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Investigation on the effectiveness of a new hoof care product to sustainably reduce and prevent Digital Dermatitis in dairy cow herds

K. Grimm,* A. Fiedler, and C. Kröger,
Hoof Health Associates (Praxisgemeinschaft für Klauengesundheit) Dres. med. vet. Fiedler, Grimm & Kröger, Munich, Germany

ABSTRACT

The regular application of hoof care and cleaning products is an important part of protocols designed to reduce burden of disease caused by the ubiquitous and multifactorial hoof disease Digital Dermatitis (DD) in dairy cows. Commonly used hoof care products such as formalin or copper sulfate applied through foot baths or by spraying hindfeet are often irritant to the skin as well as harmful to the environment or human health while scientifically proven evidence of their efficacy is scarce. Thus, in a clinical controlled trial, we investigated if the use of a hoof care product based on a mix of iron complex salts, zinc salts and aluminum designed to reduce bacterial load on the skin and to support the natural skin barrier, was able to sustainably reduce disease severity and prevent new cases in 132 cows in 2 dairy herds (n_1 = 72, n_2 = 60) in Germany. From Dec 2021 to Dec 2022 only one predefined hind foot of every cow was washed and sprayed with the product twice a week (treatment group), the other hind foot was only washed (control group). Heifers joining the herd were sprayed for at least 4 weeks beforehand according to the same treatment and control regimen. During the trial, hooves were scored for DD lesions on a monthly basis using a disease severity score (A): from 0 = no lesion, up to 60 = ulcerative lesion ≥2.5 cm and categorically with 3 categories (B): 'none', 'non-active' and 'active'. Results A: Mean area under the curve of the numerical score that summarizes development over time was substantially and statistically significantly smaller in the treatment group. Results B: Two-step regression analysis for the outcome category at evaluation day (with exclusion of the first baseline evaluation) showed that during the trial, compared with the treatment group, odds of having a lesion rather than none was 4 times higher in the control group and the odds of having an active lesion compared with an inactive one were almost 6 times higher in the control group. Additionally, spraying had a statistically significant preventive effect for the feet of heifers (n = 17) introduced to the herd during the trial on farm 1: Only one active lesion occurred in the treatment group with numerous active lesions observed in the control group and mean area under the curve of the numerical score over time was statistically significantly lower in the treatment group, too. No active lesions occurred in heifers of farm 2 (n = 12) in either of the study groups. The iron, zinc and aluminum-based product effectively reduced disease prevalence and disease severity during the one-year study period in the examined dairy herds and data from heifers suggest that the application of the product to heifers 4 weeks before entering a herd with controlled DD management measures has high potential for prevention of the disease.

INTRODUCTION

Bovine digital dermatitis (DD) is a very widespread, polymicrobial disease affecting dairy herds worldwide (Cramer et al., 2008; Holzhauer et al., 2006; Knappe-Poindecker et al., 2013; Yang et al., 2019) with *Treponema spp.* being the major pathogen (Wilson-Welder et al., 2015). It infects the skin around the hooves of cattle causing open painful, ulcerative lesions that can result in severe lameness and has welfare and economic impacts (Dolecheck and Bewley, 2018). Once introduced in a herd, it seems almost impossible to eradicate the disease, even more improbable to eradicate the causative bacteria. To our knowledge, there is no scientific report of a herd being treated until elimination of either the pathogen or the disease. One important reason being that the digestive system acts as a reservoir: The same combination of pathogens that predominates in active DD wounds (*T. denticola*, *T. maltophilum*, *T. medium*, *T. putidum*, *T. phagedenis* and *T. paraluiscuniculi*) could be found in almost all fecal samples in a study by (Zinicola et al., 2015) in 3 different DD-affected herds.

Therefore, managing the disease on a farm through a combination of preventive measures that reduce bacterial load and increase skin health, thus minimizing the

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*Katharina Grimm: Am Seefeld 26, 83119 Obing, Germany, +49 170 185 3470, grimm.k@hotmail.com

The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-24. Nonstandard abbreviations are available in the Notes.

risk of reinfection, is the goal in common protocols like the 5-point-plan for control of DD developed by international experts in the field (Geldhof et al., 2017). Next to internal and external hygiene, early lesion detection and treatment as well as documentation of hoof diseases, regular hoof trimming and setting future hoof health goals, it includes regular hoof disinfection on herd-level. For this, different methods and substances are used depending on farm structure, national regulations as well as individual guidelines and experience. Copper sulfate or formalin are the most commonly used agents (Cook et al., 2012) and scientific evidence on their efficacy compared with an untreated control group is scarce (Jacobs et al., 2019). Furthermore both agents are irritants to the skin (ECHA Europe, 2024) and can have a strong impact on either human health or the environment which is why they are classified as hazardous substances by the Globally Harmonized System of Classification and Labeling of Chemicals (United Nations, 2021). Formalin is classified as carcinogenic for humans by the Word Health Organization (International Agency for Research on Cancer (IARC), 2012) and copper sulfate was assigned to the water hazard class 3 out of 3 (highly hazardous in water) by the German Federal Environment Agency ("Rigoletto: copper-sulphate," 2024) because of its high toxicity for aquatic organisms. The ratio of (environmental) health cost and benefits should therefore be evaluated properly before use. It should also be questioned, if disinfecting the critical region with highly irritant substances on the skin of the hooves is a sensible approach, considering first, the early recontamination caused by the soiled environment the product must work in. Second, it is applied to skin which already is constantly irritated through the more or less continuous contact with manure, depending on farm hygiene.

Thus, in this trial, we tested if the use of a hoof care product containing less harmful substances could accomplish a substantial reduction of the burden of disease of DD in dairy cows in a controlled clinical trial. Its mechanism of action exceeds the effect of reducing the bacterial load on the skin: The aim is to also increase the functionality of the natural skin barrier and the skin's own ability to heal, posing a promising approach to effective and sustainable prevention of DD in affected dairy herds.

The product is to control 3 properties of the environment at the lesion site: First, the pH and sebum: When the skin, being slightly acidic primarily due to organic acids formed in sebum, gets into contact with the manure, the alkalinity of the manure breaks down the sebum of the skin which leads to dry skin with less elasticity and decreased pathogenic resistance (Vanderwolf et al., 2023). The hoof care product contains mineral acids and has a pH-value of 2,5. When sprayed onto the skin, it quickly dries out and leaves an adhesive, acidic mineral layer on the skin aimed to increase the skin resistance against the alkaline hoof environment and subsequently decrease the risk of infection.

Second, the anaerobic conditions: To preserve an anaerobic environment, the obligate anaerobic *Treponema spp*. produce hydrogen sulfide (H_2S). H_2S breaks down oxygen in the local wound environment and thus, builds up anaerobic conditions (Lai and Chu, 2008). Additionally, bacteria in DD lesions form a biofilm (Espiritu et al., 2020). This covering the wound enhances the formation of an anaerobic environment essential for the bacteria while the lack of oxygen also impairs the immune response and wound healing process which can result in chronification of the wound (Sen, 2009). The examined hoof care product contains aluminum- and iron-based minerals that coagulate and dry out the biofilm and break down H_2S (Morrison et al., 2024).

Third, the product has astringent and blood coagulating properties: Aluminum based minerals have a strong astringent effect that decreases the skin permeability of pathogens and water which reduces the risk of infection (Baskar Murthy et al., 2024; Kayarkatte and Kharghoria, 2023). Furthermore, the aluminum compounds have a blood coagulant effect supporting wound healing (Tan et al., 2024).

MATERIALS AND METHODS

The hoof care product HooFoss (Vilofoss, Denmark), containing a mix of iron complex salts, zinc salts and aluminum was tested in a clinical controlled trial on 2 dairy farms in Germany for one year from December 2021 to December 2022. Farms were chosen for a minimum DD disease prevalence of 30% active lesions taken from recent hoof trimming records of regular hoof trimming and for maximum compliance of the farmer and staff.

ANIMALS

Farm 1 lies in Lower Saxony in the Northern part of Germany. 72 cows of which 2 were Simmental and 70 were Holstein-Friesian were housed in a free stall barn with slatted flooring and rubber mattress cubicles covered with straw bedding and limestone powder twice a week each. Animal to cubicle ratio was 1:1 and animal to feeding space ratio was 1.05:1. Farm 2 is situated in Bavaria in Southern Germany. 59 Simmental cows and one Brown Swiss cow were housed in a free stall barn with rubber slatted flooring and rubber mattress cubicles covered with a thin layer of straw bedding. Animal to cubicle ratio was 1:1 and animal to feeding space ratio was 1.2:1. For about 6 weeks before calving, cows and heifers were kept in the dry-off pen and then entered the herd after calving. Young stock was raised on farm 1 on

the same facility, and farm 2 had their young stock raised at one external farm where only their youngstock was housed. The number of animals in lactation 1, 2, 3, 4 and > 5 in the study were 24, 10, 14, 13 and 6, respectively at farm 1 and 23, 15, 19, 7 and 4, respectively, at farm 2. On farm 2, cows were grazed in the warmer months. During the trial this was from 27.05.-01.10.2022.

STUDY DESIGN

The trial started with a hoof trimming of the whole herd on both farms to establish the same initial situation for every animal. Every observed hoof lesion was documented, treated, and checked upon until healing or closing of the wound. In particular, DD lesions were treated with a 66% salicylic acid paste used under a bandage that was removed and reapplied, if necessary, after 3 to 5 d. After the treatment was finished about 10 d later, the actual trial phase started. Three times a week for one month, then twice a week, both hindfeet were washed first to provide the same baseline of soiling in both study groups and then only one randomly predefined side was sprayed with the product throughout the trial serving as treatment group. The feet of the side that was only washed served as the control group. Spraying only one hindfoot allowed to have a control group with the exact same specifics as the treatment group. No blinding of the study groups for participants in the trial was implemented due to practical feasibility. The product was applied to the hindfeet from behind during milking in the milking parlor by spraying with a battery-powered backpack sprayer. Farm staff were instructed by a manufacturer's staff member on how to spray properly including the duration of spraying, amount of product to be used on each foot and regions on which the product had to be applied to. To avoid dilution of the product, feet were washed at the beginning of the milking and spray was applied at the end of the milking time to provide the maximum amount of time drying.

Heifers and dry cows were sprayed while being fixed in the feeding fence according to the same regimen during the time they spent in the dry-off pen. This means, that every heifer that entered the trial herd 4 weeks after the initial trimming was sprayed for at least 4 weeks beforehand.

DOCUMENTATION OF THE DISEASE

DD evaluation was conducted while the cows were in the trimming chute during the regular hoof trimming, which took place 4 times on farm 1 and 3 times on farm 2 during the trial phase: once at the beginning, once at the end and once or twice equally distributed in between, respectively. In between the hoof trimming dates, approximately once a month, DD lesions were evaluated during milking in the milking parlor using a telescopic mirror and a torch to be able to look closely at the typical site of the lesions around the interdigital cleft. The timeline of conducted evaluations is represented in Figure 1 and Figure 2.

Evaluations were carried out by 2 observers, the same observer throughout the whole trial on each farm, respectively. In addition to their longstanding experience in scoring DD lesions in scientific trials as well as during practice, the 2 observers aligned their scoring traits by intensive discussion over scoring one herd in the parlor together to have equal standards at the beginning of the trial and again, to readjust, after 6 mo via photographs of different types of lesions.

M-STAGES

DD-lesions were scored based on the system by (Berry et al., 2012), defining so-called M-stages, going from normal skin (M0) over erosive (M1), active and ulcerative (M2) and healing (M3) lesions up to chronic (M4) and recurring chronic (M4.1) lesions (Table 1). If different kinds of lesions were present, the most severe lesion determined the score according to the following order: M2 > M4.1 > M4 > M1 > M3.

M-SCORES

Since size of the lesion is relevant for evaluating the progression of the disease, lesion size was documented

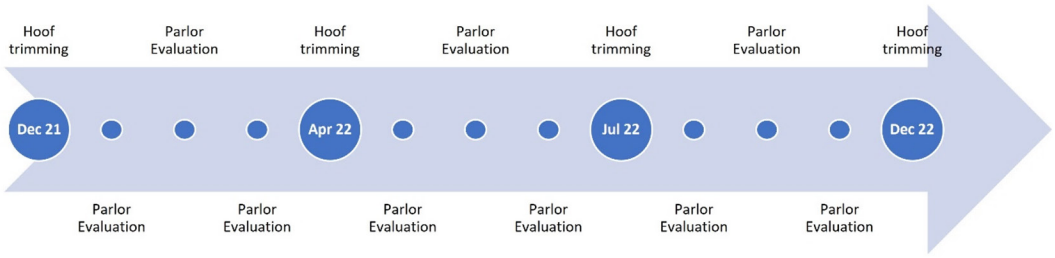

Figure 1. timeline of the trial on farm 1

Table 1. Documentation scheme for DD lesions: M-stages, assigned M-scores and classification[1]

classification	M-Stage, macroscopic pathology	description of lesion	M-Score for degrees 1, 2 and 3[2]
absent	M0 – no lesion	Normal skin. No signs of dermatitis.	0
non-active	M1 – erosive	Circumscribed, pink or gray surface, dry, matt appearance. Generally not painful.	1, 2, 3
active	M2 – ulcerative	Bright red surface, glossy, moist appearance. Can be painful.	30, 40, 60
non-active	M3 – healing	Dry, brown, scab-like surface. Not painful.	4, 5, 6
non-active	M4 – chronic	Surface is raised by tan, brown-black, irregular, thickened, proliferative or hyperkeratotic growths. Not painful.	15, 16, 17
active	M4.1 – recurring chronic	M4 with small, painful ulcerative part	20, 21, 22

[1]scheme by (Döpfer et al., 1997), amended by (Berry et al., 2012), adapted; classification according to (Solano et al., 2017b), adapted;.
[2]Score by (Döpfer, 1994) amended by (Fiedler et al., 2015): degree 1: < 1 cm, degree 2: ≥ 1 cm and <2.5 cm, degree 3: ≥ 2.5 cm.

by degrees 1, 2 and 3: < 1 cm across; ≥ 1 cm and <2,5 cm; ≥ 2,5 cm, respectively. If more than one lesion was present, diameters were added together. The M-stages at respective degrees were assigned an M-score that has originally been suggested by (Döpfer, 1994) and was amended by (Fiedler et al., 2015) to reflect disease severity numerically where smaller, less painful lesions are assigned lower values than bigger, more painful ones. Table 1 summarizes M-stages, respective M-scores and the classification as active, non-active or absent lesion which is a modification of the classification by (Solano et al., 2017b).

DATA ANALYSIS

All analyses described were performed with the software environment R (Version 4.3.3) after data were collected in Excel (Microsoft Corporation). Main data analysis was done only with data from those animals that were in the trial since the first evaluation. During preparatory data analysis a positive, statistically significant correlation between a high average M-Score and the probability of being culled or sold was found (results not shown). Because this would feign a positive effect on the disease as the high M-Score values would disappear later in the trial, only animals that were recorded at minimum 80% of the evaluations were included in the analysis. After this data cleaning, data of 100 animals, n = 54 for farm 1 and n = 46 for farm 2, were used in the analysis. Accordingly, this meant data of 200 feet being observed throughout the trial with 2288 observations in total. All animals that joined the herd later on were heifers. Since they did not go through the standard treatment protocol and all had different starting points, their development of the disease was analyzed similarly, but separately.

UNIVARIATE ANALYSIS: M-STAGES AND M-SCORES

The initial analysis compared the study groups at the start of the study. Therefore, means were compared in a Wilcoxon-Signed-Rank-Test. To facilitate the longitudinal analysis of the herd disease state M-scores, being on a numeric scale instead of the ordinal one of M-stages, were analyzed by calculating means at evaluation days and by their graphical representation. The Area Under the Curve (AUC) of the M-score of the respective legs was calculated to examine if there was a statistically significant difference between treatment and control group over the trial period using a Wilcoxon-signed-rank-test.

REGRESSION ANALYSIS

Observations of the first evaluation were excluded for the regression analysis because at the beginning of the

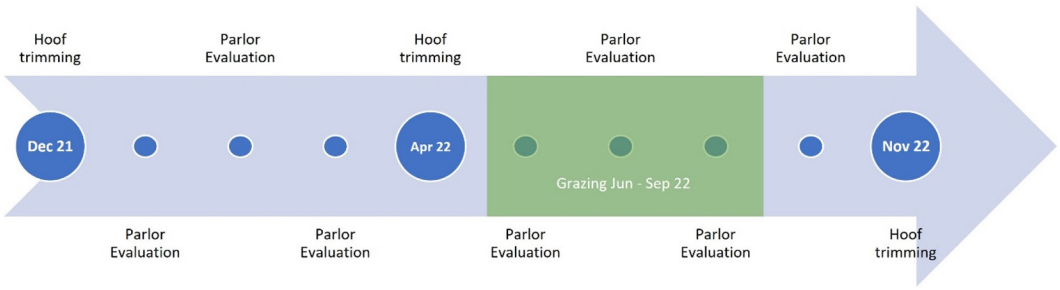

Figure 2. timeline of the trial on farm 2

trial, treatment had not started and therefore there was no effect of the spray on the disease state at this point. Two different outcome variables were used in the analysis: The M-score at each evaluation, as well as the AUC of the M-score over time of each foot.

Because of the skewed and clustered distribution of the M-score, its categorization of none, non-active and active lesion as described above was used to reduce model complexity and increase interpretability. Preliminary analysis showed that the assumption of proportional odds was not met and the use of several random effects was crucial to reflect the actual conditions, so (quasi-) Poisson or negative binomial regression were not suitable, especially with a view to interpretability. Nonetheless these methods were tested but did not result in an appropriate model fit to the data. Additionally, data were zero-inflated, this is why a 2-step logistic regression model was calculated for the day value. In step 1 a model (Model A) differentiating between cases without any lesions (none) and cases with non-active or active lesions was calculated. In step 2 the cases classified as non-active or active by model A were then classified by a new model (Model B) to differentiate between non-active and active lesions. In both steps the following fixed effects were applied: 1. side of the hind legs, thus study group, 2. evaluation type and 3. scaled M-score at first evaluation to account for different starting points of disease severity. Individual animal and farm were used as random effects in both regression models to account for similarities within observations of the same animal and of the same farm respectively. A proportional number of evaluation (ENP = evaluation number divided by total number of evaluations per farm) was calculated because of the difference in total number of evaluations between farms. It was also added to the model as a random effect. Backward step regression by excluding non-statistically significant effects and reducing the Bayesian information criterion (BIC) was performed to determine the relevant effects in the model and an ANOVA (ANOVA) of different models against the null model without random effects was conducted to examine the relevance of the individual random effects. Additionally, the intraclass correlation coefficient (ICC) for the random effects was calculated to estimate the size of the effect of the single random effects in the model. To determine model performance, a Receiver Operating Characteristic (ROC) curve analysis was performed of both models and AUC of the ROC curve as well as sensitivity and specificity at the best threshold determined by the Youden-Index (Youden, 1950) were calculated.

For an evaluation of the development of the disease over time for each foot, a linear regression analysis with random effects with the outcome AUC of the M-score over the trial period was applied. For this model additionally to backward step regression and ANOVA comparing with the null model and the ICC, marginal and conditional R-squared for generalized linear mixed models were calculated with the MuMIn package (Bartoń, 2023) to evaluate the size of the fixed effects in comparison to the random effects as well as the model fit to the data. The marginal R-squared represents the variance explained by the fixed effects and the conditional R-squared can be interpreted as variance explained by the entire model with both fixed and random effects.

RESULTS

Cows

Univariate analysis. Wilcoxon Signed-Rank-Test of the means in treatment and control group at evaluation 1 confirmed statistically that the mean M-scores were not statistically significantly different at the beginning. Whereas the treatment group's mean was slightly higher than the one of the control group on both farms (farm 1: control = 15.4 vs. treatment = 20.3, $P = 0.15$; farm 2: control = 13.3 vs. treatment = 14.7, $P = 0.70$).

Figure 3 represents the group means during the trial to represent the temporal development in the respective herds. On both farms the treatment group started out with a slightly higher mean M-score than the control group with 20.3 vs. 15.4 on farm 1 and 14.8 vs. 14.3 on farm 2, respectively. But throughout the trial phase the control group feet's mean went above and then constantly stayed higher than the one of the treatment group feet with an average difference of 6.81 (min = 3.98, max = 8.87) on farm 1 and 3.73 (min = 0.76, max = 5.42) on farm 2, respectively. The minimum distance between groups on farm 2 occurred during grazing time and right after that.

To evaluate the long-term effect of the treatment over the whole trial period numerically, the AUC of the M-score over time was analyzed for both farms. This could be done only with the data of animals that were observed at every evaluation. Thus n = 90 and n = 82 feet, meaning half the number of animals (farm 1: n = 45; farm 2: n = 41), were analyzed. Figure 4 shows a boxplot with an underlying violin plot representing the distribution of the AUC values in the 2 study groups on both farms. The mean AUC of the M-score was higher in the control group than in the treatment group on both farms (farm 1: 184.8 vs. 111.8; farm 2: 85.6 vs. 53.0), where absolute values as well as the difference were bigger on farm 1. A Wilcoxon-Signed-Rank-Test for paired values confirmed that the difference in AUC between study groups is statistically significant with p-values of 0.00 and 0.00 for farm 1 and farm 2, respectively.

Regression Analysis. After differences between the study groups were analyzed as described, a regression analysis was conducted to establish the size of the gen-

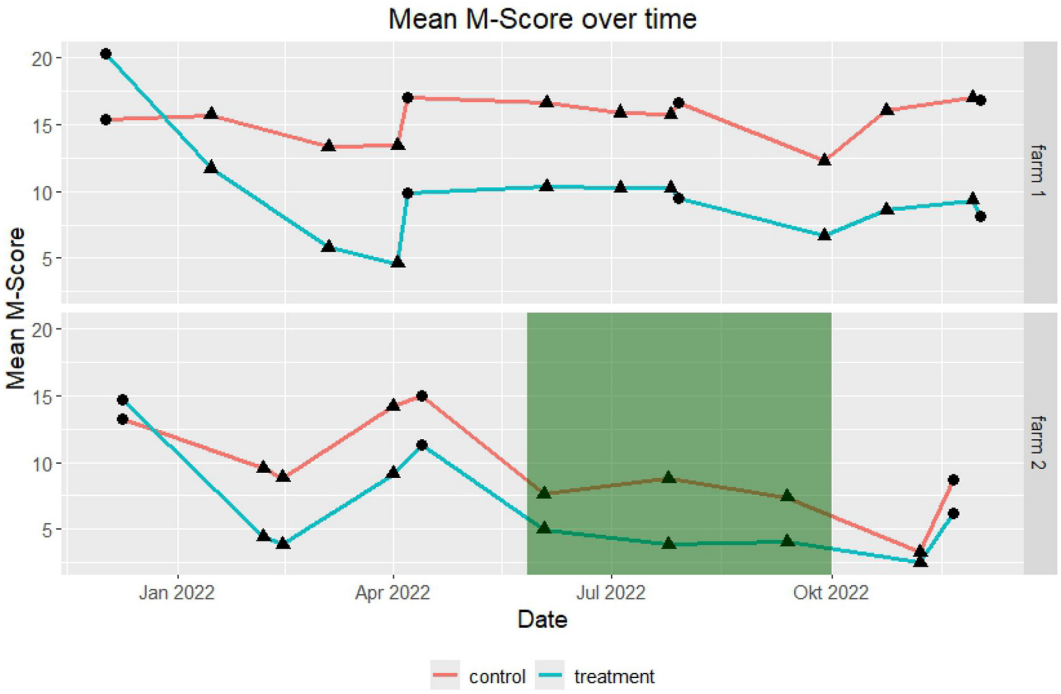

Figure 3. mean M-score over time on both farms, the green square represents grazing time on farm 2, points are hoof trimming evaluations, triangles are parlor evaluations

eral effect of the hoof care product on the disease in consideration of individual effects of M-score at starting point, evaluation number, farm, and animal.

Outcome: M-stage category at evaluation day. After backward step regression and ANOVA, the following models showed the best performance in predicting the M-stage category on an evaluation day:

M-stage category ~ study group + M-score at first evaluation + (1|ENP) + (1|animal)

Model A: none vs. non-active or active Model A predicted M-stage category none vs. any lesion with high accuracy: AUC of 0.90 (95% CI: 0.89–0.91), specificity of 0.83 (95% CI: 0.80–0.87) and sensitivity of 0.83 (95% CI: 0.79–0.86). According to the estimates of the model, the odds of having a lesion (compared with none) were 4 times (95%-CI = 3.13–5.26) higher for feet in the control group than for feet in the treatment group. An increase of 1 unit in the scaled M-score at first evaluation resulted in a probability of a foot of having a lesion rather than none by factor 1.32 (95%-CI = 1.09–1.60). Intraclass correlation coefficient of the random effects showed a high influence of the individual animal on the outcome with an ICC = 0.402 meaning that more than 40% of the variance in the data was explained by animal effect (Gelman and Hill, 2007). ENP with ICC = 0.17 explained almost a fifth of the variance in the data. In Table 2 these findings are summarized.

M-stage category ~ study group + evaluation type + (1|farm) + (1|ENP) + (1|animal)

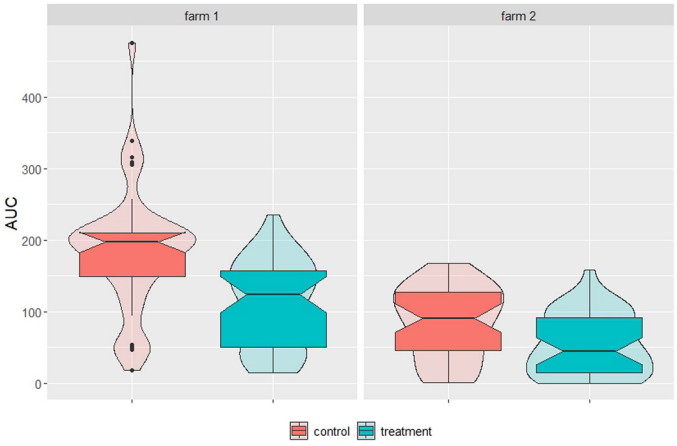

Figure 4. boxplot with underlying violin plot of Area under the Curve (AUC) for M-score of treatment and control group on both farms. The edges of the box correspond to 1st and 3rd quartile. Upper/lower whisker extends from the upper/lower hinge to the largest/smallest value no further than 1.5 * interquartile range (IQR) Dots beyond this line are outliers. The midline is at the median value. Notches extend 1.58 * IQR / sqrt(n) giving a roughly 95% confidence interval for comparing medians.

Table 2. model estimates, odds ratios and random effect variance and ICC of Model A

Fixed effects	estimate	95%-CI	Odds ratio (95%-CI)	p-value
study group (treatment)	−1.39	−1.65-(−1.14)	0.25 (0.19–0.32)	<0.001
scaled M-score at first evaluation	0.28	0.09–0.47	1.32 (1.09–1.60)	<0.01
Random effects	variance	ICC		
animal	3.10	0.40		
ENP	1.32	0.17		

Table 3. model estimates, odds ratios and random effect variance and ICC of Model B

Fixed effects	estimate	95%-CI	Odds ratio (95%-CI)	p-value
study group (treatment)	−1.78	−2.07-(−1.50)	0.17 (0.13–0.22)	<0.001
evaluation type (parlor)	−2.17	−3.35-(−1.02)	0.11 (0.04–0.36)	<0.001
Random effects	Variance	ICC		
animal	3.32	0.40		
ENP	0.90	0.11		
farm	0.74	0.09		

Model B: non-active vs. active Model B predicted M-stage category non-active vs. active under the conditions of Model A. Accuracy was slightly lower with wider confidence intervals: AUC of 0.87 (95% CI: 0.85–0.90), specificity of 0.77 (95% CI: 0.69–0.81) and sensitivity of 0.81 (95% CI: 0.86–0.93). Table 3 summarizes model parameters of the fixed and random effects. Under the conditions of model A, a foot in control group was almost 6 times (95%-CI = 4.55–7.69) more likely to have an active lesion over a non-active lesion compared with a foot in treatment group (OR_control is calculated by 1/OR_treatment). Evaluation type had an even greater effect on lesion status, albeit with less precision because of a wider CI. Again, under the conditions of Model A: Feet scored in the trimming chute were 9 times more likely to be documented with an active lesion rather than a non-active one compared with scoring in the parlor (OR_chute is calculated by 1/OR_parlor). Random effects were similar to Model A with a high explanation rate of animal effect (ICC = 0.40). However, ICC of ENP was lower (ICC = 0.11), but additionally, the factor farm explained almost a tenth of the variance in the data (ICC = 0.09). Table 4 summarizes the model parameters.

Outcome: AUC of M-score over the trial period Since the AUC of the M-score over time differs individually between animals on the 2 farms, these factors were used as random effects in a linear mixed model to evaluate the effect of study group on the M-score over time exclusively. The model was of the following form:

$$AUC_{M\text{-score}} \sim \text{study group} + \text{M-score at first evaluation} + (1|\text{animal}) + (1|\text{farm})$$

The parameter estimate for study group was −57.1 (95%-CI: −70.4 – −43.8) for treatment with a p-value <0.001 (Table 4). According to the model, this means that changing the study group from control to treatment lowered the AUC of the M-score by 57.1 points. The initial M-score had a positive influence on the outcome but with a substantially smaller absolute value of the estimate, thus smaller effect. The marginal and conditional R-squared of this generalized linear mixed model were 0.16 and 0.66, respectively. This means that the amount of explained variance of the random effects farm and animal was greater than the one of the fixed effects of study group and M-score at first evaluation. The conditional R-squared indicates a good model fit to the data.

Heifers

Univariate analysis In total 23 heifers joined the herd on farm 1 and 27 heifers were introduced on farm 2 after the first evaluation. For further analysis only the animals with at least 4 consecutive observations were included to ensure a minimum amount of time for the disease to develop and the test product to show possible effects. Thus, the number of animals analyzed was n = 29 (farm 1: n = 17, farm 2: n = 12).

As mentioned before, all heifers had been sprayed for at least 4 weeks in the dry-off pen before entering the

Table 4. model estimates and random effect variance and ICC of the linear regression model for the AUC

Fixed effects	estimate	95%-CI	p-value
Study group (treatment)	−57.1	−70.4-(−43.8)	<0.001
Scaled M-score at first evaluation	16.4	7.75–24.9	<0.001
Random effects	variance	ICC	
animal	916.8	0.16	
farm	2472.5	0.44	

CI = confidence interval, ICC = intraclass correlation coefficient.

trial. No data was collected before that. Therefore, only the disease state at observation 1, being the first parlor or hoof trimming evaluation after the day of introduction into the herd, is accessible for the heifers in contrast to the pre-evaluation data of the cows. If at this observation a treatment of DD was found to be necessary, it was administered right away according to the same protocol used at the first hoof trimming evaluation of the trial.

Wilcoxon Signed-Rank-Test of the means in treatment and control group at the first observation showed that the mean M-scores were not statistically significantly different at the point of entering the trial, whereas the treatment group's mean was slightly lower than the one of the control group on both farms (farm 1: control = 3.4 vs. treatment = 2.0, $P = 0.71$; farm 2: control = 2.6 vs. treatment = 1.3, $P = 0.37$).

Table 5 in the appendix represents the contingency table of feet being in control versus study group and having any lesion versus no lesion on each farm. At the first observation on farm 1 it was not more likely for a heifer's foot in the treatment group to have an active or non-active lesion compared with the control group: Odds ratio (Fisher's Exact Test) = 1.37 (95%-Confidence Interval (CI) = 0.62–3.04); $P = 0.43$. The same applies for an animal on farm 2: Odds ratio (Fisher's Exact Test) = 1.60 (95%-CI = 0.67–3.85; p = 0.29.

Figure 5 shows the occurrence of M-stages over the time spent in the herd, hence in the trial, for the heifers. For the first 4 observations the total number of animals was available because of the aforementioned data processing. Logically, the number of animals per observation decreases by the number of observations after that, since some animals were included in the trial later and were therefore part in only a few evaluations. It is noticeable that in the sprayed feet on farm 1 only once M2 occurred and M4.1 did not appear at all in contrast to the control group with a various number of cases. It is not known if further observations would have revealed a development of acute lesions in the treatment group because of lack of data but it is clear that at least it would only have occurred much later than in the control group. A similar effect can be observed on farm 2 but again it is much less prominent and no M2 lesions were detected in neither group.

Since the duration of a heifer being part of the trial is individually different, the maximum possible AUC for the M-score over time is also different. Therefore, to make a meaningful comparison inside the heifers' data, proportional AUC values were calculated individually with the actual AUC standardized by dividing it by the individually possible maximum AUC, meaning an M-Score of 60 at every evaluation over the time they had been in the trial.

Figure 6 shows a boxplot to represent this comparison graphically. Median proportional AUCs are higher in the control group than in the treatment group on both farms which means that over time M-score was generally higher in the control group's feet (farm 1: 0.15 vs. 0.01; farm 2: 0.03 vs. 0.00). The notches go outside the hinges of the boxes which is due to the small sample size and the resulting high confidence intervals. Still, on farm 1 the notches do not overlap, the medians are therefore most likely to be different. This is not the case on farm 2 where the variance of the M-score values was lower in both study groups and sample size is even smaller than on farm 1. Nonetheless, the positive effect the product has on the M-score is bigger on farm 1 but also seems to be present on farm 2. A Wilcoxon-Signed-Rank-Test confirmed the conclusions drawn from the boxplots with a p-value of 0.004 for farm 1 indicating a statistically significant difference and a p-value of 0.262 for farm 2 that does not provide evidence to reject the hypothesis of the means to be equal.

DISCUSSION

This clinical control trial investigated if the hoof care product HooFoss applied on hind feet of cows regularly could improve the disease state of DD on 2 German dairy farms over the period of one year. For this, only hind feet on one side were sprayed while the other side was not and therefore acted as the control group. Feet were scored for DD lesions approximately monthly in parlor and hoof trimming evaluations and statistical analysis was carried out with the data collected at these evaluations. A statistically significant effect of study group, thus spraying the feet, was found in the analysis. This effect was consistent over time and even greater when animals were sprayed before entering the herd.

STUDY DESIGN

Scoring of DD lesions Interrater agreement between the 2 observers on the 2 different farms was not examined statistically but both observers have been working

Table 5. contingency table of study group and lesion type at first observation of heifers on both farms

	lesion type	
Study group	absent	non-active or active
Farm 1		
control	14	3
treatment	13	4
Farm 2		
control	10	2
treatment	11	1

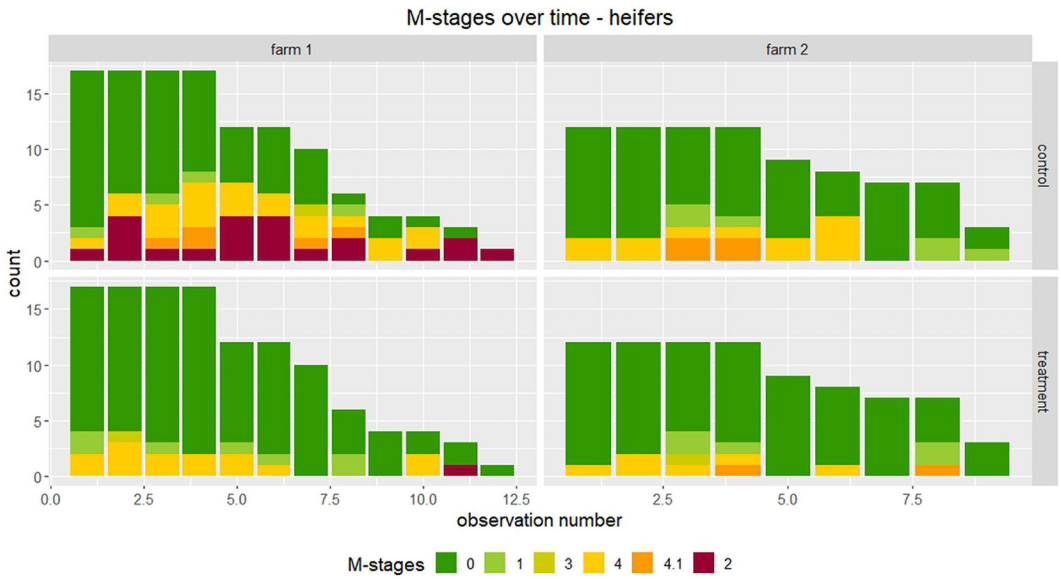

Figure 5. M-stages over time for heifers at their respective number of observation

together in the same field for over 10 years now and reevaluated their scoring methods regularly in joint evaluations of the same animals not only but especially before and again during the trial. Therefore, agreement can be expected to be very high. Nonetheless, differences between herds in M-score are most probably not only due to differences in disease state but also due to different observers. This fact has been taken into account by considering the factor "farm" during interpretation of the results.

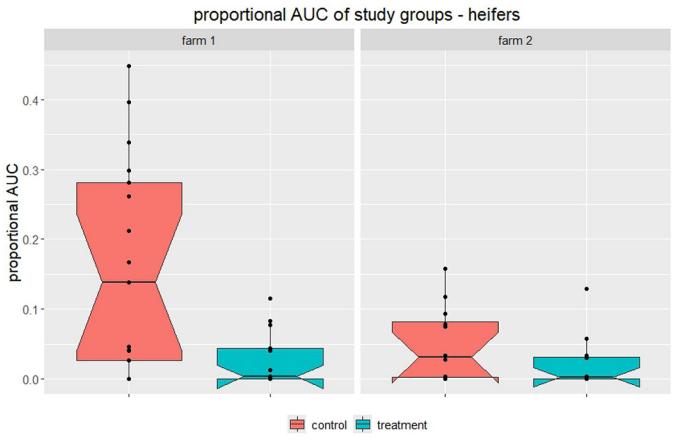

Figure 6. boxplot of proportional AUC of M-score over time for heifers on both farms. The edges of the box correspond to 1st and 3rd quartile. Upper/lower whisker extends from the upper/lower hinge to the largest/smallest value no further than 1.5 * interquartile range (IQR) Dots beyond this line are outliers. The midline is at the median value. Notches extend 1.58 * IQR / sqrt(n) giving a roughly 95% confidence interval for comparing medians. Points represent single data points.

In the scoring system by (Berry et al., 2012), DD stages M2, M3 and M4 are mostly defined by their macroscopic pathological evaluation describing an either ulcerative (M2), healing (M3) or proliferative/hyperkeratotic (M4) appearance of the lesion following the course of disease. But, since M1 and M2 are both described similarly apart from the word "ulcerative" and size, we concluded that for better delimitation, the underlying pathology could be better reflected by demarking between the 2 different stages of lesion progression: M1 defined as the erosive stage with damage only in the epithelial layers being the precursor of M2, the ulcerative stage, where the basal membrane is no longer intact and the bright red color of the dermis, strongly supplied with blood, becomes visible ("National Toxicology Program: Ulcer and Erosion," 2024; Wilson-Welder et al., 2015). Therefore, in this trial, we have used an assignment of lesions according to their apparent underlying pathological process: M0 – none, M1 - erosive, M2 - ulcerative, M3 - healing, M4 - chronic, M4.1 - reactivated chronic. This is especially important when considering M1 lesions, which in the scoring applied in this trial are consequently mostly less severe lesions than in the system it is based on where small ulcerative lesions are considered M1 instead of M2.

Evaluation types Lesions were scored during hoof trimming as well as in the milking parlor. It can be observed that scored disease severity was higher at evaluations during hoof trimming than at those conducted in the milking parlor when looking at the univariate graphs. This had been anticipated because of the much better view on the typical lesion site as well as into the interdigital cleft when the foot is lifted in the trimming chute

and the consequential possibility of more detailed observation. Nonetheless, having used only one of both evaluation types would have meant to either reduce detection accuracy when only examining in the milking parlor or to lose insight on the temporal development when only scoring at hoof trimming events (Solano et al., 2017a). To filter out this effect, the influence of the evaluation type on lesion score has been considered in both regression models. Interestingly, finding any or no lesion, as predicted by model A, was not influenced significantly by evaluation type as it was not statistically significantly relevant for model accuracy. This means that the probability of finding any or no lesion was similar in both the trimming chute and milking parlor under consideration of study group and the random effects animal and ENP. Whereas in model B, that separated between non-active and active lesions, evaluation type did have a rather great influence with active lesions being observed with much higher probability at hoof trimming evaluations than in the parlor. This suggests that DD-lesion scoring in the parlor is comparably as accurate as the gold standard of trimming chute evaluations when looking for any vs. no lesion. But that accuracy decreases in the parlor when it comes to the differentiation between lesion types active vs. non-active. This result matches the findings from (Solano et al., 2017a) as they have found that at milking parlor evaluation a higher scoring accuracy was achieved when only detection of a lesion was required compared with the differentiation between different lesion types.

Two farms The 2 study farms were very similar in respect to housing and feeding but the different breeds surely show different reactions to the same environmental changes or treatments in particular and both farms were situated in different regions resulting in different weather conditions. Also, farm 2 started out with fewer severe M-stages and a lower mean M-score. This could be one reason for the smaller effect of spraying compared with farm 1 because the potential for improvement was lower from the beginning. This effect was also observed in a study by (Solano et al., 2017b) where the use of copper sulfate was only effective in reducing active DD lesions in herds with a higher active DD-lesion prevalence at the beginning of the trial although it should be mentioned that this effect was not proven by a negative control. Additionally, farm 2 grazes the cows during summer which has a very large positive effect on hoof health (Hernandez-Mendo et al., 2007; Holzhauer et al., 2012) and most probably superimposed the treatment effect during this time as the course of the lines in Figure 3 already suggests.

Study groups

The introduction of 2 study groups within one cow at 2 different feet was a useful way to employ treatment and control group having the almost exact same environmental and individual conditions. Though it is a disadvantage only to spray one hindfoot instead of both because if the product is supposed to reduce disease severity and prevent the development of new lesions it would also reduce bacterial load and reinfection risk posed by open and chronic lesions. Therefore, spraying only one hindfoot could have impaired the preventive performance of the product on the herd-level. On the other hand, spraying one foot could have had a positive effect on the control group's feet, too: If spraying effectively reduces DD lesions on the sprayed feet, this would in turn lower the likelihood of infection in the whole herd and therefore in the control group's feet, too.

Effect of the spray on disease severity and preventive potential

At the beginning it was statistically proved that both study groups started out with the same initial situation on both farms. In the graph representing group means over time for farms 1 and 2 (Figure 3) both lines of the respective study groups follow the same pattern indicating that external and internal factors influencing the disease state have had the same impact on both treatment and control group underlining the functionality of the study design to show the effect of the spray exceeding placebo effect. The sudden decrease of mean M-score during the time grazing on farm 2 is again most probably due to the very positive effect of grazing on hoof health in general. The higher M-score of the control group at all evaluations apart from the first one though, even when grazing, can only be explained by the preventive effect of the use of the spray.

Subsequent regression analysis confirmed the assumption that spraying hind feet twice a week with the examined hoof care product substantially decreases the odds of having active DD lesions decreasing even the odds of having any lesion at all as in both steps of the model building, study group remained in the model as a statistically significant factor when predicting lesion type. The fact that random effects representing individual and farm effects had a considerable great influence on the outcome, again emphasizes that DD-management should always consist of a combination of hoof care and good management practice including smart breeding choices. The probability of having any lesion or not in Model A was influenced by the initial M-score while subsequent differentiation between active or non-active lesions was not. This matches with the findings from (Gomez et al., 2015) who have shown, that the risk of having a DD-lesion is significantly higher for cows that already had a case of DD during their rearing period compared with animals that did not. In this context, the results of the

examination of the heifers being sprayed before even entering the herd are even more interesting.

Heifers

Due to lower sample size, analysis was done mostly graphically and can only suggest what the true effect of the spray would be. Especially on farm 2 with a sample size of only n = 12 and smaller variability between study groups data can only be interpreted roughly. Additionally, effect of grazing is not the same for all individuals as the same number of observations does not account for the same evaluation dates since heifers started at different points in time. It cannot be stated that the use of the spray either had a statistically significant effect or none on the development of DD for the heifers on farm 2. Nonetheless, a positive tendency using the spray can be assumed.

In contrast, the data of farm 1 allow more robust conclusions. The bar chart of counts of lesion types shows a clear difference between control and treatment group with heifers' treatment group feet developing almost no M2 lesions and no reactivated M4 lesions at all during the time being observed. This is a sign of a very good preventive effect of the hoof care product on the rather naïve population coming into the herd of older cows with greater overall disease severity: Mean M-score was considerably lower at the initial evaluation for heifers than it was in cows (farm 1: 2.7 vs. 17.9; farm 2: 2.0 vs. 17.0). The difference of proportional AUCs between sprayed and non-sprayed feet was statistically significantly different on this farm. Additionally, the almost complete absence of M2 lesion development in the treatment group does imply that using the spray before DD even sets on could have a major impact on herd disease state in the long term.

Comparison with other hoof care products

This study evaluated the preventive effect of a hoof care product over a very long time span of one whole year compared with other studies with a maximum observation duration found being up to half a year. Therefore, long-term effect is examined to a greater extent. It started by treating all hooves medically individually which is not the common approach of studies evaluating foot bath or hoof spray efficacy as often the examined product is used as the first intervention rather than as a preventive measure although individual treatment was shown to speed healing of DD (Relun et al., 2012). Most studies in this field also do not have a negative control. The sometimes small sample sizes investigated reduce the statistical relevance even further (Thomsen, 2015). For these reasons, it was rather cumbersome to find comparable studies on hoof care products.

A recent systematic review and network meta-analysis on walk-through footbath protocols for prevention or treatment of DD though showed that only using 5% copper sulfate at least 4 times a week was better than no foot bath or water placebo (odds ratio: 4.74 (95%-CI = 1.24–21.95)) among other substances such as formalin, tensides, experimental H and experimental P, experimental T, acidified ionized copper, hypochlorite, glutaraldehyde, quaternary ammonium, organic acids and various brand products containing different combinations of these substances (Jacobs et al., 2019). Although in the particular study (Speijers et al., 2010) this was only true for differences in healing transitions between active, non-active and no lesions. In contrast to the present study, no statistically significant difference was found in the proportion of feet without any lesion between treatment groups and control. Looking at only controlled trials that evaluated effectiveness of treatment, next to the aforementioned study there were only 2 other studies proving a statistically significant odds ratio between treatment and control group: one using 0.6% acidified ionized copper with an odds ratio of 4.58 but in a rather small sample size of 24 cows and a resulting wide 95%-CI of 1.02–23.58 (Manske et al., 2002) and one using Hoof-Fit Bath (Relun et al., 2012) with odds ratios of 1.26 (95%-CI = 1.06–1.50) for prevention of active DD lesions and 3.19 (95%-CI = 1.57–7.00) for active lesions transitioning to non-active or absent lesions. Model A in this trial predicting no against any lesion found an odds ratio for treatment vs. control group of 4.00 (95%-CI = 3.13–5.26) and Model B under the conditions of Model A predicted an odds ratio for non-active against active lesions between treatment and control group of 5.88 (95%-CI = 4.55–7.69) over the whole year of the trial. This means that the examined product has a very high potential for preventing DD in dairy cows while at the same time reducing environmental impact and health hazards not only compared with many foot bath products that are commonly used so far.

So far, the product could only be compared with agents applied through a footbath or mat. Spray solutions as the one used in this trial are up to now rarely used compared with foot baths and there is few scientific research published on the prevention of DD on herd-level using a spray product. This study though was able to prove that spraying is a valid alternative. Additionally, spraying prevents potential contagion through foot baths used by a high number of cows as new product is used for every individual foot and less product is used per cow: the manufacturer reported that a volume of 100 mL per application per cow is needed twice a week whereas one footbath with about 500 l recommended volume accord-

ing to (Cook et al., 2012) for 200 cows results in 2.5 l per cow and application at for example 4 repetitions per week as stated above for 5% copper sulfate. This of course also depends on the concentration of the products used in the footbath.

CONCLUSIONS

The product analyzed in this controlled trial has shown a high efficacy in the prevention of DD in dairy cows. Using the product substantially reduced the amount not only of active lesions but even the numbers of non-active ones in the herd. This effect was shown over a comparatively long period of time of one whole year and therefore suggests a sustainable, long-term impact. This aspect was underlined by the very positive results of the heifers' population: The presented analyses emphasize how important the care of heifers is when dealing with DD on herd-level because of the very great potential that lies in the containment of the disease made possible using the spray already in young stock.

Notes

We would like to thank all family members of farm 1, farm 2 and all hoof trimmers that supported the study with their high-quality work, for their confidence in the trial, their reliability and their power of endurance. The animals in the study were kept in their usual environment and have always been housed, fed, and taken care of in accordance with the German animal protection law before during and after the trial. They were therefore never subject to any treatment or procedure requiring a report according to the German experimental animal law (Tierschutz-Versuchstierverordnung). This trial was funded by Vilofoss A/S, Ballesvej 2, DK-7000 Fredericia and Alfafarm, Strandvejen 195, DK-5500 Middelfart. Prior to the trial startup, it was agreed that the authors will have full autonomy in making decisions regarding study specifics and publications, without any obligation to adhere to instructions or opinions from Vilofoss and Alfafarm.

Digital Dermatitis, Prevention, hoof care spray, foot bath, herd health

Abbreviations: ANOVA = Analysis of Variance, AUC = Area under the curve, BIC = Bayesian Information Criterion, CI = Confidence interval, DD = Digital dermatitis, ENP = proportional evaluation number, ICC = Intraclass correlation coefficient, ROC = Receiver Operating Characteristics

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ORCIDS

K. Grimm, https://orcid.org/0000-0003-2854-1127
A. Fiedler, https://orcid.org/0009-0004-2735-4455
C. Kröger, https://orcid.org/0009-0003-2371-9384

APPENDIX