Efficacy of Digital Dermatitis treatment with non-antibiotic Hoof-Sol spray in dairy cattle

Marline van Mil
3897591
January – April 2016
University of Utrecht, Faculty of Veterinary Medicine
Supervisors: Gerrit Hooijer and Herman Barkema

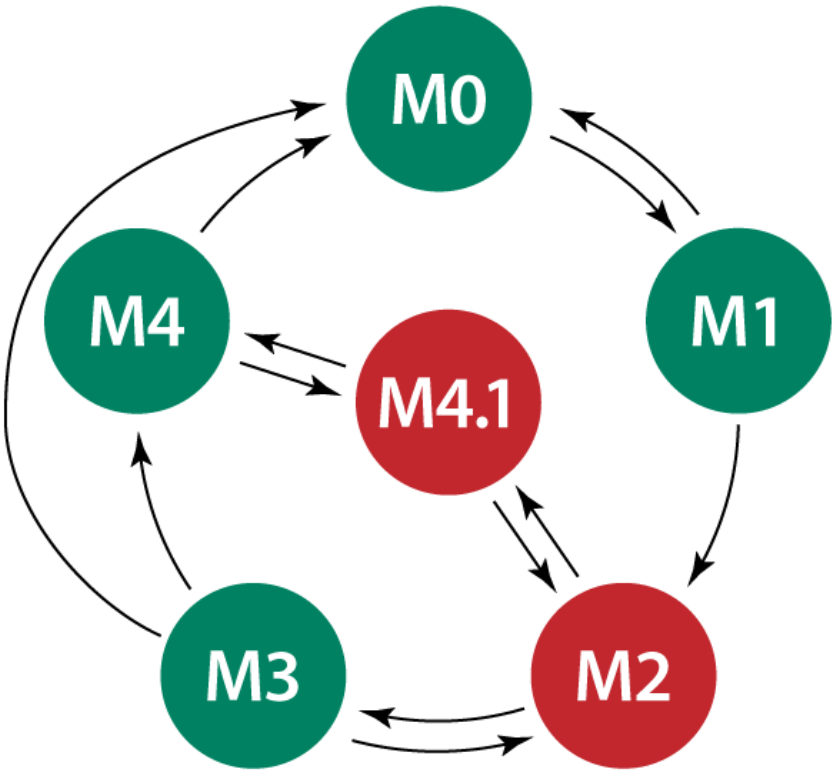

Table of Contents

Abstract

The efficacy of a topical treatment spray for digital dermatitis (DD) was compared with oxytetracycline (OTC) spray, as a positive control, and a negative control on a 566-cow, freestall dairy farm in Alberta, Canada as a sub-project in a 10-farm study. The treatment consisted of a topical spray containing aloe vera plant extracts together with copper and zinc chelates as active substances (Hoof-Sol). After cleaning of the hind feet with water, DD lesions (stages M0-4.1) were identified using a bright headlamp and a mirror on a spatula. DD lesions were sequentially randomly assigned to one of three treatments: Hoof-Sol, OTC spray and negative control, using a randomization sheet. The three treatments were administered as a spray in blinded spray bottles. At the following parlor inspection (one week later), the lesions treated in the preceding week were reevaluated. When M1-M4.1 (M+) DD lesions were still the same at the next inspection or changed to another stages (except M0), a re-treatment with the same treatment as the previous week was applied. Cure was defined as an M+ lesion transitioned in an M0 lesion. Every hind foot with an M+ lesion enrolled at week 1 was followed until week 8 and treated weekly. The proportion of M+ lesions not cured after 8 weeks of treatment was 0.86 for Hoof-Sol (95% CI: 0.76 to 0.96) and 0.61 for OTC (95% CI: 0.48 to 0.74), which showed that the efficacy of OTC is higher than Hoof-Sol (P=0.005). The proportion of remaining M+ lesions for the negative control was 0.90 (95% CI: 0.82 to 0.98). The efficacy of Hoof-Sol for treatment of DD was therefore not different from the negative control (P=0.51), whereas OTC was more effective than the negative control (P=0.0003). M+ lesions were divided in active lesions (M1, M2 and M4.1) and in chronic lesions (M3 and M4). Comparing the different treatments in week 8, no difference was found for these various lesion stages. Except for the proportion of active lesions treated with OTC. The proportion was lower than for the negative control (P=0.0121).

Introduction

Digital dermatitis (DD) is a common disease in dairy cattle. Cheli and Mortellaro (1974) first described this disease in 1974 and in the eighties, the first outbreaks were observed. DD became one of the most important claw diseases, with increasing occurrence worldwide (Blowey and Sharp, 1988). Currently, the aetiology of digital dermatitis is not fully understood. Recently, it became known that DD has a multifactorial nature with a spirochaetal bacterial component (Shearer *et al.*, 2013). Multiple studies have observed that *Treponema* spp. play an important role in the pathogenesis of DD (Gomez *et al.*, 2012). Although the pathogenesis is not clear, it becomes more important to find out about it, due to the increasing significance.

Digital dermatitis causes typical lesions above the heel bulbs, around the dew claws, or along the coronary band. The lesions are circumscribed superficial ulcerations of the skin and have a strawberry-like aspect, with long hair surrounding the lesions (Holzhauer *et al.*, 2008). Most lesions develop on the plantar epidermis of the hind feet (Blowey and Sharp, 1988; Read and Walker, 1998). These lesions can be painful and result in economic losses due to lameness, which can lead to decreased milk production, decreased fertility and increased risk of culling (Holzhauer *et al.*, 2008; Refaai *et al.*, 2013). Several systems have been developed to classify DD lesions (Döpfer *et al.*, 1997; Relun *et al.*, 2011; Berry *et al.*, 2012); the five M-stages system (Döpfer et al. 1997) is the most commonly used method. The M-stages represent the stages of DD during its

development. M0 represents DD-free claws. M1 is defined as an early, small, circumscribed lesion, with a diameter < 2 cm. The red or red-grey M2 lesion is the acute stage, with diameter >2 cm and are active ulcerative or granulomatous and typically painful. M3 is the healing stage after topical therapy where the lesion is covered by a scab. M4 is the chronic stage characterized by thickened epithelium. M4.1 is the chronically recurring stage (Holzhauer *et al.*, 2008).

There are several risk factors for increased incidence and prevalence of DD. One of the biggest risks is the introduction of infected cows, but also overcrowding, unhygienic environment, high moisture, wet floors and trauma of the digital skin are risk factors (Rodríguez-Lainz *et al.*, 1996; Holzhauer *et al.*, 2008; Gomez *et al.*, 2012). Even low parity and early lactation seem to be related to an increased prevalence of DD (Rodríguez-Lainz *et al.*, 1996; Holzhauer *et al.*, 2006). To control transmission of DD it is essential to use control strategies like regular claw trimming, footbath protocols and rapid treatments (Holzhauer *et al.*, 2011).

The aim of DD treatment is to encourage the healing of M2 lesions and preventing the development to M2 lesions. Today, oxytetracycline spray (OTC) is commonly used to treat DD. As a result of a worldwide call for reduction of antibiotics concerning the growing resistance, there is an increasing demand for alternatives. Several topical products, which do not contain antibiotics, are procurable and are already frequently used. Still, more research on the efficacy of these products is necessary. An example of these alternatives is Hoof-Sol[1], a topical spray containing aloe vera plant extracts together with copper and zinc chelates as active substances. Hoof-Sol is a product meant to treat individual hoofs. According to its manufacturer, IntraCare, the minerals used in Hoof-Sol are more stable and soluble than generally used minerals. The protocol recommends treating the lesions weekly with a 50% solution. To prepare this solution, Hoof-Sol needs to be diluted with cold water. To enlarge the skin contact, the feet can be hosed down with water before treating. Using Hoof-Sol being applied as a spray brings advantages, for example, other cows do not contaminate the solution, as it can be the case with a footbath. Compared to footbaths, less product is required and lameness can be detected by following up on the status of the feet.
The objective of this study was to determine the efficacy of Hoof-Sol for treatment of all M+ DD lesions by comparing it with OTC treatment and a negative control used weekly in the milking parlor.

Materials and Methods

Experimental design

The trial was conducted on 10 Alberta dairy farms with year-around indoor free-stall housing and DD prevalence estimated as ≥ 15%. In this report, the results at one of the farms are described. For the purpose of the experiment, accurate identification of the dairy cows in the parlor was required on the treatment days. It was also necessary that the cows did not walk through a footbath immediately after milking.

[1] Diamond Hoof Care Ltd. and Intracare BV., https://diamondhoofcare.com

For 8 weeks, the 566-cow dairy farm was visited weekly for DD inspections in the parlor. The farm had set milking times.
The hind feet were first cleaned with the parlor hose by the observer. Subsequently, DD lesions stages (M0-4.1) were identified by the observer using a bright headlamp and a mirror on a spatula (see Figure 1 and 2). This instrument ensures a better view of the heel and the interdigital space, which allowed a more accurate inspection of the lesions.

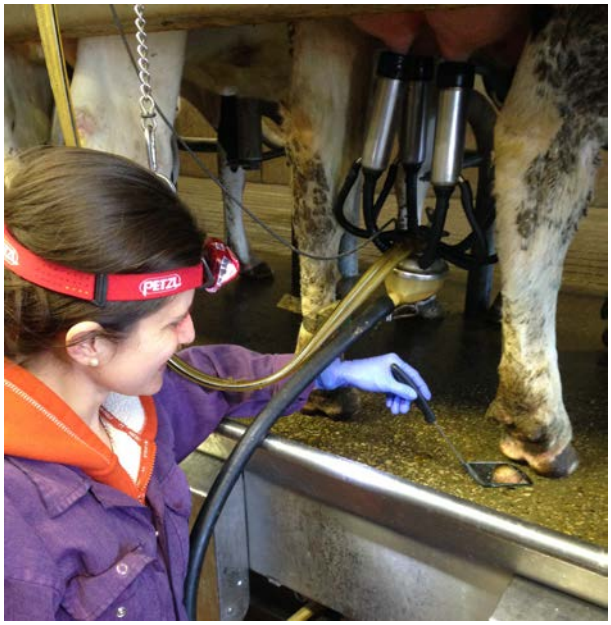

Figure 2 Equipment of the observer: a bright headlamp and a mirror on a spatula.

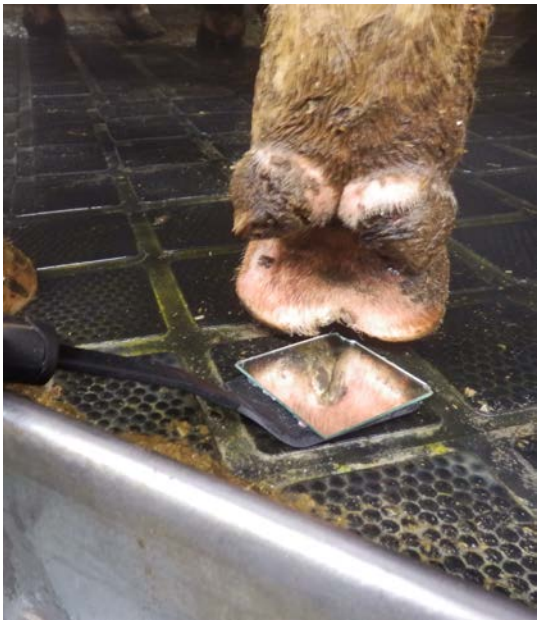

Figure 1 Mirror on spatula.

For all observations, the cow's identification number, treated foot, and the M-stage of the lesion were recorded. The observer marked the foot with an M+ DD lesion, to indicate the treatment administrator which foot should be treated. The M+ DD lesions were sequentially randomly assigned to one of three treatments: the negative control, Hoof-Sol or OTC spray using a randomization sheet. All treatments were administered as a spray, used in blinded spray bottles (500ml). Hoof-Sol was diluted with 50% of cold water, as described on the leaflet. The concentration was of the OTC spray was 25%. The OTC powder contained 250 mg/g active substance of oxytetracycline and was mixed with saline, 5g/5ml. The negative control consisted of saline with food coloring. The food coloring was used to create a solution with a similar color to the other treatments, to blind it to the farmer and researcher. After the hind feet were cleaned, scored and marked by the observer, treatment was sprayed on the lesion while the cows were standing in the parlor. Every lesion was sprayed using approximately 5 ml solution from a distance of 20-30 cm.
At the following parlor inspection (one week later), the lesions treated in the preceding week were reevaluated. Every hind foot with an M+ lesion enrolled at week 1 was followed until week 8. If M1-M4.1 lesions were still the same at the next inspection or changed to another stage (except M0), they received a re-treatment with the same treatment as the previous week. This was also applied to lesions that healed, but had developed again into a new lesion. An easy way to implement this was using treatment sheets (see Appendix 1). New identified M+ DD lesions were sequentially randomly assigned to one of the treatment groups, also by using the

randomization sheet. This sheet was also used to ensure the cows with a higher risk of DD were equally presented in all treatment groups.

After each visit, data were entered in Microsoft Excel (see Appendix 2) and new treatment sheets were prepared for the next visit. The treatments sheets contained the cow identification numbers in order with the corresponding treatments.
Between the inspections, the boots and all the equipment used in the parlor were cleaned with water and bleach. The observer and treatment administrator also changed their coveralls and gloves to ensure good hygiene measures.

A sample size calculation was done to determine the number of feet required per treatment group to detect a 15% difference between the proportion of feet with lesions in the different treatment groups. Based on these assumptions the sample size of each treatment group had to consist of 42 hind feet with an M+ lesion.

Statistical analysis

The null-hypothesis of the study was that the proportion of M+ lesions (M1, M2, M3, M4, M4.1) would be the same between all the treatment groups after 8 weeks of treatment. To demonstrate if this hypothesis could be accepted or not, the efficacy of Hoof-Sol for treatment of DD was compared with the negative control and OTC treatment. Also the negative control was compared to OTC. The experimental unit was an M+ lesion on a hind foot. An M+ lesion transitioned in an M0 was defined as healing.
A two-sample test of proportions was used to compare the proportion of M+ lesions, treated with each application (Hoof-Sol, OTC or the negative control), in week 1 with week 8. This test was also used to compare the proportion of M+ lesions in the Hoof-Sol, OTC and negative control group in week 8 (see figure 3). Finally, the two-sample test of proportions was also used to compare the different lesion stages (M0, active lesions and chronic lesions) per treatment group in week 1 with week 8 (see figure 4). In addition, the test was used for the comparison of the different lesion stages in week 8 between the different treatment groups, like figure 3 although per lesion stage (M0, active or chronic stage).
Data were analyzed in STATA version 13.1 (StataCorp, 2013, College Station, TX, USA). A P-value < 0.05 was considered statistically significant.

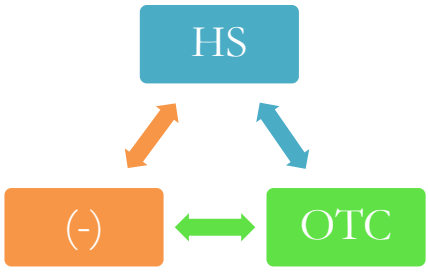

Figure 3 Comparison of the three treatment groups.

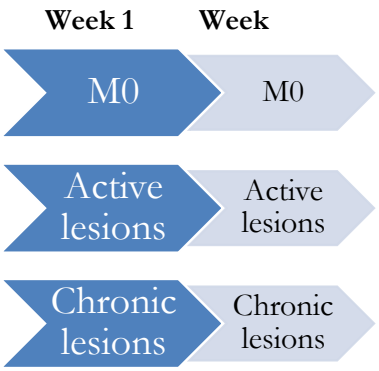

Figure 4 Proportion of the different lesion stages in week 1 compared with week 8.

Results

In week 1, cow-level DD prevalence was 51.9%. At week 1, 49 hind feet (divided over 27 cows) were treated with Hoof-Sol, 54 hind feet (divided over 35 cows) received a treatment with OTC, whereas 59 hind feet were included in the negative control group (Table 1). The actual amount of enrolled hind feet in week 1 was 63 for Hoof-Sol, 76 for the negative control and 81 for OTC,

but 14 hind feet for HS, 17 for the negative control and 27 for OTC had to be excluded from the study for different reasons such as cow being dried off or culled, or misidentification of the cow resulting in treatment with the wrong product. Table 1 illustrates the proportion of M+ lesions (M1-M4.1) in week 1 and 8 for all treatments. The proportion of M+ lesions not cured after 8 weeks of treatment was 0.86 for Hoof-Sol (43/49, 95% CI: 0.76 to 0.96) and 0.61 for OTC (45/54, 95% CI: 0.48 to 0.74), which showed that the efficacy of OTC is higher than Hoof-Sol (P=0.005). The proportion of remaining M+ lesions for the negative control was 0.90 (53/59, 95% CI: 0.82 to 0.98). The efficacy of Hoof-Sol for treatment of DD was therefore not different from the negative control (P=0.51), whereas OTC was more effective than the negative control (P=0.0003).

Table 1 Number of hind feet included and proportion of hind feet that was still affected with an digital dermatitis (M+) lesion in week 8 per treatment group.

	M+ Week 1	M+ Week 8
OTC	54	33 (61.1%) [a]
Hoof-Sol	49	42 (85.7%) [b]
Negative Control	59	53 (89.9%) [c]

[a-b] P = 0.005

[a-c] P = 0.0003

[b-c] P = 0.51

Table 2 and Figure 5 provide a more detailed insight in the different stages of DD lesions, treated with one of the treatments, that transitioned over the 8-week trial. The number of DD-negative hind feet (M0 lesions) in the OTC group increased from 0 to 11, whereas the M1, M2 and M4.1 lesions decreased after 8 weeks. The same applies for Hoof-Sol; the M1, M2 and M4.1 lesions decreased (Table 2 and Figure 5) and the amount of M0 lesions increased from 0 to 6. In the negative control group, the amount of M0 lesions increased with 10.1 %. However, the active lesions increased.

Table 2 M-lesions in week 1 and 8 per treatment group.

	OTC		Hoof-Sol		Negative control	
	Week 1	Week 8	Week 1	Week 8	Week 1	Week 8
M0	0 (0%)	11 (20%)	0 (0%)	6 (12%)	0 (0%)	6 (10%)
M1	3 (6%)	2 (4%)	6 (12%)	5 (10%)	4 (7%)	6 (10%)
M2	3 (6%)	1 (2%)	4 (8%)	1 (2%)	3 (5%)	5 (8%)
M3	5 (9%)	2 (4%)	11(22%)	3 (6%)	13 (22%)	6 (10%)
M4	40 (74%)	38 (70%)	24 (49%)	34 (69%)	38 (64%)	34 (58%)
M4.1	3 (6%)	0 (0%)	4 (8%)	0 (0%)	1 (2%)	2 (3%)
Total	54	54	49	49	59	59

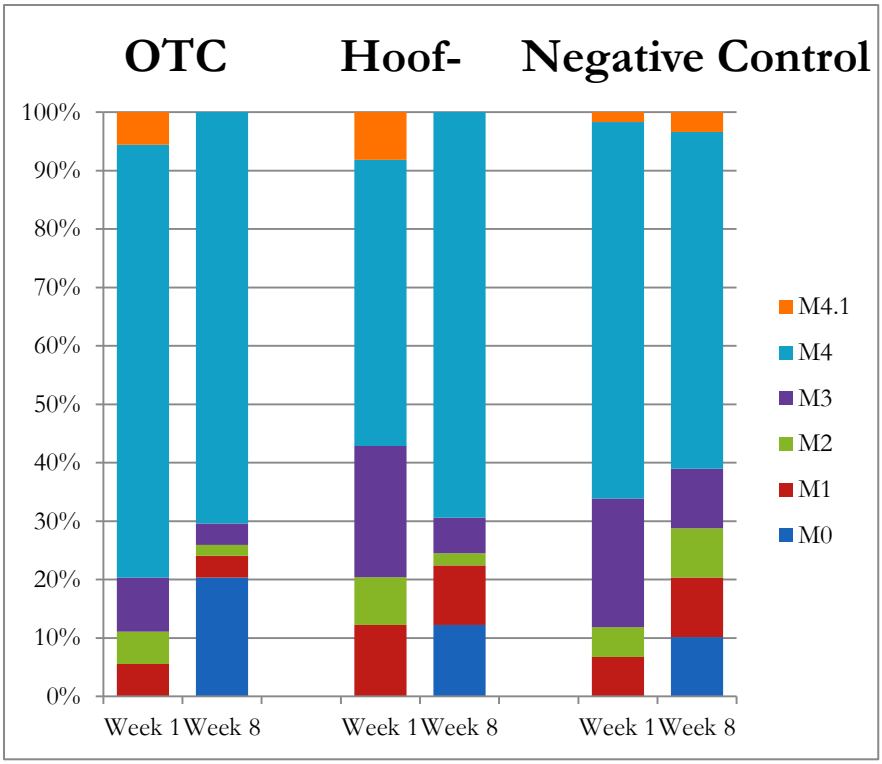

Figure 5 Proportion of different stages of digital dermatitis lesion in the OTC, Hoof-Sol and negative control treatment group, week 1 compared to week 8.

M+ lesions were divided in active lesions (M1, M2, M4.1) and in chronic lesions (M3 and M4). In figures 6, 7 and 8 the development of the M0, active and chronic lesions is presented per treatment group. As presented in figure 6, an increase of the proportion of M0 lesions was observed in the OTC treatment group (P=0.0005), whereas the proportion of active lesions did not change (P=0.07). The same applied to the chronic lesions (P=0.24).

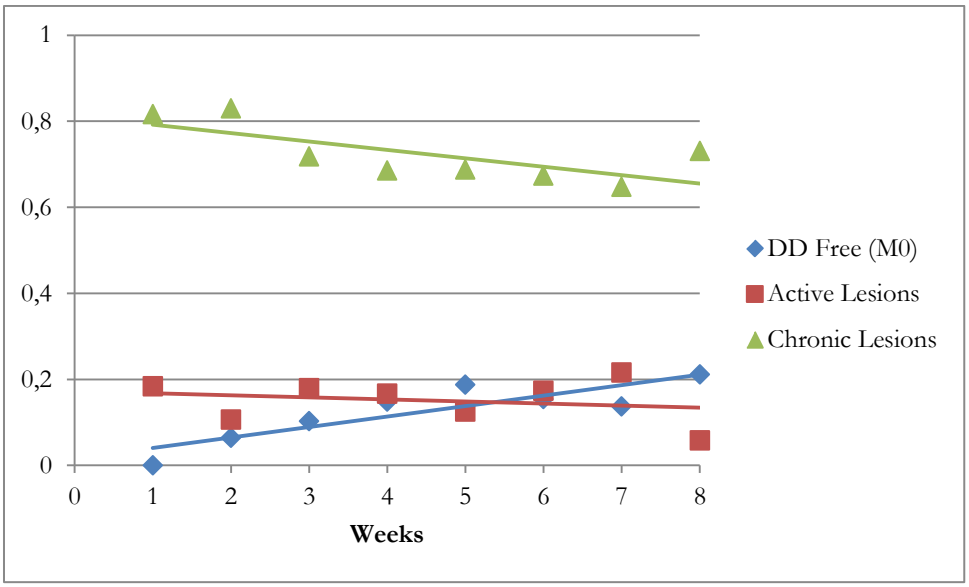

Figure 6 Development of M0, active and chronic lesions in the OTC treatment group.

For Hoof-Sol the M0 lesions increased (P=0.01) and active lesions decreased (P=0.045). The proportion of chronic lesions did not change after 8 weeks (see figure 7).

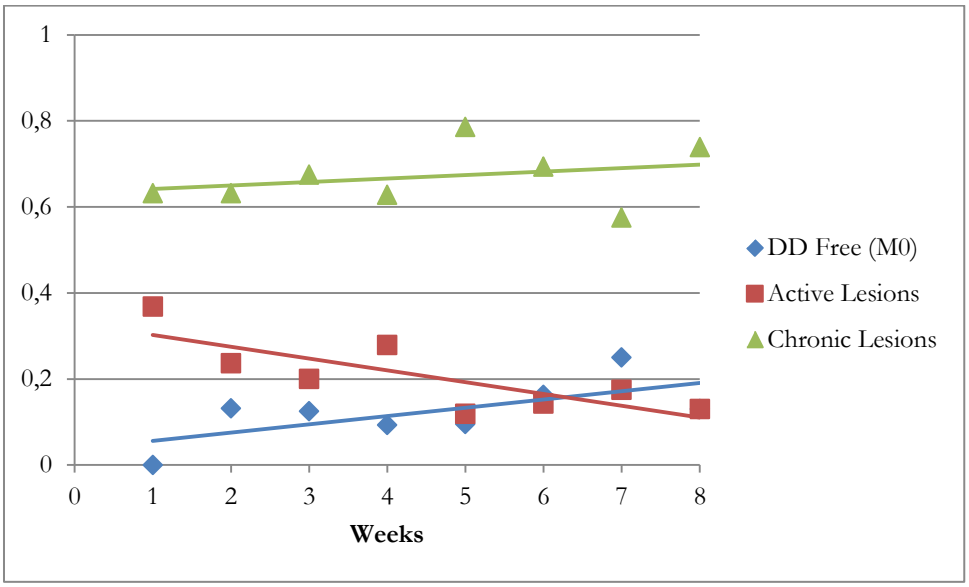

Figure 7 Development of M0, active and chronic lesions in the Hoof-Sol treatment group.

For the negative control, M0 increased (P=0.01) and the chronic lesions decreased (P=0.02). The significant difference between the chronic lesions in the negative control group between week 1 and week 8 was unexpected (see figure 8).

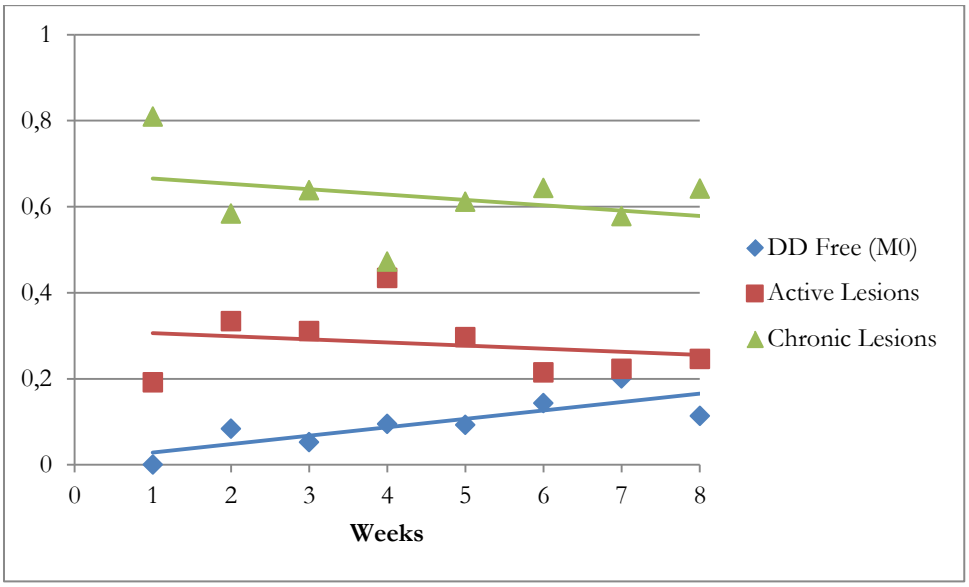

Figure 8 Development of the M0, active and chronic lesions in the negative control treatment group.

Comparing the different treatments in week 8, lead to no difference for the lesion stages. Except for the proportion of active lesions treated with OTC. The proportion was lower than for the negative control (P=0.01).

Discussion

The objective of the study was to determine the efficacy of weekly Hoof-Sol treatment of M+ DD lesions compared to weekly topical OTC treatment and a negative control. Studies on various individual topical treatments showed that OTC is effective in treatment of DD (Laven and Logue, 2006). There are several studies on topical treatments containing antibiotics. Most of these showed high cure rates (Laven and Logue, 2006). Berry et al. 2010 found no difference between topical treatment with 10 g of lincomycin or 10 g of OTC administered on days 1 and 2, both providing 68% cure on day 30. Laven & Logue 2006 also reviewed studies on alternative topical sprays compared with treatments containing antibiotics. Topical treatment with OTC appeared to be equally effective as a commercial formulation of soluble copper, peroxide compound, and a cationic agent. However, the other alternatives where significant less effective (Shearer, Elliott and Hernandez, 1999). Nevertheless, use of treatments containing antibiotics was not always more effective. Another study found that the efficacy of OTC was less than the non-antibiotic modified formulation of Victory (with reduced soluble copper and peroxide compound, but increased levels of cationic agent) (Shearer and Hernandez, 2000). This was based on the proportion of painful M2 lesions after 4 weeks. The low efficacy of OTC was considered as a result of antimicrobial resistance (Shearer and Hernandez, 2000). These products were administered in the milking parlor, similar to this study. On this farm OTC was more effective than Hoof-Sol. Table 1 demonstrates that the amount of M+ lesions in week 8 decreased 39% for OTC, 15% for Hoof-Sol and 10% for the negative control. The outcome that OTC had a higher cure rate is similar to what most of the other studies reported, except for the study of Shearer & Hernandez (2000), in which a lower efficacy for OTC was found. Most of the studies on topical treatments for DD were not performed in the milking parlor (Shearer, Elliott and Hernandez, 1999; Holzhauer *et al.*, 2011; Berry *et al.*, 2012), so more research on this way of application needs to be done.

A point of consideration is the intra-observer disagreement. Despite the training the observers received, lesions could be determined different by the observer during the study. This could provide various outcomes as regards to scoring. Some stages are hard to define, especially the small ones. Thereby, some feet could not be cleaned enough to be sure which kind of M-stage it was. Studies on scoring DD in the milking parlor showed that this method is still reliable (Rodriguez-Lainz *et al.*, 1998; Relun *et al.*, 2011). Not only scoring, but also the application of the treatments in milking parlor was sometimes difficult. The treatments were sprayed on the lesions with approximately 5 ml from a distance of 20-30 cm. However, the goal was to spray every lesion with 5 ml, although sometimes fluid rebounded or dripped off the feet. Thereby, the interdigital space could be missed easily. By using a sprayer system like Spray Pro[2] invented by Hoof-Sol, this problem was reduced.

[2] https://diamondhoofcare.com/products/spray-pro/

In this report, only data of one farm were included, because the data of other farms were not available yet. The sample size was large enough to determine a difference of 15% between the treatment groups, although a larger sample size would increase the accurately of the study. Hoof-Sol presented a numerical decrease in active lesions in week 8. However, this was not significant compared to OTC and the negative control. This may be due to the lack of power in this study. The use of a paired test might increase the power of the study. Due to the relative large number of cows dried off or culled and new introduced cows during the trial, no paired test was used. In this analysis some aspects were not included. For example, only the transformation to M0 was defined as cure. A transformation of an M2 to an M3 lesion could be regarded as an improvement (Döpfer et al. 1997). Another point raised by Döpfer et al. (1997) was that an M4 lesion could still be an M4, but a less proliferative one which would be regarded as an improvement, but not included in this analysis. Thereby, the cow factor was not considered and DD lesions on both hind feet of a cow were analyzed as independent lesions, although genetic susceptibility for DD exists and clustering within cow can therefore be expected (Scholey *et al.*, 2010).
Additionally, cow characteristics like parity, lactation stage and hoof health (interdigital dermatitis, heal erosion) were not included. Several studies have shown that these factors influence the risk for DD (Argaez-Rodríguez *et al.*, 1997; Somers *et al.*, 2005). As described before a randomizing sheet was used to ensure that cows with a higher risk were equally presented in both treatment groups. However, future studies should take the lactation stage and parity into account.

Conclusions

On this dairy farm, weekly use of the topical OTC spray during 8 weeks was more effective as treatment for the M+ lesions of DD than Hoof-Sol. Hoof-Sol treatment of DD lesions was not more effective than a negative control. However, Hoof-Sol presented a numerical decrease in active lesions, compared to OTC and the negative control group; this was however not significant, possibly due to the lack of power in this study.

Acknowledgements

I acknowledge the farmers involved in this study, for their participation and assistance. I want to thank Dr. Barkema and Dr. Hooijer for the opportunity doing my research internship in Canada and for their help. I want to thank Casey Jacobs for her help, her effort made this project to a success. Finally, I want to thank Casey Jacobs, Charlotte Pickel, Joanna Wong and Marlieke Pronk, due to them and the hospitality of Dr. Barkema and Casey Jacobs, I enjoyed working on this project.

References

Argaez-Rodríguez, F. J., Hird, D. W., de Anda, J. H., Read, D. H. and Rodriguez-Lainz, A. (1997) 'Papillomatous digital dermatitis on a commercial dairy farm in Mexicali, Mexico: Incidence and effect on reproduction and milk production', *Preventive Veterinary Medicine*, 32, pp. 275–286.

Berry, S. L., Read, D. H., Famula, T. R., Mongini, A. and Döpfer, D. (2012) 'Long-term observations on the dynamics of bovine digital dermatitis lesions on a California dairy after topical treatment with lincomycin HCl', *The Veterinary Journal*. Elsevier Ltd, 193(3), pp. 654–658.

Berry, S. L., Read, D. H., Walker, R. L. and Famula, T. R. (2010) 'Clinical, histologic, and bacteriologic findings in dairy cows with digital dermatitis (footwarts) one month after topical treatment with lincomycin hydrochloride or oxytetracycline hydrochloride.', *Journal of the American Veterinary Medical Association*, 237(5), pp. 555–560.

Blowey, R. W. and Sharp, M. W. (1988) 'Digital dermatitis in dairy cattle', *The Veterinary record*, 122, pp. 505–508.

Cheli, R. and Mortellaro, C. (1974) 'La dermatite digitale del bovino', in *Proceedings of the 8th International Conference on Diseases of Cattle*, pp. 208–213.

Döpfer, D., Koopmans, A., Meijer, F. A., Szakáll, I., Schukken, Y. H., Klee, W., Bosma, R. B., Cornelisse, J. L., van Asten, A. J. and ter Huurne, A. A. (1997) 'Histological and bacteriological evaluation of digital dermatitis in cattle, with special reference to spirochaetes and Campylobacter faecalis.', *The Veterinary record*, 140(24), pp. 620–623.

Gomez, A., Cook, N. B., Bernardoni, N. D., Rieman, J., Dusick, A. F., Hartshorn, R., Socha, M. T., Read, D. H. and Döpfer, D. (2012) 'An experimental infection model to induce digital dermatitis infection in cattle.', *Journal of dairy science*. Elsevier, 95(4), pp. 1821–30.

Holzhauer, M., Bartels, C. J., van Barneveld, M., Vulders, C. and Lam, T. (2011) 'Curative effect of topical treatment of digital dermatitis with a gel containing activated copper and zinc chelate', *The Veterinary record*, 169(21), pp. 555–555.

Holzhauer, M., Bartels, C. J. M., Döpfer, D. and van Schaik, G. (2008) 'Clinical course of digital dermatitis lesions in an endemically infected herd without preventive herd strategies', *Veterinary Journal*, 177(2), pp. 222–230.

Holzhauer, M., Hardenberg, C., Bartels, C. J. M. and Frankena, K. (2006) 'Herd- and Cow-Level Prevalence of Digital Dermatitis in The Netherlands and Associated Risk Factors', *Journal of dairy science*. Elsevier, 89(2), pp. 580–588.

Laven, R. A. and Logue, D. N. (2006) 'Treatment strategies for digital dermatitis for the UK.', *Veterinary journal (London, England : 1997)*, 171(1), pp. 79–88.

Read, D. H. and Walker, R. L. (1998) 'Papillomatous Digital Dermatitis (Footwarts) in California Dairy Cattle: Clinical and Gross Pathologic Findings', *Journal of Veterinary Diagnostic Investigation*, 10(1), pp. 67–76.

Refaai, W., Van Aert, M., Abd El-Aal, A. M., Behery, A. E. and Opsomer, G. (2013) 'Infectious diseases causing lameness in cattle with a main emphasis on digital dermatitis (Mortellaro disease)', *Livestock Science*, 156(1-3), pp. 53–63.

Relun, A., Guatteo, R., Roussel, P. and Bareille, N. (2011) 'A simple method to score digital dermatitis in dairy cows in the milking parlor.', *Journal of dairy science*. Elsevier, 94(11), pp. 5424–34.

Rodríguez-Lainz, A., Hird, D. W., Carpenter, T. E. and Read, D. H. (1996) 'Case-control study of papillomatous digital dermatitis in Southern California dairy farms', *Preventive Veterinary Medicine*, 28, pp. 117–131.

Rodriguez-Lainz, A., Melendez-Retamal, P., Hird, D. W. and Read, D. H. (1998) 'Papillomatous digital dermatitis in Chilean dairies and evaluation of a screening method.', *Preventive veterinary medicine*, 37(1-4), pp. 197–207.

Scholey, R. A., Ollier, W. E. R., Blowey, R. W., Murray, R. D. and Carter, S. D. (2010) 'Determining host genetic susceptibility or resistance to bovine digital dermatitis in cattle', *Advances in Animal Biosciences*. Cambridge University Press, 1(01), p. 2.

Shearer, J. K., Elliott, J. B. and Hernandez, J. (1999) 'Comparison of topical application of oxytetracycline and four nonantibiotic solutions for treatment of papillomatous digital dermatitis in dairy cows.', *Journal of the American Veterinary Medical Association*, 214, pp. 688–690.

Shearer, J. K. and Hernandez, J. (2000) 'Efficacy of two modified nonantibiotic formulations (Victory) for treatment of papillomatous digital dermatitis in dairy cows.', *Journal of dairy science*, 83(4), pp. 741–745.

Shearer, J. K., Stock, M. L., Van Amstel, S. R. and Coetzee, J. F. (2013) 'Assessment and management of pain associated with lameness in cattle.', *The Veterinary clinics of North America. Food animal practice*, 29(1), pp. 135–56.

Somers, J. G. C. J., Frankena, K., Noordhuizen-Stassen, E. N. and Metz, J. H. M. (2005) 'Risk factors for digital dermatitis in dairy cows kept in cubicle houses in The Netherlands.', *Preventive veterinary medicine*, 71(1-2), pp. 11–21.

Appendix 1

FARM:

DATE:

TREATMENT ADMINISTRATOR:

COW ID		ADMINISTERED	
	TX (0, 1, 2, 3, 4)	LEFT	RIGHT

Appendix 2

FARM NAME:					
DATE of visit:					
VISIT Number:					
Scorer name:					
Treater name:					
Data inputter name:					
COW ID	**FOOT (L/R)**	**DD SCORE**	**Treatment Group**	**Applied (0/1)**	