**INTERPRETIVE SUMMARY**

Title

Vanhoudt

RUNNING HEAD:

**Working title 1 (JW): The effect of an enzyme alginogel or copper and zinc chelates gel treatment on the M-score and wound healing progress of active digital dermatitis lesions in a randomized clinical field trail**

**Working title 2: Variation in M-score and wound healing aspects following randomized treatment of ulcerative digital dermatitis lesions with an enzyme alginogel or copper and zinc chelates gel**

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**ABSTRACT**

OBJECTIVES: Bovine digital dermatitis (DD) is a contagious disease and an important cause of lameness in cattle worldwide. A common therapy on herd level is the use of footbaths, which can contain formalin or multicompounds such as copper sulfate. A common individual treatment strategy is the use of an antimicrobial topical tetracyclin spray, or non-antibiotic products such as copper and zinc chelates, glutaraldehyde or salicylic acids. These treatment strategies and products are all focused on the bacterial component of DD because of their antimicrobial or antibacterial action. The focus of our study was to treat M1- and M2-lesions of DD as an open wound and stimulate wound healing, instead of treating DD as an infectious dermatitis. We therefore compared an enzyme alginogel (EAG), a wound-healing product, with an antimicrobial product containing copper and zinc chelates (CZG).   
MATERIALS: 536 lactating cows from seven different dairy farms were claw-trimmed and examined for the presence of DD using the M-score. Feet with M1- or M2-lesions were included in the trial. Feet were alternately allocated to the EAG or CZG treatment group and photographed, treated and bandaged on day 0 and 3. On day 7, lesions were photographed and received a third treatment and bandage if the lesion was still an open wound. All treated lesions were photographed for examination on day 10. Clinical improvement of DD lesions was defined as lesions that transferred from being classified as M1-lesions to M0, M3, M4 or M4.1, or M2-lesions that transferred to M0, M1, M3, M4 or M4.1. The effect of EAG and CZG on clinical improvement was analyzed using logistic regression models. During a follow up period of 10 weeks, hind feet were examined using the M-score every two weeks in the milking parlor.   
RESULTS: 202 hind feet were treated and included in the analysis. The clinical improvement rate of feet treated with EAG was 29.3% (29/99, CI 20.0 – 38.0%) and with CZG 93.2% (99/103, CI 88.0 – 98.0%). Feet treated with EAG were less likely (OR: 0.03, *p* < 0.001) to improve than feet treated with CZG. The covariable ‘Farm’ was identified as a significant risk factor for clinical improvement of the treated DD-lesions in the logistic regression.   
CONCLUSIONS: EAG was inferior to CZG. Considering the lower M-score clinical improvement rate and the higher recurrence risk of EAG, compared to CZG, EAG is not likely a favorable treatment product for M1- and M2-lesions of DD.

Key words: dairy cow, digital dermatitis, randomized clinical trial, topical treatment, wound healing

INTRODUCTION

Digital dermatitis (DD) is considered a contagious disease and an important cause of lameness in dairy cattle worldwide. It is characterized by hyperkeratotic or ulcerative lesions, typically located on the plantar or palmar aspect of the foot immediately proximal to the interdigital cleft. Lesions are mostly present on the hind feet and associated with lameness, reduced milk production, a diminished reproductive performance, and decreased animal welfare (Bruijnis et al., 2012; Higginson Cutler et al., 2013; Dolecheck and Bewley, 2018).

In general, alongside herd level DD control through foot bathing, cows with ulcerative DD lesions receive a topical treatment with or without bandage (Plummer and Krull, 2017). These ulcerative lesions are commonly grouped as active lesions and consist of the M1, M2, and M4.1 stage lesions (ADD REF). Topical treatments contain either antibiotics, e.g. broad spectrum tetracyclines, or a non-antibiotic active compound, e.g. copper and zinc chelates. Products based on copper and zinc chelates are widely used in the Netherlands and reported as an effective treatment option for DD when compared with topical tetracyclines, with clinical cure rates of 85-90% and 45-55% respectively (Holzhauer et al., 2011; Dotinga et al., 2017a). These clinical cure rates however don’t necessarily imply return to normal unaffected skin, but mostly resemble progress of the lesion to a chronic, non-ulcerative, hyperkeratotic stage. With this approach the disease is kept in a manageable state (Döpfer and Bonino Morlán, 2008).

Alternatively, DD lesions can also be considered chronic, non-healing wounds with a bacterial infection. Management of chronic, open wounds is based on the TIME principle (tissue debridement, infection control, moisture balance, and edges of the wound) and aims at return to normal unaffected skin (Schultz et al., 2003; Leaper et al., 2012; Bowers and Franco, 2020). Novel products and strategies have emerged since its first introduction to aid in achieving the TIME principle, with enzymatic alginates being one of them. Recently, an enzyme alginogel appeared effective in the treatment of udder cleft dermatitis, an ulcerative dermatitis, in dairy cows (van Werven et al., 2018). Alginates are known for their absorbing capacity of debris and exudate (passive debridement), and for keeping the wound moist through their gelling capacity (moisture balance). The antimicrobial enzyme system, glucose oxidase, lactoperoxidase, and guaiacol (**GLG-enzyme system**), in the enzyme alginogel studied by van Werven et al. (2018) results in the controlled release of reactive oxygen species which selectively disrupt bacterial cell walls (infection control) (De Smet et al., 2009).

We hypothesized that an enzyme alginogel is an effective topical treatment for active DD lesions. To test this hypothesis, we compared the enzyme alginogel with the standard non-antibiotic topical treatment in the Netherlands, i.e. a copper and zinc chelates gel. Effectiveness of treatment was evaluated using both the M-score for DD (Döpfer et al., 1997; Berry et al., 2012) and wound healing criteria (REF Equine Wound Management 3rd ed).

MATERIALS AND METHODS

***Ethical Statement***

This study was performed conform European law concerning the protection of animals kept for farming purposes (Council Directive 98/58/EC) and was not considered an animal experiment under Dutch legislation. The farmers participated in the research based on an informed consent statement.

***Study Design***

This open label, randomized clinical trial with positive control was set up as an intention to treat, non-inferiority trial to compare the treatment of active DD lesions with an enzyme alginogel [treatment group (**alginogel**), BoTop, Flen Health, Esch-sur-Alzette, Luxembourg] or with a copper and zinc chelates gel [control group (**coppergel**), Intra Hoof-fit Gel, Intracare, Veghel, the Netherlands]. For welfare reasons, an untreated negative control group was not included in the study design. Sample size was calculated using the Farrington-Manning Score test for proportion difference in SAS (version 9.4 M5) with a difference greater than 10% indicating inferiority. The expected clinical improvement rate of the coppergel was set at 92%. Considering a 95% confidence interval (**CI**) and a power of 80%, resulted in a calculated sample size of at least 104 active DD lesions in each group.

***Scorer Training***

Four students in veterinary medicine were trained in applying the M-score (Döpfer et al., 1997; Berry et al., 2012) by studying the literature, classroom training (39 digital color photographs of cattle feet with varying M-stages), and one in-parlor M-scoring of washed hind feet of approximately 50 dairy cows together with AV (Relun et al., 2011; Solano et al., 2017).

***Herd Selection***

Veterinarians working in the Utrecht area, were contacted and asked to suggest dairy farms that would meet the selection criteria for the study: (1) herds estimated to have a high (> 20%) prevalence of cows with active DD lesions, (2) presence of a safe and functional trimming chute on the farm, (3) lactating herd size of at least 50 cows, and (4) willingness to participate in the study. This resulted in a convenience sample of 7 farms. Farms were recruited between January 20 and February 20, 2019.

***Treatment Protocol***

Upon recruitment (day ‘zero’, **D0**), on all farms but one, the hind feet of all lactating cows were given a foot trim according to the Dutch five step method (REF Toussaint R.E. Trimming. In: Toussaint R.E., editor. Cattle Foot Care and Claw Trimming. Farming Press; Ipswich, UK: 1989. pp. 75–94. Chapter 3.) by the herd’s regular foot trimmer and all feet of these cows were given an M-score by a trained veterinary student. On one farm, the farmer selected 43 cows for foot trimming, because they were lame, likely to have an active DD lesion, or both. Hind feet with an active DD lesion, i.e. M1, M2, or M4.1 stage lesions (ADD REF), were selected for topical treatment. The first case on a farm was allocated to the alginogel or coppergel treatment group by flipping a coin. Thereafter, cows were alternately allocated to the alginogel or coppergel treatment group. All active DD lesions within one cow received the same treatment in order to exclude a potential systemic effect of the treatment product. Before applying a treatment, lesions were cleaned with cold water, a paper towel, or both and a photograph was taken (Sony Cybershot DSC-W830, Sony). The treatment product was applied directly to the lesion and then the treated lesion was covered with an impregnated, non-aqueous, non-linting gauze dressing (Cuticerin gauze dressing, 10 x 10 cm, Smith and Nephew, United Kingdom). The foot was then bandaged with soft padding (Cellona®, Lohmann & Rauscher, Neuwied, Germany), vetwrap (EickWrap, Eickenmeyer®, Culemborg, the Netherlands), and tape (Leukoplast®, BSN Medical, Luxembourg) (Figure 1). On day 3 (**D3**), the bandage was removed in a trimming chute, the foot was gently rinsed with cold water and dried with a paper towel. The lesion was then given an M-score, photographed and treated under bandage with the same product that was used on D0 as described above. On day 7 (**D7**) this procedure was repeated. Feet without active DD lesions on D7 were only photographed but received no further treatment nor bandage. All feet recruited on D0 were given a final M-score and photographed on day 10 (**D10**), after rinsing with cold water and drying with a paper towel. Day 10 was considered the endpoint of the treatment period. For welfare reasons, lesions that were still active at D10 were treated according to the farm treatment protocol, which was mostly a tetracycline spray or the coppergel, but no bandage was applied. Due to logistical reasons, the D10 evaluation took place on day 9 on farm 5. The students were aware of the treatment allocation in such a way that they applied and recorded the initial treatment on D0 and could see the different color of the treatment products when replacing the bandage. They would always first give an M-score to the lesion and then check the treatment allocation of the foot for further treatment according to the protocol. The farmers were blinded to the treatment allocation and were allowed to continue their regular control of DD for cows that were not in the trial, but asked not to run the cows through a footbath during the 10 days trial period.

***Treatment Outcomes***

***M-Score.*** Clinical improvement between D0 and D0 (**MS0-10**, Table 1) was the primary outcome and investigated using an M-score transition matrix. Cure was defined as a transition to the M0 stage.

***M-Score Follow-Up.*** On day 21 (**D21**) and day 35 (**D35**), all lactating cows were M-scored in the milking parlor by the veterinary students. Hind feet were hosed and inspected using a mirror glued on a spatula and headlamp (Figure 2). This method is considered to be practical and accurate for M-scoring DD lesions (Relun et al., 2011; Stokes et al., 2012; Solano et al., 2017). These M-scores were used to calculate D21 and D35 clinical improvement (**MS0-21** and **MS0-35**, respectively). Farmers were allowed to carry out their routine control measures for DD for all cows during the follow-up period. Two farms were not included in the follow-up period, because they used a milking system which made in-parlor M-scoring impossible.

***Wound Healing Progress.*** An expert in veterinary wound healing (JW), who was blinded for the treatment allocation, examined all photographs made during the treatment period for skin necrosis, granulation tissue, granulation tissue level in comparison with the surrounding skin, granulation tissue necrosis, wound contraction, and epithelization. Sequential pairs of photographs of the lesions [i.e. D0-D3 (**WHP0-3**), D3-D7 (**WHP3-7**), D7-D10 (**WHP7-10**), and D0-D10 (**WHP0-10**, primary outcome)] were given an evaluation of wound healing progress: improved, equal, worsened, or unable to score. Unable to score wound healing progress was specified as presence of crust materials, presence of fecal contamination, poor image quality, or other. The end of the treatment period (D10) was the endpoint of the wound healing assessment.

***Statistical Analysis***

Data were analyzed using the statistical software package R (ADD REF). The experimental unit was an M1, M2, or M4.1 stage lesion on a hind foot at recruitment (D0). A Pearson’s chi-square test of contingencies (with α = 0.05) was used to investigate if there was an association between treatment and MS0-21 and MS0-35.

***M-Score.*** The outcome for the logistic regression analysis was MS0-10 (yes, no). To enable the assessment of the effect of different time periods under bandage (7 versus 10 days), a variable ‘Bandage’ was created. First univariable logistic regression models with ‘Farm’ as random effect were applied to assess the effect of ‘Treatment’, ‘M-score on D0’, and ‘Bandage’ respectively on the MS0-10. This was followed by a linear mixed effects (**LME**) model with ‘Treatment’, ‘M-score on D0’ and ‘Bandage’ as independent variables, and ‘Farm’ as random effect. The final reduced model was based on the lowest Akaike information criterion using a backward elimination approach. *The Hosmer-Lemeshow Goodness of Fit-test showed that the final model had a good fit to the data as P = 0.92 (> 0.05).*

***Wound Healing Progress.*** The outcome for the logistic regression analysis was WHP0-10 [improved, not improved (i.e. equal, worsened, and unable to score)]. First univariable logistic regression models with ‘Farm’ as random effect were applied to assess the effect of ‘Treatment’, ‘M-score on D0’, ‘Bandage’, ‘WHP0-3’, ‘WHP3-7’, and ‘WHP7-10’ respectively on WHP0-10. This was followed by an LME model with ‘Treatment’, ‘M-score on D0’, ‘Bandage’, ‘WHP0-3’, ‘WHP3-7’, and ‘WHP7-10’ as independent variables, and ‘Farm’ as random effect, using the same backward elimination procedure as for the M-score. *The Hosmer-Lemeshow Goodness of Fit-test showed that the final model had a good fit to the data as P = 0.92 (> 0.05).*

**RESULTS**

***REFLECT Study Flow***

***M-Score.*** On D0, we examined a total of 1086 hind feet from 543 cows, divided over seven different dairy farms. Of these, 728 feet (67%) were scored as M0 (i.e. no DD). The remaining 358 feet (33%) had DD, with 158 feet (15%) being classified as M2 stage lesions, 112 feet (10%) as M4 stage lesions, 46 feet (4%) as M1 stage lesions, 21 feet (2%) as M3 stage lesions, 20 feet (2%) as M4.1 stage lesions, and one foot with DD but unable to M-score. The control group contained 117 feet (23 M1, 83 M2, and 11 M4.1 stage lesions). Five feet (one M1 and four M2 stage lesions) from the control group were excluded from analysis, one foot due to loss of follow-up, two cows were unsafe to handle, and two feet had lost their bandage prior to assessment days. The treatment group contained 107 feet (23 M1, 75 M2, and 9 M4.1 stage lesions). Five feet (two M1 and three M2 stage lesions) from the treatment group were excluded from analysis, two feet due to missing a bandage change at D3 and three feet had lost their bandage prior to assessment days. For the analysis of clinical improvement and cure rates, 214 feet were eligible, 112 feet (from 88 cows) were in the control group and 102 feet (from 85 cows) were in the treatment group. In this total sample size of 173 cows, 132 cows had one foot affected and 41 cows had two feet affected.

***M-Score Follow-Up.*** After exclusion of the feet from cows on the two farms with an automatic milking system, 130 feet (67 feet in the control group and 63 feet in the treatment group) remained in the study. M-scores were missing for 20 feet on D21 and for 25 feet on D35. On D21, six feet were unable to M-score and on D35 this were four feet. As a result, the M-scores of 104 and 101 feet, respectively, were available for the calculation of D21 and D35 clinical improvement rates.

***Wound Healing Progress.*** From the 214 feet available for M-score clinical improvement and cure rates analyses on D10, for one foot all the photographs were missing and for tree feet the photograph from D10 were missing. This resulted in a total of 210 feet available for analysis of the primary outcome (WHP0-10). Of these, one foot had the photograph of D3 missing and 2 feet had the photograph of D7 missing, resulting in 209 feet available for analysis of WHP0-3, 207 feet for the analysis of WHP3-7, and 208 feet available for the analysis of WHP7-10.

***Lactating Herd DD Prevalence on D0***

The lactating herd prevalence of active DD lesions at feet level on D0 ranged between 10 and 45% (median 17%, Q1 16%, Q3 26%, Figure 3)

***Treatment outcomes***

***M-Score at D10.*** The overall MS0-10 was 27% for the alginogel group and 94% for the coppergel group (Table 2). Only 7 feet (3%) were cured by D10, with 2% cured in the alginogel group and 4% cured in the coppergel group. The MS0-10 per farm is provided in the Appendix (Table A1). In the alginogel group, M1 stage lesions mainly remained an M1 stage lesion (33%) or became an M2, M3, or M4.1 stage lesion by D10, while the majority of M2 stage lesions remained an M2-lesion (77%). In the coppergel group, however, active DD lesions were most likely to become an M3 stage lesion (75%) by D10. The full transition matrix for D10 M-scores in relation to D0 M-scores is given in the Appendix (Table A2).

***M-Score Follow-up.*** The overall MS0-21 was 74% for the alginogel group and 84% for the coppergel group, while MS0-35 was 71% for alginogel group and 81% for the coppergel group (Table 2). There was no association observed between treatment group and MS0-21 or MS0-35 (χ2(1) = 0.81, *P* > 0.05 and χ2(1) = 0.75, *P* > 0.05, respectively).

***Wound Healing Progress.*** Of the lesions treated with the alginogel 63% improved between D0 and D10, whereas of the lesions treated with the coppergel 20% improved (Table 3). The WHP0-10 for the majority of the lesions treated with the coppergel could not be assessed (69%), mostly due to the presence of crust materials (Table 4). The M-scores on D10 for those feet not being able to score WHP0-10 and the WHP0-10 per farm are provided in the Appendix (Tables A3 and A4, respectively).

***Logistic regression analyses***

***M-Score.*** The results of the univariate logistic regression analyses are provided in the appendix (Table A5). In the final multivariable LME model, lesions treated with the alginogel had a decreased likelihood for MS0-10 [AOR: 0.06, 95% CI: 0.02 – 0.14, Table 5].

***Wound Healing Progress.*** To deal with the large proportion of lesions unable to score WHP0-10, these lesions were first classified as ‘not improved’ for the logistic regression analyses, followed by classification as ‘improved’ and repetition of the logistic regression analyses. The results of the univariate logistic regression analyses are provided in the appendix (Table A5). In the final multivariable LME model, with lesions unable to score classified as ‘not improved’, lesions treated with the alginogel had an increased likelihood for WHP0-10 (AOR: 2.41, 95% CI: 1.09 – 5.30, Table 5). In the final multivariable LME model, with lesions unable to score classified as ‘improved’, lesions treated with the alginogel had a decreased likelihood for WHP0-10 (AOR: 0.36, 95% CI: 0.10 – 1.21, Table 5).

**DISCUSSION**

This study investigated the effect of two non-antibiotic topical treatment products on active DD lesions using M-score clinical improvement and wound healing progress as outcome measures. At the end of the 10 days treatment trial, M-score clinical improvement was high for lesions treated with the coppergel, with most of the active lesions transitioned into chronic DD lesions, whereas M-score clinical improvement was low for lesions treated with the alginogel, with most of the lesions remaining active DD lesions. The high clinical improvement of lesions treated with the coppergel is in line with findings by others (REFS). Follow-up M-score clinical improvement ranged between 70 and 85% with the alginogel performing 10% less in comparison with the coppergel, both 21 and 35 days after recruitment. During the follow-up period, farmers were allowed to continue their regular DD control, which in the Netherlands most commonly consists of topical treatment with the coppergel with or without a light bandage for 3 days or a tetracycline spray without bandage, whether or not combined with routine biocide footbaths. Thereby, those lesions that remained active DD lesions during the treatment trial, were exposed to the effects of different topical treatment products which most often result in chronic DD lesions (results not presented). In the final LME model, lesions treated with the alginogel had a decreased likelihood for M-score clinical improvement and a shorter period under bandage had an increased likelihood for M-score clinical improvement. The M-score clinical improvement results can be explained by the effect of both treatment products on wounds. Copper has an astringent effect which dries the wound and stimulates crust formation (REF). The alginogel on the other hand, has a debriding and moisturizing effect which likely keeps the wounds as active DD lesions during a treatment period of only 10 days (REF). The effect of a shorter period under bandage is likely explained by the fact that lesions not bandaged for the full treatment trial duration, were only those that had become chronic DD lesions by D7 and hence already considered to be clinically improved. Only 4% of these chronic lesions reversed to active DD lesions, but not the M2 stage, between D7 and D10 (results not presented). This is in line with the x days average time for recurrence of treated M2 stage lesions (REF).

More lesions in the alginogel group had improved wound healing progress at the end of the 10 days treatment trial in comparison with lesions in the coppergel group. However, for a large proportion of lesions in the coppergel group wound healing progress could not be evaluated due to the presence of crust materials. Crusts hamper the assessment of wounds as removal of the crust might reveal a healthy healing would or an infected wound (REF). To deal with those lesions that were unable to score for wound healing progress due to the presence of crust material, we assumed that their wound healing progress had either improved or not in the logistic regression analyses. In the final LME model, assuming that the lesions under crusts did not improve, lesions in the alginogel group were more likely to have an improved wound healing progress. This effect reversed in the final LME model, assuming lesions under crusts to be improved (albeit losing significance). Lesions with improved wound healing progress at the intermediate assessments were more likely to have improved wound healing progress at the end of the treatment trial, regardless of how was dealt with lesions unable to score for wound healing progress.

M-score is based on histological appearance of lesions and then translated to macroscopic appearance. This classification differs from wound healing aspects.

DD should be considered as an infected wound. Once the infection is dealt with, body can deal with the wound. The immune system of the cow needs aid from antimicrobial in treatment product. Copper has good antimicrobial properties but is to aggressive for the wound (REF). The GLG-enzyme system in the alginogel has the potential to deal with the infection but treatment duration in this study was too short to achieve complete closure of the wound. Only achieved under optimal hygienic conditions with daily alginogel and bandage replacement for ulcers of comparable size in humans (REF). For both copper and GLG-enzyme system efficacy against pathogenic Treps in DD still needs to be demonstrated both in vitro and in vivo.

In our study we looked at two different aspects in dealing with DD. Either M-score clinical improvement which results in a manageable state of the disease and the majority of the lesions residing in the chronic state. This does not result in eradication of disease and M4 stage lesions play a major role in the epidemiology of DD. On the other hand, we looked at wound healing aspects of topical DD treatment, aimed at achieving M0 stage as treatment outcome.

*Ter verwerking in discussie.*

*Treps slow down wound healing (EBCR Mirjam)*

*Invloed koeien met letsels aan 2 poten op resultaat waarvoor niet gecorrigeerd is.*

It should be mentioned that feet on day 10 were scored in a trimming chute, which makes it possible to inspect feet closely and the time to inspect was not limited. This differs from in-parlor M-scoring.

To date, this study is the first that investigated the effect of an enzyme alginogel with an GLG-complex on DD-lesions and showed a clinical improvement rate of 29.3% on day 10. There are several studies available in the field of human medicine, that investigated the effect of the enzyme alginogel on open wounds such as pressure ulcers (Durante, 2012; Bernaerts et al., 2012; de la Brassinne et al., 2006). The alginogel containing the GLG-complex used in the current study, only registered for human use (Flaminal®, Flen Pharma), was tested in these studies. These studies showed that the gel was effective in reducing the surface area of wounds, after treating daily in a range from 28 days up to 5 months. Van Werven et al. (2018) showed that the enzyme alginogel containing the GLG-complex significantly improves udder cleft dermatitis in dairy cows; in this study cows were treated daily for 12 weeks. These studies /study?? suggest that our treatment period, a maximum of three treatments within seven days, was probably not long enough to establish a high clinical improvement rate for this alginogel. The treatment protocol used in the current study was based on the summary of product characteristics of the copper and zinc chelates gel and appeared to be successful for the copper and zinc chelates gel in this study, with a clinical improvement rate of 93.2%. However, with the above-mentioned studies in mind, this may indicate that the duration to establish a high clinical improvement rate may differ for alginogels. For future research, it is strongly recommended to lengthen the treatment period with the enzyme alginogel.

The clinical improvement rate of the enzyme alginogel on day 10 lies within a range of 0.0% to 60.0% on farm-level. These differences between herds are probably due to management factors, such as type of housing. All farms included in this study housed their cows in cubicles during the treatment period, however, the length and width of cubicles are associated with the prevalence of DD within a herd (Somers et al., 2005). Cubicle bedding, herd size, flooring system, the use of a manure scraper and feed management are also associated with DD (Somers et al., 2005). Such management factors are not taken into account in this study but might influence the improvement rate of DD. Individual susceptibility factors such as genetics, morphological, physiological and behavioral factors also may influence the improvement rate (Palmer and O’Connell, 2015).

The clinical improvement rate of the copper and zinc chelates gel on day 10 was 93.2% within a range of 84.6 – 100% on farm-level. These results are in accordance with previous studies that investigated the efficacy of copper and zinc chelates on DD (Dotinga et al., 2017b). Holzhauer et al. (2011) reported an improvement rate (defined as a transition from M2 to M0, M1, M3 or M4) of 92% with a range of 86.0 to 100% within herds, after 28 days. Dotinga et al. (2017) reported an improvement rate of 86.8% after 10 days, with a range of 61.5 to 100% within herds. After 10 days, 71% of the M2-lesions transitioned to M3, which is in accordance with the results of the current study.

The M-scores in the follow up period were obtained in the milking parlor, done by four master students of Veterinary Medicine. These students received the same training in M-scoring, however, differences in interpreting the type of DD-lesions could exist (Vanhoudt et al., 2019). In the milking parlor M3 is often confused with M4 (Relun et al., 2011) and M2 with M4.1 (Solano et al., 2017). M1 is often confused with M2 and M4 (Relun et al., 2011). This might have influenced the calculated recurrence to M2-lesions, since the recurrence rate is highly influenced by a given M1- or M2-score in the follow up period. These stages only differ from each other in diameter, and the time to inspect the feet is limited during in-parlor M-scoring. Adding gridlines on the mirror could have helped to minimize misclassification of lesions by size (Relun et al., 2011). An analysis to test the interobserver agreement was not performed in this study but could have provided an indication of agreement between the master students. Thereby, only a low number of feet were used to calculate the recurrence, since this analysis only included feet that were considered to be clinically improved at day 10. Also, feet on day 10 were scored in a trimming chute instead of the milking parlor. This makes it possible to inspect feet closely and without a restricted examination time, in contrast to M-scoring in the milking parlor. The M-scoring in the trimming chute is therefore probably more accurate than the M-scoring in the milking parlor.

When DD-lesions where found as an active lesion (i.e. M1- or M2) during the examination on day 10, these lesions were, because of animal welfare reasons, treated according to the treatment protocol of the farmer. This means that these lesions were treated with tetracyclin spray or the copper and zinc chelates gel, but no bandage was applied. Further treatment of these lesions was on responsibility of the farmer and were not taken into account in the follow up period but might have influenced the recurrence to M2-lesions during this period. A possible underestimated recurrence found in this study, could be clarified because at some farms the cattle were put on pasture during the follow up period. Keeping cows at pasture is associated with a lower prevalence of DD in a herd (Rodriguez-Lainz et al., 1999; Olmos et al., 2009).

As in this study, non-antibiotic products are mainly tested on active DD-lesions (i.e. M1- and M2-lesions) (Holzhauer et al., 2011; Dotinga et al., 2017; Jacobs et al., 2018). These lesions are painful and therefore important to treat regarding animal welfare (Mumba et al., 1999). The aim of such trials is to cure the lesion, or a transition of the lesion to a chronic stage. It is previously concluded that M4-lesions determine more than 88% of the reproduction ratiobecause 70% of the infectious time is spent in this stage of disease (Biemans et al., 2018). Dopfer et al. (2012) considered both M2- and M4-lesions as infectious, based on clinical experience. It is paramount not only to treat active lesions, but also to reduce the transmission rate of DD and therefore the prevalence of DD within a herd. Given that M2- and M4-lesions both play an important role in the reproduction rate of DD, the symptoms and transmission of DD could be maximal reduced by prevention of these two stages. Additional research that investigates the treatment of chronic DD-lesions (i.e. M4-lesions) is therefore recommended. The broad range of clinical improvement rates of the enzyme alginogel on farm-level highlight that management factors likely do play an important role in the success of treatment of active DD lesions. Treatment and prevention of DD in a herd should be therefore also focused on management of the farm, and not only on treatment on cow-level.

# CONCLUSION

The clinical improvement rate of the enzyme alginogel on day 10 was 29.3% and therefore inferior to the copper and zinc chelates gel which had an improvement rate of 93.2% on day 10. The recurrence to M2-lesions in the follow-up period…. Considering these results, the enzyme alginogel is not likely a favorable treatment product for M1- and M2-lesions of DD.

# CONFLICTS OF INTEREST

None of the authors has a personal or financial relationship with organizations, pharmacists or people that could influence or bias the content of this study. The enzyme alginogel was kindly donated by Flen Health.

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*Describe lesions (including histology?) and discuss from wound management perspective.* The typical ulcerative DD lesions have been histologically described as complete loss of the stratum corneum, rete ridge formation at the dermo-epidermal border with micro-abscesses in both epidermis and dermis, and pronounced perivascular infiltration in the dermis (Dopfer et al. 1997 Vet Rec)

*histological findings M2-stage lesion Dopfer et al. 1997 Vet Rec: Stratum comeum completely lost over the extension of the lesion; pronounced haemorrhage in its remainders at the lesion borders: homy columns, ballooning degeneration and acanthosis; stratum basale 0-11 mf/1 0 hpf in more than one cell layer, less mf than class 1; rete ridge formation with broad-based tips at the dermo-epidermal border with microabscesses in both epidermis and dermis; pronounced perivascular infiltration in the dermis; neutrophils and eosinophils predominating in the epidermis with occasional plasma cells*

*histological findings M1-stage lesion Dopfer et al. 1997 Vet Rec: Epithelium 2-3x as high as controls; rete ridge formation; plaque with partial loss of the epithelium and parakeratosis; areas of ballooning degeneration filled with islands of fibrin; horny columns as papillary projections towards the skin surface ending in microabscesses and haemorrhages; stratum corneum hyperplastic; stratum spinosum acanthotic; stratum basale has more than one cell layer and shows 4-11 mitotic figures per 10 high power fields (mf/1 0 hpf) compared to the controls; perivascular infiltration in the dermis; mononuclear cells and neutrophils with occasional eosinophils present in the dermis and epidermis*

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**TABLES**

**Table 1.** Definitions of clinical improvement and cure following topical treatment using the M-scores (REFS) at the start (D0) and end (D10) of the treatment trial

|  |  |  |
| --- | --- | --- |
| M-Score on D0 | M-Score on D10 |  |
|  | Clinical Improvement | Cure |
| M1 | M0, M3, and M4 | M0 |
| M2 | M0, M1, M3, M4, and M4.1 | M0 |
| M4.1 | M0, M1, M3, and M4 | M0 |

**Table 2.** The M-score clinical improvement of active digital dermatitis lesions treated with an enzyme alginogel (alginogel) or copper and zinc chelates gel (coppergel), at different evaluation days

|  |  |  |  |
| --- | --- | --- | --- |
| Evaluation Day | M-Score on Day 0 | Clinically Improved, N (%) | |
|  |  | Alginogel | Coppergel |
| Day 10 | M1 | 6 (29) | 21 (95) |
|  | M2 | 17 (24) | 73 (92) |
|  | M4.1 | 5 (56) | 11 (100) |
|  | Total | 28 (27) | 105 (94) |
| Day 21 | M1 | 12 (100) | 10 (91) |
|  | M2 | 27 (69) | 29 (81) |
|  | M4.1 | 2 (50) | 2 (100) |
|  | Total | 41 (74) | 41 (84) |
| Day 35 | M1 | 9 (90) | 10 (91) |
|  | M2 | 24 (69) | 29 (76) |
|  | M4.1 | 2 (50) | 3 (100) |
|  | Total | 35 (71) | 42 (81) |

Day 0 and day 10 M-scores were determined from lifted feet in the trimming chute, whereas day 21 and day 35 M-scores were determined from standing feet in the milking parlor.

**Table 3.** Overview of wound healing progress per treatment group, percentages add up vertically per evaluation pair

|  |  |  |  |
| --- | --- | --- | --- |
| Evaluation Pair | Wound Healing Progress | Alginogel  N (%) | Coppergel  N (%) |
| WHP0-10 | N hind feet | 101 (100) | 109 (100) |
|  | Improved | 64 (63) | 22 (20) |
|  | Equal | 27 (27) | 10 (9) |
|  | Worsened | 2 (2) | 2 (2) |
|  | Unable to score | 8 (8) | 75 (69) |
| WHP0-3 | N hind feet | 101 (100) | 108 (100) |
|  | Improved | 30 (30) | 14 (13) |
|  | Equal | 38 (37) | 11 (10) |
|  | Worsened | 1 (1) | 0 |
|  | Unable to score | 32 (32) | 83 (77) |
| WHP3-7 | N hind feet | 100 (100) | 107 (100) |
|  | Improved | 31 (31) | 9 (8) |
|  | Equal | 33 (33) | 8 (7) |
|  | Worsened | 2 (2) | 1 (1) |
|  | Unable to score | 34 (34) | 89 (84) |
| WHP7-10 | N hind feet | 100 (100) | 108 (100) |
|  | Improved | 49 (49) | 7 (6) |
|  | Equal | 23 (23) | 8 (7) |
|  | Worsened | 7 (7) | 3 (3) |
|  | Unable to score | 21 (21) | 90 (84) |

WHP0-10: primary outcome, wound healing progress between day 0 and day 10

WHP0-3: wound healing progress between day 0 and day 3

WHP3-7: wound healing progress between day 3 and day 7

WHP7-10: wound healing progress between day 7 and day 10

**Table 4.** Overview of reasons for not being able to score wound healing progress between day 0 and day 10 per treatment group

|  |  |  |  |
| --- | --- | --- | --- |
|  | Alginogel  (N = 101)  N (%) | Coppergel  (N = 109)  N (%) | Total  (N = 210)  N (%) |
| N hind feet unable to score | 8 (100) | 75 (100) | 83 (100) |
| Presence of crust materials | 0 | 52 (69) | 52 (63) |
| Presence of fecal contamination | 1 (13) | 17 (23)\* | 18 (22) |
| Poor photograph quality | 6 (74) | 4 (5) | 10 (12) |
| Other | 1 (13) | 2 (3) | 3 (3) |

\* *Of these 17 feet X were not bandaged between D7 and D10 - evt over total 18*

**Table 5.** Associations between M-score clinical improvement and wound healing progress outcome variables and different explanatory variables using linear mixed effects model with farm as random effect and backward elimination using lowest Akaike information criterion

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **M-score Clinical Improvement** | | **Wound Healing Progress** | | | |
|  |  | **Lesions unable to score classified as ‘not improved’** | | **Lesions unable to score classified as ‘improved’** | |
| **Variable** |  | **AOR** | **95% CI** | **AOR** | **95% CI** | **AOR** | **95% CI** |
| Intercept |  | 4.37 |  | 0.15 |  | 0.62 |  |
| Treatment group | Coppergel |  |  |  |  |  |  |
|  | Alginogel | 0.06 | 0.02 – 0.14 | 2.41 | 1.09 – 5.30 | 0.36 | 0.10 – 1.21 |
| M-score on day 0 | M1 |  |  |  |  |  |  |
|  | M2 |  |  |  |  |  |  |
|  | M4.1 |  |  |  |  |  |  |
| Period under bandage | 10 days |  |  |  |  |  |  |
|  | 7 days | 14.81 | 5.06 – 54.47 |  |  | 0.25 | 0.06 – 0.94 |
| WHP0-3\* | Not improved |  |  |  |  |  |  |
|  | Improved |  |  |  |  | 3.28 | 1.10 – 10.14 |
| WHP3-7\* | Not improved |  |  |  |  |  |  |
|  | Improved |  |  | 7.56 | 2.65 – 24.09 |  |  |
| WHP7-10\* | Not improved |  |  |  |  |  |  |
|  | Improved |  |  | 25.46 | 8.88 – 93.11 | 30.51 | 11.66 – 89.93 |

\* Variables WHP0-3, WHP3-7, and WHP7-10 were not included in the M-score clinical improvement linear mixed effects model

**FIGURE LEGENDS**

**Vanhoudt Figure 1.** a) The DD-lesion was fully covered with the treatment product (on this picture enzyme alginogel) and covered with a gauze. The foot was then bandaged with a b) soft padding and with c) vetwrap and tape.

**Vanhoudt Figure 2.**Examination of a hind foot in the milking parlor, using a mirror glued on a spatula.

**Vanhoudt Figure 3.** REFLECT flow chart

**Vanhoudt Figure 4.** Lactating herd feet level DD prevalence at recruitment

**FIGURES**

**Vanhoudt Figure 1.**

*A group of different types of food

Description automatically generated*

**Vanhoudt Figure 2.**

*A picture containing ground, outdoor

Description automatically generated*

**Vanhoudt Figure 4.**

**APPENDIX**

**Table A1.** The trimming chute M-score clinical improvement of active digital dermatitis lesions\* treated with an enzyme alginogel (alginogel) or copper and zinc chelates gel (coppergel), per farm and for the primary outcome (MS0-10)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Alginogel |  | Coppergel |  |
|  | Treated (N) | Clinically Improved N (%) | Treated (N) | Clinically Improved N (%) |
| Primary outcome (MS0-10) | 102 | 28 (25) | 112 | 105 (94) |
| Farm 1 | 11 | 1 (9) | 14 | 12 (86) |
| Farm 2 | 24 | 5 (21) | 27 | 26 (96) |
| Farm 3 | 7 | 1 (14) | 5 | 5 (100) |
| Farm 4 | 15 | 8 (53) | 18 | 18 (100) |
| Farm 5 | 13 | 3 (23) | 16 | 14 (88) |
| Farm 6 | 18 | 6 (33) | 19 | 19 (100) |
| Farm 7 | 14 | 4 (29) | 13 | 11 (85) |

**Table A2.** Trimming chute M-score transition matrix of active digital dermatitis lesions between day 0 (D0) and day 10 (D10) following treatment with an enzyme alginogel (alginogel) or a copper and zinc chelates gel (coppergel); for each treatment group, percentages add up horizontally per M-score on D0

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Treatment Group | M-Score on D0 | M-Score on D10, N (%) | | | | | | |
| M0 | M1 | M2 | M3 | M4 | M4.1 | Total |
| Alginogel | M1 | 0 | 7 (33) | 4 (19) | 4 (19) | 2 (10) | 4 (19) | 21 (100) |
|  | M2 | 0 | 11 (15) | 55 (77) | 2 (3) | 3 (4) | 1 (1) | 72 (100) |
|  | M4.1 | 2 (22) | 2 (22) | 2 (22) | 1 (12) | 0 | 2 (22) | 9 (100) |
|  | total | 2 (2) | 20 (19) | 61 (60) | 7 (7) | 5 (5) | 7 (7) | 102 (100) |
| Coppergel | M1 | 4 (18) | 1 (4) | 0 | 12 (55) | 5 (23) | 0 | 22 (100) |
|  | M2 | 1 (1) | 6 (8) | 6 (8) | 64 (81) | 2 (2) | 0 | 79 (100) |
|  | M4.1 | 0 | 1 (9) | 0 | 8 (73) | 2 (18) | 0 | 11 (100) |
|  | total | 5 (4) | 8 (7) | 6 (6) | 84 (75) | 9 (8) | 0 | 112 (100) |

**CHECK! Table A3.** Overview of the M-scores on day 10 for the feet with photographs classified as ‘unable to score’ for wound healing progress per treatment group

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | Control group  (N = 110) |  |  |  | Treatment group  (N = 102) |  |  |  | Total  (N = 212) |  |
| N feet unable to score |  |  | 75 |  |  |  | 8 |  |  |  | 83 |  |
| Reason unable to score |  | N | M-score D10 | N |  | N | M-score D10 | N |  | N | M-score D10 | N |
| Presence of crust materials |  | 52 | M0 | 1 |  | 0 | M0 | 0 |  | 52 | M0 | 1 |
|  |  |  | M1 | 4 |  |  | M1 | 0 |  |  | M1 | 4 |
|  |  |  | M2 | 1 |  |  | M2 | 0 |  |  | M2 | 1 |
|  |  |  | M3 | 46 |  |  | M3 | 0 |  |  | M3 | 46 |
|  |  |  | M4 | 0 |  |  | M4 | 0 |  |  | M4 | 0 |
|  |  |  | M4.1 | 0 |  |  | M4.1 | 0 |  |  | M4.1 | 0 |
| Presence of fecal contamination |  | 16 | M0 | 0 |  | 1 | M0 | 0 |  | 17 | M0 | 0 |
|  |  |  | M1 | 0 |  |  | M1 | 1 |  |  | M1 | 1 |
|  |  |  | M2 | 0 |  |  | M2 | 0 |  |  | M2 | 0 |
|  |  |  | M3 | 17 |  |  | M3 | 0 |  |  | M3 | 17 |
|  |  |  | M4 | 0 |  |  | M4 | 0 |  |  | M4 | 0 |
|  |  |  | M4.1 | 0 |  |  | M4.1 | 0 |  |  | M4.1 | 0 |
| Poor photograph quality |  | 4 | M0 | 0 |  | 6 | M0 | 0 |  | 10 | M0 | 0 |
|  |  |  | M1 | 2 |  |  | M1 | 0 |  |  | M1 | 2 |
|  |  |  | M2 | 0 |  |  | M2 | 3 |  |  | M2 | 3 |
|  |  |  | M3 | 2 |  |  | M3 | 1 |  |  | M3 | 3 |
|  |  |  | M4 | 0 |  |  | M4 | 2 |  |  | M4 | 2 |
|  |  |  | M4.1 | 0 |  |  | M4.1 | 0 |  |  | M4.1 | 0 |
| Other |  | 3 | M0 | 0 |  | 1 | M0 | 0 |  | 4 | M0 | 0 |
|  |  |  | M1 | 0 |  |  | M1 | 0 |  |  | M1 | 0 |
|  |  |  | M2 | 0 |  |  | M2 | 1 |  |  | M2 | 1 |
|  |  |  | M3 | 1 |  |  | M3 | 0 |  |  | M3 | 1 |
|  |  |  | M4 | 1 |  |  | M4 | 0 |  |  | M4 | 1 |
|  |  |  | M4.1 | 0 |  |  | M4.1 | 0 |  |  | M4.1 | 0 |

**CHECK! Table A4.** Overview of wound healing progress between day 0 and day 10 (WHP0-10) per farm and treatment group (percentages add up vertically per farm)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | WHP0-10 | Control group  N (%) | Treatment group  N (%) | Total  N (%) |
| Primary | N hind feet | 110 (100) | 102 (100) | 212 (100) |
| outcome | Improved | 23 (21) | 65 (64) | 88 (42) |
|  | Equal | 10 (9) | 27 (26) | 37 (17) |
|  | Worsened | 2 (2) | 2 (2) | 4 (2) |
|  | Unable to score | 75 (68) | 8 (8) | 83 (39) |
|  |  |  |  |  |
| Farm 1 | N hind feet | 14 (100) | 11 (100) | 25 (100) |
|  | Improved | 1 (7) | 3 (27) | 4 (16) |
|  | Equal or worsened | 1 (7) | 5 (46) | 6 (24) |
|  | Unable to score | 12 (86) | 3 (27) | 15 (60) |
| Farm 2 | N hind feet | 26 (100) | 24 (100) | 50 (100) |
|  | Improved | 1 (4) | 16 (67) | 17 (34) |
|  | Equal or worsened | 2 (8) | 8 (33) | 10 (20) |
|  | Unable to score | 23 (88) | 0 | 23 (46) |
| Farm 3 | N hind feet | 5 (100) | 7 (100) | 12 (100) |
|  | Improved | 0 | 5 (71) | 5 (42) |
|  | Equal or worsened | 0 | 2 (29) | 2 (16) |
|  | Unable to score | 5 (100) | 0 | 5 (42) |
| Farm 4 | N hind feet | 17 (100) | 15 (100) | 32 (100) |
|  | Improved | 2 (12) | 9 (60) | 11 (34) |
|  | Equal or worsened | 3 (18) | 4 (27) | 7 (22) |
|  | Unable to score | 12 (70) | 2 (13) | 14 (44) |
| Farm 5 | N hind feet | 16 (100) | 13 (100) | 29 (100) |
|  | Improved | 7 (44) | 10 (77) | 17 (59) |
|  | Equal and worsened | 4 (25) | 3 (23) | 7 (24) |
|  | Unable to score | 5 (31) | 0 | 5 (17) |
| Farm 6 | N hind feet | 19 (100) | 18 (100) | 37 (100) |
|  | Improved | 3 (16) | 16 (89) | 19 (51) |
|  | Equal and worsened | 1 (5) | 2 (11) | 3 (8) |
|  | Unable to score | 15 (79) | 0 | 15 (41) |
| Farm 7 | N hind feet | 13 (100) | 14 (100) | 27 (100) |
|  | Improved | 9 (69) | 6 (43) | 15 (56) |
|  | Equal and worsened | 1 (8) | 5 (36) | 6 (22) |
|  | Unable to score | 3 (23) | 3 (21) | 6 (22) |

**Table A5.** Associations between M-score clinical improvement and wound healing progress outcome variables and different explanatory variables using univariable logistic regression analysis with farm as random effect

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **M-score Clinical Improvement** | | **Wound Healing Progress** | | | |
|  |  | **Lesions unable to score classified as ‘not improved’** | | **Lesions unable to score classified as ‘improved’** | |
| **Variable** |  | **OR** | **95% CI** | **OR** | **95% CI** | **OR** | **95% CI** |
| Treatment group | Coppergel | 1 | referent | 1 | referent | 1 | referent |
|  | Alginogel | 0.02 | 0.01 – 0.05 | 7.65 | 4.05 – 15.13 | 0.31 | 0.14 – 0.63 |
| M-score on day 0 | M1 | 1 | referent | 1 | referent | 1 | referent |
|  | M2 | 0.88 | 0.43 – 1.77 | 1.31 | 0.63 – 2.79 | 1.88 | 0.83 – 4.09 |
|  | M4.1 | 2.42 | 0.72 – 9.89 | 1.63 | 0.51 – 5.21 | 2.27 | 0.61 – 10.97 |
| Period under bandage | 10 days | 1 | referent | 1 | referent | 1 | referent |
|  | 7 days | 40.29 | 15.48 – 138.45 | 0.19 | 0.09 – 0.36 | 1.61 | 0.80 – 3.37 |
| WHP0-3\* | Not improved |  |  | 1 | referent | 1 | referent |
|  | Improved |  |  | 4.06 | 2.00 – 8.57 | 5.03 | 2.43 – 10.56 |
| WHP3-7\* | Not improved |  |  | 1 | referent | 1 | referent |
|  | Improved |  |  | 12.50 | 5.23 – 34.94 | 5.96 | 2.81 – 12.82 |
| WHP7-10\* | Not improved |  |  | 1 | referent | 1 | referent |
|  | Improved |  |  | 45.12 | 16.98 – 157.14 | 34.27 | 14.33 – 89.31 |

\* Variables WHP0-3, WHP3-7, and WHP7-10 were not included in the M-score clinical improvement logistic regression analysis

**Table A.** Associations between M-score clinical improvement and wound healing progress outcome variables and different explanatory variables using univariable logistic regression analysis with farm as fixed effect

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **M-score Clinical Improvement** | | **Wound Healing Progress** | | | |
|  |  | **Lesions unable to score classified as ‘not improved’** | | **Lesions unable to score classified as ‘improved’** | |
| **Variable** |  | **OR** | **95% CI** | **OR** | **95% CI** | **OR** | **95% CI** |
| Treatment group | Coppergel | 1 | referent | 0.25 | referent | 8.08 | referent |
|  | Alginogel | 0.03 | 0.01 – 0.06 | 6.84 | 3.74 – 12.92 | 0.31 | 0.14 – 0.63 |
| M-score on day 0 | M1 | 1 | referent | 0.62 | referent | 2.50 | referent |
|  | M2 | 0.87 | 0.43 – 1.74 | 1.14 | 0.57 – 2.34 | 1.88 | 0.83 – 4.09 |
|  | M4.1 | 2.37 | 0.72 – 9.39 | 1.33 | 0.45 – 3.93 | 2.27 | 0.61 – 10.97 |
| Period under bandage | 10 days | 1 | referent | 1.20 | referent | 3.41 | referent |
|  | 7 days | 40.29 | 15.49 – 138.47 | 0.25 | 0.13 – 0.45 | 1.61 | 0.80 – 3.37 |
| WHP0-3 | Not improved |  |  | 0.51 | referent | 1.38 | referent |
|  | Improved |  |  | 4.17 | 2.08 – 8.71 | 5.03 | 2.43 – 10.56 |
| WHP3-7 | Not improved |  |  | 0.45 | referent | 1.20 | referent |
|  | Improved |  |  | 12.53 | 5.29 – 34.82 | 5.96 | 2.81 – 12.82 |
| WHP7-10 | Not improved |  |  | 0.29 | referent | 0.41 | referent |
|  | Improved |  |  | 45.12 | 16.98 – 157.12 | 34.27 | 14.33 – 89.12 |
| Farm | 1 | 1 | referent | 0.19 | referent | 0.19 | referent |
|  | 2 | 1.43 | 0.54 – 3.78 | 2.70 | 0.86 – 10.39 | 2.70 | 0.86 – 10.39 |
|  | 3 | 0.92 | 0.23 – 3.72 | 3.75 | 0.79 – 19.34 | 3.75 | 0.79 – 19.34 |
|  | 4 | 3.43 | 1.12 – 11.27 | 2.75 | 0.80 – 11.21 | 2.75 | 0.80 – 11.21 |
|  | 5 | 1.31 | 0.44 – 3.89 | 7.00 | 2.04 – 29.06 | 7.44 | 2.18 – 30.75 |
|  | 6 | 1.92 | 0.68 – 5.55 | 5.54 | 1.71 – 21.88 | 5.54 | 1.71 – 21.88 |
|  | 7 | 1.15 | 0.39 – 3.47 | 6.13 | 1.75 – 25.68 | 6.56 | 1.90 – 27.37 |

**Table A.** Associations between M-score clinical improvement and wound healing progress outcome variables and different explanatory variables using multivariable logistic regression analysis with backward elimination using Akaike information criterion and farm as fixed effect

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **M-score Clinical Improvement** | | **Wound Healing Progress** | | | |
|  |  | **Lesions unable to score classified as ‘not improved’** | | **Lesions unable to score classified as ‘improved’** | |
| **Variable** |  | **AOR** | **95% CI** | **AOR** | **95% CI** | **AOR** | **95% CI** |
| Intercept |  | 0.88 |  | 0.10 |  | 0.14 |  |
| Treatment group | Coppergel |  |  |  |  |  |  |
|  | Alginogel | 0.04 | 0.01 – 0.11 | 2.51 | 1.08 – 5.86 | 0.98 | 0.33 – 3.02 |
| M-score on day 0 | M1 |  |  |  |  |  |  |
|  | M2 | 3.10 | 0.79 – 15.87 |  |  |  |  |
|  | M4.1 | 16.57 | 2.30 – 148.36 |  |  |  |  |
| Period under bandage | 10 days |  |  |  |  |  |  |
|  | 7 days | 17.83 | 4.64 – 98.57 |  |  |  |  |
| Farm | 1 |  |  |  |  |  |  |
|  | 2 | 1.63 | 0.38 – 7.44 | 0.79 | 0.17 – 4.05 | 1.56 | 0.26 – 9.49 |
|  | 3 | 2.11 | 0.23 – 17.46 | 0.82 | 0.05 – 7.21 | 2.88 | 0.22 – 44.83 |
|  | 4 | 8.56 | 1.54 – 52.77 | 2.14 | 0.47 – 10.43 | 0.43 | 0.06 – 2.64 |
|  | 5 | 1.39 | 0.25 – 8.13 | 1.31 | 0.20 – 7.90 | 2.92 | 0.42 – 23.28 |
|  | 6 | 3.09 | 0.58 – 17.84 | 2.76 | 0.61 – 14.21 | 0.60 | 0.08 – 4.50 |
|  | 7 | 2.07 | 0.34 – 13.36 | 2.19 | 0.46 – 11.73 | 0.79 | 0.11 – 5.73 |
| WHP0-3\* | Not improved |  |  |  |  |  |  |
|  | Improved |  |  |  |  | 3.67 | 1.19 – 11.75 |
| WHP3-7\* | Not improved |  |  |  |  |  |  |
|  | Improved |  |  | 8.72 | 2.72 – 31.87 |  |  |
| WHP7-10\* | Not improved |  |  |  |  |  |  |
|  | Improved |  |  | 27.16 | 8.75 – 108.18 | 41.14 | 13.56 – 153.44 |

\* Variables WHP0-3, WHP3-7, and WHP7-10 were not included in the M-score clinical improvement model

**Table A.** Overview of wound healing progress between day 0 and day 10 (WHP0-10) per M-score on day 0 (D0) and treatment group

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  |  |  |  |
| M-score on D0 | WHP0-10 | Alginogel (N = 101)  N (%) | Coppergel (N = 109)  N (%) | Total  (N = 210)  N (%) |
| M1 | N hind feet | 21 (100) | 21 (100) | 42 (100) |
|  | Improved | 11 (52) | 5 (24) | 16 (38) |
|  | Equal | 6 (29) | 4 (19) | 10 (24) |
|  | Worsened | 1 (5) | 1 (5) | 2 (5) |
|  | Unable to score | 3 (14) | 11 (52) | 14(33) |
| M2 | N hind feet | 71 (100) | 77 (100) | 148 (100) |
|  | Improved | 46 (65) | 15 (19) | 61 (41) |
|  | Equal | 19 (27) | 5 (7) | 24 (16) |
|  | Worsened | 1 (1) | 1 (1) | 2 (2) |
|  | Unable to score | 5 (7) | 56 (73) | 61 (41) |
| M4.1 | N hind feet | 9 (100) | 11 (100) | 20 (100) |
|  | Improved | 7 (78) | 2 (18) | 9 (45) |
|  | Equal | 2 (22) | 1 (9) | 3 (15) |
|  | Worsened | 0 | 0 | 0 |
|  | Unable to score | 0 | 8 (73) | 8 (40) |

**Table.** M-score transition matrix of active digital dermatitis lesions between day 0 (D0) and day 21 (D21) following treatment with an enzyme alginogel (alginogel) or a copper and zinc chelates gel (coppergel); D0 M-scores were given in the trimming chute and D21 M-scores were given in the milking parlor; for each treatment group, percentages add up horizontally per M-score on D0

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Treatment Group | M-Score on D0 | M-Score on D21  N(%) | | | | | | |
|  |  | M0 | M1 | M2 | M3 | M4 | M4.1 | Total |
| Treatment | M1 | 3 (25) | 0 | 0 | 5 (42) | 4 (33) | 0 | 12 (100) |
|  | M2 | 7 (18) | 3 (8) | 12 (30) | 14 (36) | 3 (8) | 0 | 39 (100) |
|  | M4.1 | 1 (25) | 0 | 1 (25) | 1 (25) | 0 | 1 (25) | 4 (100) |
|  | total | 11 (20) | 3 (5) | 13 (24) | 20 (36) | 7 (13) | 1 (2) | 55 (100) |
| Control | M1 | 5 (45) | 0 | 1 (9) | 3 (29) | 2 (18) | 0 | 11 (100) |
|  | M2 | 7 (19) | 1 (3) | 7 (19) | 10 (28) | 9 (25) | 2 (6) | 36 (100) |
|  | M4.1 | 0 | 0 | 0 | 2 (100) | 0 | 0 | 2 (100) |
|  | total | 12 (24) | 1 (2) | 8 (16) | 15 (31) | 11 (22) | 2 (4) | 49 (100) |

**Table.** M-score transition matrix of active digital dermatitis lesions between day 0 (D0) and day 35 (D35) following treatment with an enzyme alginogel (alginogel) or a copper and zinc chelates gel (coppergel); D0 M-scores were given in the trimming chute and D35 M-scores were given in the milking parlor; for each treatment group, percentages add up horizontally per M-score on D0

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Treatment Group | M-Score on D0 | M-Score on D35  N(%) | | | | | | |
|  |  | M0 | M1 | M2 | M3 | M4 | M4.1 | Total |
| Treatment | M1 | 3 (30) | 0 | 1 (10) | 0 | 6 (60) | 0 | 10 (100) |
|  | M2 | 7 (20) | 0 | 11 (31) | 8 (23) | 8 23() | 1 (3) | 35 (100) |
|  | M4.1 | 0 | 0 | 1 (25) | 1 (25) | 1 (25) | 1 (25) | 4 (100) |
|  | total | 10 (20) | 0 | 13 (27) | 9 (18) | 15 (31) | 2 (4) | 49 (100) |
| Control | M1 | 4 (36) | 0 | 0 | 5 (46) | 1 (9) | 1 (9) | 11 (100) |
|  | M2 | 4 (11) | 2 (5) | 9 (23) | 11 (29) | 8 (21) | 4 (11) | 38 (100) |
|  | M4.1 | 0 | 0 | 0 | 2 (67) | 1 (33) | 0 | 3 (100) |
|  | total | 8 (15) | 2 (4) | 9 (17) | 18 (35) | 10 (19) | 5 (10) | 52 (100) |

**Table.** Overview of wound healing progress (WHP) on D10 per period under bandage and treatment group

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Control group | (N = 110) |  |  | Treatment group | (N = 102) |  |
| Period under bandage (N) | WHP | N |  | Period under bandage (N) | WHP | N |
| 7 days (79) | Improved | 17 |  | 7 days (13) | Improved | 5 |
|  | Equal | 7 |  |  | Equal | 5 |
|  | Worsened | 1 |  |  | Worsened | 1 |
|  | Unable to score | 54 |  |  | Unable to score | 2 |
| M4.1 (4) | Improved | 0 |  | M4.1 (5) | Improved | 4 |
|  | Equal | 1 |  |  | Equal | 1 |
|  | Worsened | 0 |  |  | Worsened | 0 |
|  | Unable to score | 3 |  |  | Unable to score | 0 |
| 10 days (27) | Improved | 6 |  | 10 days (84) | Improved | 56 |
|  | Equal | 2 |  |  | Equal | 21 |
|  | Worsened | 1 |  |  | Worsened | 1 |
|  | Unable to score | 18 |  |  | Unable to score | 6 |

**Output statistiek in R**