Without them this study would not have been possible. We would also like to thank Sofie Simon (Dechra Veterinary Products A/S, 7171 Uldum, Denmark), Jörg M. D. Enemark (Elanco Animal Health 2730 Herlev, Denmark) and the Department of Veterinary Clinical Sciences (University of Copenhagen) for sponsoring the products and materials used. For the preparation and processing of our tissue samples we would like to thank Heidi Elise Holm from the laboratory at the Department of Veterinary Clinical Sciences Section for

Veterinary Reproduction and Obstetrics (University of Copenhagen). Finally, we would like to thank supervisors Nynne Capion (Department of Veterinary Clinical Sciences, University of Copenhagen) and Jörg M. D. Enemark (Elanco Animal Health 2730 Herlev, Denmark) for critical comments and guidance throughout this veterinary master thesis.

REFERENCES

Berry, S.L., D.H. Read, T.R. Famula, A. Mongini, and D. Döpfer. 2012. Long-term observations on the dynamics of bovine digital dermatitis lesions on a California dairy after topical treatment with lincomycin HCl. Vet. J. 193:654–658. https://doi.org/doi:10.1016/j.tvjl.2012.06.048.

Berry, S.L., D.H. Read, R.L. Walker, and T.R. Famula. 2010. Clinical, histologic, and bacteriologic findings in dairy cows with digital dermatitis (footwarts) one month after topical treatment with lincomycin hydrochloride or oxytetracycline hydrochloride. J. Am. Vet. Med. Assoc. 237:555–560. https://doi.org/doi:10.2460/javma.237.5.555.

Biemans, F., P. Bijma, N.M. Boots, and M.C.M. de Jong. 2018. Digital Dermatitis in dairy cattle: The contribution of different disease classes to transmission. Epidemics 23:76–84. https://doi.org/doi:10.1016/j.epidem.2017.12.007.

Blowey, R.W., S.. Done, and W. Cooley. 1994. Observations on the pathogenesis of digital dermatitis in cattle. Vet. Rec. 135:115–117. https://doi.org/doi:10.1002/anie.199300921.

Britt, J.B., and J. McClure. 1998. Field trials with antibiotic and non-antibiotic treatments for papillomatous digital dermatitis. Bov. Pract. 32:25–28.

Campbell, R.E., and P.D. Rosahn. 1950. The morphology and staining characteristics of the Treponema pallidum. Review of the literature and description of a new technique for staining the organism in tissues. Yale J. Biol. Med. 22:527–543.

Capion, N., E.K. Larsson, and O.L. Nielsen. 2018. 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. 101:1325-1333. https://doi.org/doi:10.3168/jds.2017-13622.

Cha, E., J.A. Hertl, D. Bar, and Y.T. Gröhn. 2010. The cost of different types of lameness in dairy cows calculated by dynamic programming. Prev. Vet. Med. 97:1–8. https://doi.org/doi:10.1016/j.prevetmed.2010.07.011.

Christensen, H., and A.Ø. Larsen. 2016. Sammenligning af behandlingseffekt for Cyclospray og Repidermaspray på Digital Dermatitis hos køer og kvier. Dansk Veterinær Tidsskr. 4:28–30.

Cohen, J. 1960. A coefficient of agreement for nominal scales. Educ. Psychol. Meas. 20:37–46.

Cutler, J.H.H., G. Cramer, J.J. Walter, S.T. Millman, and D.F. Kelton. 2013. 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. 96:7550–7557. https://doi.org/doi:10.3168/jds.2012-6384.

Demirkan, I., S.D. Carter, R.D. Murray, R.W. Blowey, and M.J. Woodward. 1998. The frequent detection of a treponeme in bovine digital dermatitis by immunocytochemistry and polymerase chain reaction. Vet. Microbiol. 60:285–292. https://doi.org/doi:10.1016/S0378-1135(98)00146-1.

Demirkan, I., R.L. Walker, R.D. Murray, R.W. Blowey, and S.D. Carter. 1999. Serological Evidence of Spirochaetal Infections Associated with Digital Dermatits in Dairy Cattle. Vet. J. 157:69–77.

Döpfer, D., A. Koopmans, F.A. Meijer, I. Szakáll, Y.H. Schukken, W. Klee, R.B. Bosma, J.L. Cornelisse, A.J.A.M. Van Asten, and A.A.H.M. Ter Huurne. 1997. Histological and bacteriological evaluation of digital dermatitis in cattle, with special reference to spirochaetes and Campylobacter faecalis. Vet. Rec. 140:620–623. https://doi.org/doi:10.1136/vr.140.24.620.

Ettema, J., S. Østergaard, and A.R. Kristensen. 2010. Modelling the economic impact of three lameness causing diseases using herd and cow level evidence. Prev. Vet. Med. 95:64–73. https://doi.org/doi:10.1016/j.prevetmed.2010.03.001.

921 Fiedler, A., C. Sauter-Louis, and J. Maierl. 2015. Polyurethane dressing, tetracycline and
922 salicylic acid use for treatment of digital dermatitis in cattle: A comparative study. Tierarztl.
923 Prax. Ausgabe G Grosstiere - Nutztiere 43:350–358. https://doi.org/doi:10.15653/TPG-
924 140751.

925 Gomez, A., N.B. Cook, N.D. Bernardoni, J. Rieman, A.F. Dusick, R. Hartshorn, M.T. Socha,
926 D.H. Read, and D. Döpfer. 2012. An experimental infection model to induce digital
927 dermatitis infection in cattle. J. Dairy Sci. 95:1821–1830.
928 https://doi.org/doi:10.3168/jds.2011-4754.

929 Hernandez, J., and J.K. Shearer. 2000. Efficacy of oxytetracycline for treatment of papillomatous
930 digital dermatitis lesions on various anatomic locations in dairy cows. J. Am. Vet. Med.
931 Assoc. 216:1288–1290. https://doi.org/doi:10.2460/javma.2000.216.1288.

932 Hernandez, J., J.K. Shearer, and J.B. Elliott. 1999. Comparison of topical application of
933 oxytetracycline and four nonantibiotic solutions for treatment of papillomatous digital
934 dermatitis in dairy cows.. J. Am. Vet. Med. Assoc. 214:688–90.

935 Holzhauer, M., C.J. Bartels, M. Van Barneveld, C. Vulders, and T. Lam. 2011. Curative effect of
936 topical treatment of digital dermatitis with a gel containing activated copper and zinc
937 chelate. Vet. Rec. 169:555. https://doi.org/doi:10.1136/vr.d5513.

938 Jacobs, C., K. Orsel, S. Mason, and W. Barkema. 2018. Comparison of effects of routine topical
939 treatments in the milking parlor on digital dermatitis lesions. J. Dairy Sci. 101:5255-5266.
940 https://doi.org/doi:10.3168/jds.2017-13984.

941 Kofler, J., C. Innerebner, R. Pesenhofer, A. Hangl, and A. Tichy. 2015. Effectiveness of salicylic
942 acid paste for treatment of digital dermatitis in dairy cows compared with tetracycline spray
943 and hydrotherapy. Berl. Munch. Tierarztl. Wochenschr. 128:326–334.
944 https://doi.org/doi:10.1017/CBO9781107415324.004.

945 Krull, A.C., J.K. Shearer, P.J. Gorden, V.L. Cooper, G.J. Phillips, and P.J. Plummera. 2014.
946 Deep sequencing analysis reveals temporal microbiota changes associated with
947 development of bovine digital dermatitis. Infect. Immun. 82:3359–3373.
948 https://doi.org/doi:10.1128/IAI.02077-14.

949

950 Krull, A.C., J.K. Shearer, P.J. Gorden, H.M. Scott, and P.J. Plummer. 2016. Digital dermatitis:
951 Natural lesion progression and regression in Holstein dairy cattle over 3 years. J. Dairy Sci.
952 99:3718–3731. https://doi.org/doi:10.3168/jds.2015-10535.

953 Landis, J.R., and G.G. Koch. 1997. The measurement of observeragreement for categorical data.
954 Biometrics 33:159–174. https://doi.org/doi:10.2307/2529310.

955 Laven, R.A., and D.N. Logue. 2006. Treatment strategies for digital dermatitis for the UK. Vet.
956 J. 171:79–88. https://doi.org/doi:10.1016/j.tvjl.2004.08.009.

957 Manske, T., J. Hultgren, and C. Bergsten. 2002. Topical treatment of digital dermatitis associated
958 with severe heel-horn erosion in a Swedish dairy herd. Prev. Vet. Med. 53:215–231.
959 https://doi.org/doi:10.1016/S0167-5877(01)00268-9.

960 McGavin, M.D., and J.F. Zachary. 2007. Pathological Basis of Veterinary Disease 4th Ed..
961 Mosby Elsevier, St. Louis, MO.

962 Moore, D.A., S.L. Berry, M.L. Truscott, and V. Koziy. 2001. Efficacy of a nonantimicrobial
963 cream administered topically for treatment of digital dermatitis in dairy cattle. J. Am. Vet.
964 Med. Assoc. 219:1435–1438. https://doi.org/doi:10.2460/javma.2001.219.1435.

965 Nishikawa, A., and K. Taguchi. 2008. Healing of digital dermatitis after a single treatment with
966 topical oxytetracycline in 89 dairy cows. Vet. Rec. 163:574–576.
967 https://doi.org/doi:10.1136/vr.163.19.574.

968 Orsel, K., P. Plummer, J. Shearer, J. De Buck, S.D. Carter, R. Guatteo, and H.W. Barkema.
969 2018. Missing pieces of the puzzle to effectively control digital dermatitis. Transbound.
970 Emerg. Dis. 65:186–198. https://doi.org/doi:10.1111/tbed.12729.

971 Read, D.H., and R.L. Walker. 1998. Papillomatous digital dermatitis (footwarts) in California
972 dairy cattle: Clinical and gross pathologic findings. J. Vet. Diagnostic Investig. 10:67–76.
973 https://doi.org/doi:10.1177/104063879801000112.

974 Read, D.H., R.L. Walker, A.E. Castro, J.P. Sundbergh, and M.C. Thurmond. 1992. An invasive
975 spirochaete associated with interdigital papillomatosis of dairy cattle. Vet. Rec. 130:59–60.
976 https://doi.org/doi:10.1136/vr.130.3.59.

977

978 Relun, A., R. Guatteo, P. Roussel, and N. Bareille. 2011. A simple method to score digital
979 dermatitis in dairy cows in the milking parlor. J. Dairy Sci. 94:5424–5434.
980 https://doi.org/doi:10.3168/jds.2010-4054.

981

982 Relun, A., A. Lehebel, M. Bruggink, N. Bareille, and R. Guatteo. 2013. Estimation of the
983 relative impact of treatment and herd management practices on prevention of digital
984 dermatitis in French dairy herds. Prev. Vet. Med. 110:558–562.
985 https://doi.org/doi:10.1016/j.prevetmed.2012.12.015.

986 Schultz, N., and N. Capion. 2013. Efficacy of salicylic acid in the treatment of digital dermatitis
987 in dairy cattle. Vet. J. 198:518–523. https://doi.org/doi:10.1016/j.tvjl.2013.09.002.

988 Shearer, J.K., and J. Hernandez. 2000. Efficacy of Two Modified Nonantibiotic Formulations
989 (Victory) for Treatment of Papillomatous Digital Dermatitis in Dairy Cows. J. Dairy Sci.
990 83:741-745. https://doi.org/doi:10.3168/jds.S0022-0302(00)74936-8.

991 Solano, L., H.W. Barkema, C. Jacobs, and K. Orsel. 2017. Validation of the M-stage scoring
992 system for digital dermatitis on dairy cows in the milking parlor. J. Dairy Sci. 100:1592–
993 1603. https://doi.org/doi:10.3168/jds.2016-11365.

994 Thomsen, P.T., I.C. Klaas, and K. Bach. 2008. Short Communication: Scoring of Digital
995 Dermatitis During Milking as an Alternative to Scoring in a Hoof Trimming Chute. J. Dairy
996 Sci. 91:4679–4682. https://doi.org/doi:10.3168/jds.2008-1342.

997 van Amstel, S. R., S. van Vuuren, and C. L. C. Tutt. 1995. Digital dermatitis: Report of an
998 outbreak. J. S. Afr. Vet. Assoc. 66:177–181.

999 Wells, S.J., L.P. Garber, and B.A. Wagner. 1999. Papillomatous digital dermatitis and associated
1000 risk factors in US dairy herds. Prev. Vet. Med. 38:11–24.
1001 https://doi.org/doi:10.1016/S0167-5877(98)00132-9.