



## Evaluation of a biocide footbath solution in the occurrence and healing of digital dermatitis lesions in dairy cows: A clinical trial

Juan Manuel Ariza Chacon, Nathalie Bareille, Anne Lehebel, Kenny Oberle, Anne Relun, Raphaël Guatteo

### ► To cite this version:

Juan Manuel Ariza Chacon, Nathalie Bareille, Anne Lehebel, Kenny Oberle, Anne Relun, et al.. Evaluation of a biocide footbath solution in the occurrence and healing of digital dermatitis lesions in dairy cows: A clinical trial. Preventive Veterinary Medicine, 2019, 163, pp.58-67. 10.1016/j.prevetmed.2018.12.017 . hal-02064431

**HAL Id: hal-02064431**

**<https://hal.science/hal-02064431>**

Submitted on 19 Oct 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

**Evaluation of a biocide footbath solution in the occurrence and healing of digital dermatitis lesions in dairy cows. A clinical trial.**

J.M. Ariza<sup>1,2,a</sup>, N. Bareille<sup>1,a</sup>, A. Lehebel<sup>1,a</sup>, K. Oberle<sup>2,b</sup>, A. Relun<sup>1,a</sup>, and R. Guatteo<sup>1,a</sup>

<sup>1</sup> *BIOEPAR, INRA, Oniris, 44307, Université Bretagne Loire, Nantes, France.*

<sup>2</sup> *Qalian, Neovia group, Segré, 49500, France.*

<sup>a</sup> *Present address: BIOEPAR, ONIRIS, Atlanpole-Chantrerie*

*CS 40706, 44307 Nantes cedex 3, France.*

<sup>b</sup> *Present address: Qalian, Neovia group, 34 Rue Jean Monnet, 49500 Segré, France.*

Corresponding author: Raphaël Guatteo. Email: [raphael.guatteo@oniris-nantes.fr](mailto:raphael.guatteo@oniris-nantes.fr)

**ABSTRACT**

The objective of this study was to evaluate the effect of the implementation of different footbathing practices using a new biocide solution (Pink-Step™, Qalian, France) in the healing and the occurrence of bovine digital dermatitis (bDD) lesions. The investigation was conducted through a controlled within cow clinical trial in which the hind feet of cows from each farm were allocated either to the control group or to a moderate (MR) or (IR) intensive footbath-regimen groups. The trial involved 1036 cows (2072 feet) from 10 dairy farms located in western France where bDD was endemic. Split-footbaths were placed at the exit of the milking parlor of each farm, allowing the biocide to be administered to one side of the cows while using the other empty side as a negative control. The administration frequency for MR was of 2 days/week/1<sup>st</sup>-month, then 2 days/2 weeks/2<sup>nd</sup>-month, and then 2 days/month, and for IR was of 2 days/week/1<sup>st</sup>-2<sup>nd</sup> months, and then 2 days/2 weeks. Footbaths were administered during 140 days (approx.), and feet were evaluated for the at least once a month

in the milking parlor. Nested survival models were used to estimate the relative impact of the footbath regimens and other concomitant risk factors on the time that bDD lesions occurred or healed. No effect of Pink-Step™ was evidenced on the bDD occurrence during the trial. The risk for bDD occurrence was increased by poor feet cleanliness at both the cow (HR 1.69, CI 1.21–2.39) and farm level (HR 2.06, CI 1.44–2.94). Otherwise, the use of Pink-Step™ in an IR was effective in improving the healing of bDD lesions (HR 1.79, CI 1.12–2.88). The time to healing was also improved in inactive lesions (HR 2.19, CI 1.42–3.37). Conversely, the time to healing was delayed in feet receiving hoof-trimming (HR 0.41, CI 0.26–0.62), in cows with a contralateral bDD lesion (HR 0.32, CI 0.22–0.46) or in late lactation (HR 0.61, CI 0.43–0.85), and finally in farms with more than 100 cows (HR 0.48, CI 0.34–0.67). These findings reinforce the crucial role of hygiene in bDD dynamics and highlight the importance of implementing multiple control measures simultaneously, such as hygiene improvements in the barn, early detection and treatment of bDD lesions and the correct usage of individual and collective treatments. The implementation of Pink-Step™ represents a promising strategy for reducing the persistence of bDD lesions in affected herds.

**Key words:** Bovine digital dermatitis, biocide, footbaths, clinical trial, risk factors.

## INTRODUCTION

Bovine digital dermatitis (bDD) is currently among the principal cause of infectious lameness in dairy cows (Sogstad et al., 2005; Cramer et al., 2008; Refaai et al., 2013; Solano et al., 2016). bDD raises important economic, public-health and animal-welfare concerns. Indeed, bDD is associated with different challenges such as reduced farmers' incomes, increased use of antibiotics and, more importantly, lameness and thereby animal pain, impaired milk

51 production and reproduction (Relun et al. 2013; Ettema et al. 2010; Bruijnis et al. 2010). bDD  
52 can affect 96% of herds and between 7% to 30% of cows within a herd (Solano et al., 2016;  
53 Cramer et al., 2008). The disease is characterized by the occurrence of ulcerative lesions in  
54 the skin of the interdigital cleft which may persist or evolve to chronic forms (Read and  
55 Walker, 1998). Diseased cattle act as reservoirs and thereby as potential sources for outbreaks  
56 (Döpfer et al., 2012). Although the precise cause of bDD has not been completely elucidated,  
57 it is known that certain farming practices have a notable impact on the environment in which  
58 the disease develops (Somers et al., 2005). Therefore, bDD is considered to be a multifactorial  
59 disease consistently associated with unhygienic and wet conditions which mainly alter the  
60 integrity of feet skin. However, for clinical lesions to develop, the presence of specific  
61 *Treponema* species on feet suffering from cutaneous maceration is essential (Gomez et al.,  
62 2012). Control strategies consequently aim to limit exposure to factors which might impact  
63 the spread of bDD. In practice, the control of bDD frequently relies on the individual  
64 treatment of active lesions and on the collective administration of disinfectant solutions  
65 through footbaths for prophylactic and healing purposes. However, evidence supporting the  
66 effectiveness of collective solutions remains scarce, mainly due to the limitations posed by  
67 small samples and design weaknesses (Ariza et al., 2017).

68

69 The banning of antimicrobial use in footbaths is a priority to respond to a growing  
70 antimicrobial resistance threat at human and animal levels (Holzhauer et al., 2017; Hyde et  
71 al., 2017). Moreover, other common products used in footbaths, in some cases, represent an  
72 environmental risk, such as copper sulfate (Ippolito et al., 2010), or a harmful practice for  
73 farmers, such as formaldehyde, which has been recognized as carcinogenic (Cogliano et al.,  
74 2005). Several footbath solutions that claim to be effective and safe are currently available on  
75 the market without major scientific evidence supporting these claims (Ariza et al., 2017).

Furthermore, the currently available footbath solutions are not adapted to all farming conditions. Indeed, significant differences between farms, for example, in terms of hygiene, housing system, herd size or lameness prevalence, may have an important impact on the implementation and effectiveness of footbathing practices (Relun et al., 2012; Relun et al., 2013), and often this impact is ignored in controlled trials (Ariza et al., 2017). Additionally, the increasing bDD prevalence and the development of non-healing lesions are raising concerns that highly pathogenic or resistant strains are emerging (Evans et al., 2011). Therefore, new solutions for the collective disinfection of feet must consist of efficient and safe practices that can be easily adapted to the complex setting of each farm. Pink-step™ (Qalian, France) is a biocide that represents a potential alternative for bDD control. This is a safe and biodegradable disinfectant solution of confirmed bactericidal efficacy under soil conditions designed for the footbath administration (European Commission, 2016; European Chemicals Agency, 2017).

A clinical trial was developed to investigate the effectiveness of this new footbath biocide solution in delaying the occurrence of bDD lesions and in enhancing the healing of existent bDD lesions. In order to obtain reliable results, the main possible risk factors present at the cow and farm levels were concomitantly included in the trial analyses.

## **MATERIAL AND METHODS**

This investigation is reported following the recommendations of the CONSORT statement, extension to within-person trials (Pandis et al., 2017). All procedures were carried out under the agreement of the Ethics Veterinary Committee in Clinical Research and Epidemiology

from the Veterinary School of Nantes, France (CERVO, France) (registered number: CERVO-2016-12-V.)

### **Trial design**

The trial was designed to be a controlled within cow clinical trial in which the hind feet of cows from each farm were allocated either to the control group or to one of two footbath regimen groups using Pink-step™.

### **Study population**

The trial was conducted on 10 dairy farms in western France from October 2016 to June 2017. Farms were selected from a list provided by hoof-trimmers and veterinarians according to the trial protocol (Supplementary material S1). These farms were known to have experienced bDD during the preceding two-year period. However, none of the farms had administered footbaths during the two months preceding the trial. The herds were composed of Holstein cows to reduce the potential effects of breed on bDD. Cows were milked in a rotatory or conventional milking-parlor (location for bDD scoring). Additionally, to minimize possible imbalances between farms, after the pre-study visits, only farms with a herd prevalence  $\geq$  15% of active bDD lesions were included. Farmers milked on average 90 cows (range: 45–145) twice a day. Cows were mostly housed in cubicles (9 farms), and only one farm had no access to pasture during the spring and summer seasons.

### **Footbath regimen groups and concomitant treatments**

The footbathing procedure consisted in placing a footbath at the milking parlor exit and administering a disinfectant solution over a complete 5-month period. A split walk-through footbath was used to administer the disinfectant solution. The footbath consisted of 2 baths

separated by a grill which partially avoids contamination of the footbath by cow feces (Intra-Bath™, Intracare). Each bath measured 233 cm long × 32.5 cm wide × 19 cm high. The combined volume of the 2 sides of the footbath was 160 L. No pre-footbath foot wash was used in the study. The disinfectant solution administered, named Pink-step™, was a new biocide with recognized *in-vitro* efficacy (European Commission, 2016; European Chemicals Agency, 2017). Pink-step™ solution is composed of lactic acid (30%; w/w) and glycolic acid (10%; w/w) as active substances, anionic and non-ionic surfactants, and other excipients as a dye. The dose recommended by the manufacturer for footbathing was a 5% (v/v) solution in water. The split footbath made it possible to concurrently administer the Pink-step™ solution in one side of the footbath whilst the other side of the footbath remained empty and was used as a control. The feet of the lactating cows enrolled in the trial thus were allocated to three different groups, consisting of two different regimens of footbath administration frequencies and the empty bath (control group). According to the willingness of the farmers, farms were allocated to the moderate or intensive regimen. The Moderate Regimen (MR) was planned to resemble current field practices and consisted in footbath administration for 2 days (4 consecutive milkings) every week for the first month, then every two weeks for the second month, and then once a month until the end of the trial. The Intensive Regimen (IR) aimed to evaluate the possible advantages of increasing the frequency of footbathing over time, and consisted in footbath administration for 2 days every week for the first 2 months, and then every fortnight until the end of the trial. For both regimens studied, the biocide solution was expected to be renewed every 100 cow passages following the guidelines for the use of the solution.

Individual concomitant treatments were allowed during the trial for ethical and welfare reasons. Therefore, during the trial, farmers were expected to individually detect and treat all

ulcerative-active bDD lesions using 2 applications of oxytetracycline (30 mg/ml) (Oxytetrin™, MSD) 2 days apart, regardless of whether the cow foot treated was allocated to the control or treatment groups.

#### **Follow-up, data collection, and outcomes measures**

Farms were visited by 3 investigators trained through practical lessons before the trial start to fill out the questionnaires and conduct the overall feet scoring. Each visit followed 3 steps: (1) scoring the hind feet of all lactating cows for bDD and feet hygiene during milking, (2) checking compliance with the protocol, and (3) checking any changes in herd management practices. The investigators filled out a questionnaire which included: i) the covariates presented in Table 1, ii) items related to the protocol compliance including assessments of the quantities of product used by month, and iii) items related to management practices such as the implementation of individual treatments or trimming according to two additional sheets prepared for these events. Baseline records on the prevalence and other covariates of the participant farms were recorded during pre-study visits which took place before the start of the trial.

Digital dermatitis status was assessed during milking using a swiveling mirror based on the methodology described by Relun et al. (2011). The hind feet of all lactating cows were washed using tap water before the examination. The hind feet then were recorded using the M scoring system, modified from Döpfer et al. (1997) and Berry et al. (2012). In this system, the M0 stage corresponds to healthy feet without bDD lesions; M1 is considered as an early-stage ulcerative lesion (0–2 cm diameter); M2 represents painful ulcerative lesions with a diameter >2 cm; M3 is the healing stage with a lesion covered by a scab; M4 is the chronic stage characterized by dyskeratosis or surface proliferation; and M4.1 consists in a chronic lesion



with a small area of ulceration. In addition, lesion scores were gathered into 2 different categories, inactive lesions (M3 - 4) and active lesions (M1 - 2 - 4.1). Otherwise, feet dirtiness (tarsus (hock); metatarsus and digit (phalanges)) of the entire herd was assessed in the milking parlor prior to washing the feet for the bDD lesion diagnosis. The hind feet of each cow were scored using a 3-point nominal scale, varying from clean (score 1) to dirty (score 3), as described by Guatteo et al. (2013) (Figure 1). For each cow, the higher of the two foot scores (one for the left foot, the other for the right) was retained. The first scoring was performed immediately before the start of the administration of footbaths. Consecutive visits at intervals no longer than 30 days were made during the trial period. Additionally, for ethical and welfare concerns, farmers were informed about the overall prevalences of bDD lesions one week after the visit but without specifying which animals were affected. This was to avoid influencing the owners' perceptions of the study protocol and their decision-making process for individual treatment.

Two different outcome measures were recorded for each foot of the observed cows. A first outcome studied the healing effect and evaluated the healing of bDD lesions; the time in days to heal a bDD lesion was measured, counting from the first date of observation until the first date without any bDD lesion. The second outcome studied and evaluated the delay in the occurrence of bDD lesions, counting the time in days from the first observation of a foot without any bDD lesion until the first date of occurrence of a bDD lesion.

### **Sample size**

Sample sizes were calculated for both outcomes studied using the formula for sample size estimation in clinical trials with clustered survival times as the primary outcome (Xie and Waksman, 2003). The sample size implemented in the trial was based on the second outcome

because the detectable differences in the target effect between the treatment and control group were smaller (10%) compared with the healing outcome (20%). Therefore, a larger number of animals was needed to achieve statistical power for the occurrence outcome than the healing outcome. Due to the lack of previous data to account for the within cow correlation, a classical intra-cluster correlation coefficient (ICC) was set at 0.05 (Adams et al., 2004). The number of farms included was calculated based on the average French herd size of 60 lactating cows and on the occurrence rates (4 cases for 100 feet-months at risk) reported in a previous study following a similar approach (Relun et al., 2013). Therefore, with a type I error risk of 0.05 ( $\alpha=0.1$ ), at least 264 cows by each footbath regimen were necessary to guarantee 80% power ( $\beta=0.2$ ) to detect the target difference between control feet and footbath feet (10%). These calculations led to the recruitment of 10 average French farms (n= 60 lactating cows) for the inclusion of at least 528 cows (2 footbath regimen) and accounting for 15% of cows lost during the follow up of the trial.

#### **Treatment group allocation**

The side allocation (left or right) of the control group was balanced between the farms. Masked envelopes containing the side allocation were prepared and chosen randomly just before the first footbath administration. During the recruitment process and before any lesion scoring, half of the farms were allocated to the IR according to the farmers' willingness to spend more time administering the footbaths. The footbaths were administered by the farmers and therefore they were aware of the side containing the biocide product. Likewise, due to the pink color of the biocide substance, it might be possible that investigators were aware of the feet being treated during the trial.

#### **Data analysis**

All data were initially entered into a Microsoft Access database (Microsoft Corp., Redmond, WA). New covariates were built from the raw data using R (R Core Team, 2017). The effectiveness of both treatment regimens was evaluated on the clinical healing of bDD lesions and the reduced occurrence of bDD lesions through a survival analysis with a hind foot as the statistical unit. Survival analyses were carried out using the Frailtypack package in R (Rondeau et al., 2017). Nested survival models (Rondeau et al., 2012) were applied including a nested random effect for cows grouped in farms to adjust for clustering within observations, thereby feet in the models were considered as independent observations clustered at the farm level and subclustered at the cow level. Factors considered as potential effect modifiers of the healing and the occurrence of bDD lesions at the herd, cow and feet levels were included as covariates in the models (Table 1). The temporality between exposure and the outcomes studied was taken into account in the models, and when recurrent events were recorded time-dependent covariates were constructed. The survival analysis was planned in 3 steps: (i) treatment regimens and all covariates were tested in univariate analyses. Those covariates which contributed to the model at a 20% significance level were selected for multivariable analysis including the 2 x 2 interactions between these covariates (Dohoo et al., 2003). (ii) The proportional hazards assumption and the goodness-of-fit of the final model were checked by graphic procedures and the Schoenfeld residuals test (Schoenfeld, 1982). (iii) The multivariable models were checked for confounding for every covariate using backward stepwise selection with footbath regimen group forced into the model. Confounding was assumed to occur when the estimates changed by more than 20%.

For the purpose of analysis, when evaluating the healing outcome, and to ensure the true healed status of a lesion, only feet initially scored with an active or inactive lesion were considered to be healed in the models if in subsequent visits an M0 (“Healthy stage”) score

was noted on at least 2 consecutive visits. Likewise, for the outcome evaluating the occurrence of bDD lesions, only feet conserving the same M0 score during the 2 consecutive initial visits were included, ensuring the real absence of any lesion. The occurrence of a lesion was considered in the model if the included feet suffered any bDD lesion (active or inactive) during the trial period on 2 consecutive visits, to ensure the true lesion occurrence in the feet. For both outcomes, feet with visits spaced more than 45 days apart were removed from the analysis, on the fact that bDD lesions may be completely healed within 1 month (Holzhauer et al., 2008; Nielsen et al., 2012). Results of the models are presented as hazard ratios (HR) with their respective confidence intervals (CI), estimated for each covariate from the hazard function by taking the exponent of the estimates of effects. Therefore, the HR calculated for the occurrence outcome measures the instantaneous risk for a foot free of lesion to experience a bDD lesion being treated with one of the regimens versus being untreated. Meanwhile, the HR calculated for the healing outcome measures the instantaneous risk for a foot with a lesion to become healed being treated with one of the regimens versus being untreated. Finally, for the outcome evaluating the bDD occurrence, HR measures the instantaneous risk for the occurrence of a lesion in a foot being treated with one of the regimens versus being untreated.

## RESULTS

Farms were recruited between October and December 2016. For each farm, hind feet were inspected between 1 and 6 times at a median frequency of 30 days from January to June. Table 2 presents the main characteristics of the farms before the regimen side allocation. The baseline characteristics of the feet are summarized in Table 3. In total, 2,072 feet were allocated into one of the three groups, precisely, 394 in the MR group, 642 in the IR group,

and 1,036 in the control group (Figure 2). During the follow-up period, there were no deviations from the trial protocol or adverse effects reported or observed.

### **Effect on Occurrence**

At the start of the trial, 852 hind feet (41%) out of 2,072 hind feet (1,036 cows) were free of active bDD lesions during two consecutive visits. Then, 109 feet were excluded from the dataset because the interval between visits was longer than 45 days. Finally, 743 hind feet of 468 cows from 10 herds were included in the analysis (Figure 2, total initial feet without lesion). Only 275 cows shared the same bDD-free score in both hind feet at the start of the trial. Significant baseline differences were found between the feet allocation groups for cow feet hygiene, initial prevalence, proportion of heifers, herd size and farm feet hygiene covariates (Table 3).

Among all of the feet included in the analyses, 161 (21%) experienced a bDD lesion during the trial period. Inactive lesions (142) were more prone to occur than active lesions (19). The median time before the occurrence of a bDD lesion was 80 days (37 to 142 days), and the mean incidence rate was 6 cases for 100 feet-months at risk. After the analyses, only the poor feet cleanliness at the cow level and the farm level were significantly associated with a high risk of bDD occurrence in the multivariable analysis (Figure 3). No effect of the Pink-Step™ solution was evidenced on the bDD occurrence during the trial. None of the other covariates included in the multivariable model or their interactions were statistically significant in the multivariable model. Finally, the estimated variance of the cluster effect at the farm level was 0.0073 (CI 0.0010 - 0.0132, p value=0.0126), and at the cow level 3.65 (CI 2.29 – 5.00, p value<0.001).

## **Effect on Healing**

Initially, 1,107 hind feet (53%) out of 2,072 hind feet (1,036 cows) were affected with active or inactive bDD lesions. However, of these feet, 300 were excluded from the dataset because the interval between their visits was longer than 45 days. Therefore, 807 hind feet of 508 cows from 10 herds were included in the analysis (Figure 2, total initial feet with lesion). Among these cows, 299 had bDD lesions on both hind feet at the start of the trial. Significant baseline differences were found between the feet allocation groups for cow feet hygiene, preventive hoof-trimming, initial prevalence, proportion of heifers and herd size (Table 3).

Among all of the feet included in the analyses, 186 (23%) achieved the healing either of an active lesion (74) or an inactive lesion (112). The median time before the healing of a bDD lesion was 46 days (30 to 140 days), and on average the healing rate was 5% between two visits. According to the group allocation, the mean healing rates by month were 4%, 8% and 5% in the control, MR, and IR, respectively. From the multivariable analyses, six covariates were significantly associated with the risk of bDD healing (Figure 4). Thus, the time to healing was improved in inactive lesions and by the use of Pink-Step™ footbaths in IR. Otherwise, in feet trimmed during the trial period, the time to healing was reduced. Lesions in cows which either had a contralateral lesion or were at late lactation were identified to be at a higher risk of persisting. Moreover, in larger herds, the risk of lesion persistency was increased. A single interaction between the initial lesion aspect and the allocation footbath group was detected, indicating that feet with an active lesion in the IR group have a reduced time to healing compared to active lesions on feet allocated to the MR and control group. None of the others covariates included in the multivariable model or their interactions were

statistically significant in the multivariable model. Finally, the estimated variance of the cluster effect at the farm level was 0.0073 (CI 0.0010 - 0.0132, p value=0.012), and at the cow level 1.52 (CI 0.97 - 2.06, p value= 0.004).

## DISCUSSION

The findings of this investigation indicate that the collective disinfection of herd feet using Pink-step™ footbaths significantly improved the healing of bDD lesions when administered at an intensive frequency. The time to healing of bDD lesions was increased importantly in feet with active lesions, in trimmed feet, in cows in late lactation, in cows with contralateral lesions, and especially in larger herds. Otherwise, the occurrence of bDD lesions was mainly affected by feet cleanliness at the cow and farm level, and no effect on delaying the occurrence of the footbath solution (Pink-step™) was evidenced.

The originality of our investigation was to conduct a controlled clinical trial allocating the feet of the same cow into the control and the intervention groups. This approach allowed a drastic reduction (over threefold) in the trial's sample size, which is important in veterinary studies (Ariza et al., 2017). Furthermore, all of the feet shared the same risk whatever group they were allocated to (control or footbath), thereby reducing the farm effect. The findings of this investigation also enabled the estimation of the correlation between the feet of a same cow regarding bDD status, a parameter which has been largely suspected but not until now reported, to support the importance of developing and implementing prophylactic collective strategies. The trial was conducted on 10 farms in an effort to encompass the diversity of local herd management practices. Likewise, the multiple observations recorded over a long trial period were conceived to increase the precision of the measurements and to capture

differences in the farming environment over time (Ariza et al., 2017). Therefore, due to the major role of the farm factors and the dynamic nature of bDD, the trial was designed for a survival analysis, which enables one to adjust for covariates that change over time, such as feet cleanliness or lactation stage. Additionally, the nested survival model used for the analyses accounted for the heterogeneity caused by unmeasured covariates at the farm and cow level in the same model. In turn, due to the high prevalence of bDD lesions and the high frequency of observations planned, a scoring methodology which had no impact on daily farming practices had to be adopted even if it was less accurate than bDD scoring on restrained cows in a trimming chute ( $Se \geq 0.90$ ;  $Sp \geq 0.80$ ) (Relun et al., 2011). Nevertheless, the inter-digital space remains hard to approach using this methodology and therefore score misclassifications might lead to underestimating the hazards for all the covariates (Dohoo et al., 2003). Additionally, although the tree investigators received the same lesion scoring training, our trial protocol did not take into account the inter-observer agreement. However, as each farm was monitored entirely by a single investigator, the random farm effect might have reduced this bias effect. To reduce the risk of over or underestimation effects due to a misdiagnosis of “M1” or “M3” stages (Cramer et al., 2017), for the data analyses the “M5” stages were gathered into active, inactive and healthy stages. Consequently, the healing or the occurrence of bDD lesions was mainly determined by the presence or the absence of a healthy stage. In contrast with prior studies, and to avoid a potential overestimation of the footbath effect, the “M3” and “M4” stages were considered in the models as a diseased status. This original approach is one of the important features of the present trial. Especially when taking into consideration that the M4 stage might represent the most infective stage in which diseased animals spend more time (Biemans et al., 2017). Finally, the likelihood of obtaining beneficial effects by the implementation of a footbath might be enhanced by the usage of disinfectants tested in-vitro that mimic field soil conditions.



Few high-quality trials have reported footbath solutions to be effective (Thomsen, 2015). To our knowledge, the only peer-reviewed controlled trial that found good results was that of Solano et al., (2017), which used standardized footbaths of copper sulfate at 5% administered once a week. Another study also has reported successful results using copper sulfate at 5% for the healing of bDD lesions, however, the solution was administered by collective spraying (Relun et al., 2012). The scarce evidence related to footbaths may indicate the difficulties entailed in the design and evaluation of such clinical trials, or the small effect of footbaths in practice when farm conditions are far from ideal for their implementation. The present trial also reports a beneficial effect of footbaths using a safe and a biodegradable solution. Beneficial effects were only evidenced in the healing of bDD lesions. Beyond the bactericidal effect of footbaths on bDD lesions, a potential mechanism of Pink-step™ for improving skin healing may be the presumed dermal regenerative effects of the glycolic acids present in the biocide solution (Green et al., 2009). Otherwise, it is important to note that due to the design and duration of this trial, it was not possible to record lesion recurrence, a phenomenon already described in individual treatment trials which should be of interest when evaluating the long-term effectiveness of footbaths. Therefore, future studies must focus on the possible recurrence or recrudescence of bDD lesions and the effective healing of active lesions. The increased intensity in footbathing has previously been noted as beneficial by other studies (Holzhauer et al., 2012; Solano et al., 2017). These benefits were also evidenced in this study by the healing rates recorded during the first months and the healing efficacy evidenced by the IR group. To resemble field conditions, the administration frequencies implemented in the present trial changed over the time to mimic current in-field practices where the footbaths usage is reduced during the summer season. Therefore, additional studies are necessary to clarify the relation between the intensity of footbathing, the influence of seasons, and the effectiveness of such measures. Moreover, as noted in a previous investigation, future studies

must implement standardized footbath dimensions to ensure the optimal performance of the disinfectant products studied and to enable reliable and comparable results (Solano et al., 2017).

An association between footbathing practices and a reduced risk of bDD occurrence was not evidenced in this trial. The lack of effectiveness of both footbathing regimens to delay the occurrence of bDD lesions might be related to the weak effect of the footbath solution, which was probably below what was expected when the sample size necessary for the trial was calculated. Likewise, as the correlation between feet was unknown before this trial, the current sample size was probably inferior to that needed to evidence small effects on bDD occurrence. Additionally, in this trial all of the feet were cleaned for the scoring at each visit, and therefore every healthy foot periodically received what can be perceived as a prophylactic intervention. Consequently, the disinfectant efficacy of a solution over an already cleaned foot might be imperceptible, in other words, the effectiveness of disinfectants on delaying the occurrence of bDD lesions might be roughly equivalent to regular cleaning with water (Thomsen et al., 2012). Despite the evidenced lack of effectiveness on delaying the occurrence of bDD lesions, the time to healing was enhanced in inactive lesions compared to active lesions, and footbathing practices thus might act as a potential protective measure, controlling bDD reservoirs and the recrudescence of their lesions. One of the important limitations of this trial was related to the bias produced by the absence of investigator blinding during the follow-up. While this bias could be avoided by using a colored solution instead of an empty bath, the split-bath design implemented in this trial restrained this approach for practical reasons and for the risk of invert the solutions during their preparation. Although an objective methodology was implemented to score the lesions, investigators could not be blinded to the footbath allocation of feet due to the distinctive pink color of the solution

studied. Future clinical trials in bDD control should aim to blind the investigators to ensure an objective assessment of the lesions.

Otherwise, the split-body design of this trial might lead to some limitations. Although all of the feet had the same baseline probability of developing or healing a bDD lesion, significant differences between the allocation groups were detected after randomization. The imbalances between the baseline characteristics of the feet groups highlight the importance of considering confounding by adjusting for all potential effect modifiers in the data analysis. Another limitation related to the split-body design is the possible carry across effects within feet. On one hand, the pathogens in untreated feet might have remained undisturbed during the trial and thus may have increased the infection pressure in the environment. On the other hand, the disinfectant effect of footbaths might have reduced to an important degree the densities of environmental pathogens, enhancing as well the healing rates and decreasing the risk of lesion occurrence in untreated feet. In both scenarios, an under-estimation of the true effect of footbaths was expected. However, these methodological limitations inherent to this design might be compensated by the reduced sample size of the design. In this trial, the small random effect at the farm level reflected the homogeneous sample analyzed, being coherent with the strict inclusion parameters applied. Otherwise, the large random effect at the cow level suggests a greater correlation of the survival times for feet belonging to the same cow. This strong correlation was evidenced, for example, by the reduced risk of bDD healing in cows which have contralateral lesions. Future studies implementing the split-body design must consider the implications of this choice, such as establishing an appropriate data analysis and including a smaller number of farms or just one farm but with a large herd.

The individual treatment of active lesions was rare in the data set. Although their effectiveness is supported by scientific literature (Apley, 2015), in this trial the time to healing

was not improved by individual treatments. A possible explanation may lie in how the farmer's decision to treat was altered by the trial environment which involved a close follow-up by the veterinarians involved in the trial. Similarly, it may be possible that only the most severe cases of bDD capture the attention of farmers, and such lesions are frequently less responsive to treatment (Evans et al., 2011). Another explanation for the lack of efficacy of Oxytetracycline treatment involves an incorrect or incomplete implementation of the protocol (Sawant et al., 2005; Relun et al., 2013a). Likewise, during the trial, the feet were trimmed mainly for therapeutic reasons instead of prophylactic reasons. Therefore, the healing benefits of trimming might be missed in those severe cases which persisted longer than the trial period. Similarly, the presumed preventive influence of trimming was not evidenced in the trial, probably because the impact of dirty conditions was more important than that of the implementation of prophylactic measures on the farms studied.

As has been consistently noted in other epidemiological studies (Relun et al., 2013b), poor feet hygiene was confirmed as the most important factor influencing bDD lesion occurrence at both the cow and farm levels. However, although previous studies have suggested that poor feet hygiene might delay the healing of bDD lesions (Relun et al., 2012), we were unable to identify a relation between feet cleanliness and time to bDD healing. Experimental studies have confirmed that dirty and wet environmental conditions are the main determinants for the occurrence of bDD lesions (Gomez et al., 2012). Similarly, field studies have identified different factors which may alter environmental hygiene and thereby increase the risk of bDD, such as housing in cubicles, grooved concrete floors, and reduced manure scraping rates (Somers et al., 2005; Barker et al., 2009; Oliveira et al., 2017). In practice, feet hygiene is a measure of the impact of several factors that condition the farming environment in which cows stand. Therefore, as the bDD lesions and the treatments implemented were centered at

the foot level, focusing cleanliness observations on the foot rather than the leg might improve the precision concerning the association of the different factors which may affect feet hygiene and bDD lesions (Guatteo et al., 2013). Otherwise, other studies have reported an important seasonal effect on the risk of bDD (Argaez-Rodriguez et al., 1997) and other feet disorders (Murray et al., 1996). Although this factor is mostly related to a limited access to pasture, this association was not evidenced in the present study in relation to grazing practices or their impact on feet cleanliness and bDD healing or occurrence.

Another limitation of this trial is related to the potential selection bias on the allocation process due that the footbath frequency regimen (MR or IR) was assigned base on the willingness of the farmers. Farmers who accepted to participate only if included in the IR were likely more conscientious of the disease problem than those opting for the MR and the involvement of the farmers on the trial could therefore be different. Beyond the potential impact of different farm characteristics and farm management factors recorded during the trial, the heterogeneity within cows indicates that other factors affecting the bDD dynamics differ between cows and their feet. Further investigation at the cow level therefore might enhance current understanding of bDD, highlighting, for example, the role of skin microbiota or the immune response in the disease outcome.

## CONCLUSION

This investigation confirmed that multiple factors interact in the dynamics of bDD lesions determining their occurrence and persistence. Strategies to control the bDD therefore must rely on the simultaneous implementation of multiple measures to improve the environment of feet, and to reduce the severity of lesions and the presence of infected cows. The results of this study revealed the utility of footbathing practices for improving the time to healing of

bDD lesions when the Pink-step™ solution was administered at an intensive frequency. Finally, to limit bDD lesion occurrence, trial findings confirmed the crucial importance of implementing efficient measures to improve feet hygiene.

## ACKNOWLEDGMENTS

The authors acknowledge the farmers and veterinarians who have contributed to this study. Additionally, we thank G. Puel (Oniris, Nantes, France) and Y. Quenet (INRA, Nantes, France) for helpful participation as investigators, for their time and motivation.

**Funding:** This study was supported as part of a Ph.D. studentship by Qalian (Neovia group, Segré, France) and the “Association Nationale de la Recherche et de la Technologie” (ANRT, Paris, France).

## REFERENCES

- Adams G, Gulliford MC, Ukoumunne OC, Eldridge S, Chinn S and Campbell MJ 2004. Patterns of intra-cluster correlation from primary care research to inform study design and analysis. *Journal of Clinical Epidemiology* 57, 785–794.
- Apley MD 2015. Clinical Evidence for Individual Animal Therapy for Papillomatous Digital Dermatitis (Hairy Heel Wart) and Infectious Bovine Pododermatitis (Foot Rot). *Veterinary Clinics of North America: Food Animal Practice* 31, 81–95.
- Argaez-Rodriguez FJ, Hird DW, Hernandez de Anda J, Read DH and Rodriguez-Lainz A 1997. Papillomatous digital dermatitis on a commercial dairy farm in Mexicali, Mexico: incidence and effect on reproduction and milk production. *Preventive Veterinary Medicine* 32, 275–286.
- Ariza JM, Relun A, Bareille N, Oberle K and Guatteo R 2017. Effectiveness of collective treatments in the prevention and treatment of bovine digital dermatitis lesions: A systematic review. *Journal of Dairy Science* 100, 7401–7418.
- Barker ZE, Amory JR, Wright JL, Mason S a, Blowey RW and Green LE 2009. Risk factors for increased rates of sole ulcers, white line disease, and digital dermatitis in dairy cattle from twenty-seven farms in England and Wales. *Journal of dairy science* 92, 1971–1978.
- Biemans F, Bijma P, Boots NM and de Jong MCM 2017. Digital Dermatitis in dairy cattle:

The contribution of different disease classes to transmission. *Epidemics*.

Bruijnis MRN, Hogeveen H and Stassen EN 2010. Assessing economic consequences of foot disorders in dairy cattle using a dynamic stochastic simulation model. *Journal of dairy science* 93, 2419–2432.

Cogliano VJ, Grosse Y, Baan RA, Straif K, Secretan MB, El Ghissassi F, Andrae U, Burge S, Chhabra R, Cocker J, Coggon D, Conolly R, Demers P, Eastmond D, Faustman E, Feron V, Gérin M, Goldberg M, Goldstein B, Grafström R, Hansen J, Hauptmann M, Hughes K, Junghans T, Krewski D, Olin S, Reynier M, Shaham J, Soffritti M, Stayner L, Stewart P and Wolf D 2005. Meeting report: Summary of IARC Monographs on formaldehyde, 2-butoxyethanol, and 1-tert-butoxy-2-propanol. *Environmental Health Perspectives* 113, 1205–1208.

Cramer G, Lissemore KD, Guard CL, Leslie KE and Kelton DF 2008. Herd- and cow-level prevalence of foot lesions in Ontario dairy cattle. *Journal of dairy science* 91, 3888–3895.

Cramer G, Winders T, Solano L and Kleinschmit DH 2017. Evaluation of agreement among digital dermatitis scoring methods in the milking parlor, pen, and hoof trimming chute. *Journal of dairy science*, 1–9.

Dohoo IR, Martin W and Stryhn HE 2003. *Veterinary Epidemiologic Research*. University of Prince Edward Island, Charlottetown, P.E.I.

Döpfer D, Holzhauser M and Boven M Van 2012. The dynamics of digital dermatitis in populations of dairy cattle: Model-based estimates of transition rates and implications for control. *Veterinary Journal* 193, 648–653.

Ettema J, Østergaard S and Kristensen AR 2010. Modelling the economic impact of three lameness causing diseases using herd and cow level evidence. *Preventive Veterinary Medicine* 95, 64–73.

European Chemicals Agency 2017. *Guidance on the Biocidal Products Regulation: Volume II Efficacy - Assessment and Evaluation (Parts B+C)*. European Chemicals Agency, Helsinki, FI.

European Commission 2016. *EU dairy farms report 2013 based on FADN data*.

Evans NJ, Blowey RW, Timofte D, Isherwood DR, Brown JM, Murray R, Paton RJ and Carter SD 2011. Association between bovine digital dermatitis treponemes and a range of ‘non-healing’ bovine hoof disorders. *The Veterinary record* 168, 214.

Gomez A, Cook NB, Bernardoni ND, Rieman J, Dusick a F, Hartshorn R, Socha MT, Read DH and Döpfer D 2012. An experimental infection model to induce digital dermatitis infection in cattle. *Journal of dairy science* 95, 1821–30.

Green BA, Yu RJ and Van Scott EJ 2009. Clinical and cosmeceutical uses of hydroxyacids. Clinics in Dermatology 27, 495–501.

Guatteo R, Arnoult A, Ménard J-L and Bareille N 2013. Elaboration of a scoring grid to assess feet cleanliness in dairy cattle and identification of risk factors in the winter period. In Rencontres Recherches Ruminants. Vol 20., pp. 379–382.

Holzhauer M, Bartels CJ, Bergsten C, van Riet MMJ, Frankena K and Lam TJGM 2012. The effect of an acidified, ionized copper sulphate solution on digital dermatitis in dairy cows. Veterinary Journal 193, 659–663.

Holzhauer M, Bartels CJM, Döpfer D and van Schaik G 2008. Clinical course of digital dermatitis lesions in an endemically infected herd without preventive herd strategies. The Veterinary Journal 177, 222–230.

Holzhauer M, Ploegmakers-Van Deventer R, Smits D and Swart W 2017. Comparing the curative efficacy of topical treatment with thiamphenicol and oxytetracycline on digital dermatitis lesions in dairy cattle. Veterinary Record 180, 500.

Hyde RM, Remnant JG, Bradley AJ, Breen JE, Hudson CD, Davies PL, Clarke T, Critchell Y, Hylands M, Linton E, Wood E and Green MJ 2017. Quantitative analysis of antimicrobial use on British dairy farms. Veterinary Record, vetrec-2017-104614.

Ippolito JA, Ducey T and Tarkalson D 2010. Copper impacts on corn, soil extractability, and the soil bacterial community. Soil Science 175, 586–592.

Murray RD, Downham DY, Clarkson MJ, Faull WB, Hughes JW, Manson FJ, Merritt JB, Russell WB, Sutherst JE and Ward WR 1996. Epidemiology of lameness in dairy cattle: Description and analysis of foot lesions. VETERINARY RECORD 138, 586–591.

Nielsen BH, Thomsen PT, Green LE and Kaler J 2012. A study of the dynamics of digital dermatitis in 742 lactating dairy cows. Preventive Veterinary Medicine 104, 44–52.

Oliveira VHS, Sørensen JT and Thomsen PT 2017. Associations between biosecurity practices and bovine digital dermatitis in Danish dairy herds. Journal of Dairy Science 100, 8398–8408.

Pandis N, Chung B, Scherer RW, Elbourne D and Altman DG 2017. CONSORT 2010 statement: extension checklist for reporting within person randomised trials. Bmj 2835, j2835.

Read DH and Walker RL 1998. Papillomatous digital dermatitis (footwarts) in California dairy cattle: clinical and gross pathologic findings. Journal of veterinary diagnostic investigation: official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc 10, 67–76.

Refaai W, Van Aert M, Abd El-Aal a. M, Behery a. E and Opsomer G 2013. Infectious



diseases causing lameness in cattle with a main emphasis on digital dermatitis (Mortellaro disease). *Livestock Science* 156, 53–63.

Relun, Guatteo, Auzanneau and Bareille 2013a. Farmers' practices, motivators and barriers for adoption of treatments of digital dermatitis in dairy farms. *animal* 7, 1542–1550.

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

Relun A, Lehebel A, Bareille N and Guatteo R 2012. Effectiveness of different regimens of a collective topical treatment using a solution of copper and zinc chelates in the cure of digital dermatitis in dairy farms under field conditions. *Journal of dairy science* 95, 3722–35.

Relun, Lehebel, Bruggink, Bareille and Guatteo 2013b. Estimation of the relative impact of treatment and herd management practices on prevention of digital dermatitis in French dairy herds. *Preventive veterinary medicine* 110, 558–62.

Relun a, Lehebel a, Chesnin a, Guatteo R and Bareille N 2013c. Association between digital dermatitis lesions and test-day milk yield of Holstein cows from 41 French dairy farms. *Journal of dairy science* 96, 2190–200.

Rondeau V, Gonzalez JR and Mazroui Y 2017. R frailtypack: general frailty models: shared, joint and nested frailty models with prediction.

Rondeau V, Mazroui Y and Gonzalez JR 2012. frailtypack: An R Package for the Analysis of Correlated Survival Data with Frailty Models Using Penalized Likelihood Estimation or Parametrical Estimation. *Journal of Statistical Software* 47, 1–28.

Sawant AA, Sordillo LM and Jayarao BM 2005. A Survey on Antibiotic Usage in Dairy Herds in Pennsylvania. *Journal of Dairy Science* 88, 2991–2999.

Schoenfeld D 1982. Partial Residuals for The Proportionnal Hazards Regression Model. *Biometrika* 69, 239–241.

Sogstad Å M, Fjeldaas T, Østerås O and Forshell KP 2005. Prevalence of claw lesions in Norwegian dairy cattle housed in tie stalls and free stalls. *Preventive Veterinary Medicine* 70, 191–209.

Solano L, Barkema HW, Mason S, Pajor EA, LeBlanc SJ and Orsel K 2016. Prevalence and distribution of foot lesions in dairy cattle in Alberta, Canada. *Journal of Dairy Science* 99, 6828–6841.

Solano L, Barkema HW, Pickel C and Orsel K 2017. Effectiveness of a standardized footbath protocol for prevention of digital dermatitis. *Journal of Dairy Science* 100, 1295–1307.

Somers JG CJ, Frankena K, Noordhuizen-Stassen EN and Metz JHM 2005. Risk factors for digital dermatitis in dairy cows kept in cubicle houses in The Netherlands. *Preventive*




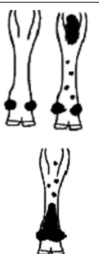


Veterinary Medicine 71, 11–21.

Thomsen PT 2015. Short communication: Efficacy of copper sulfate hoof baths against digital dermatitis—Where is the evidence? *Journal of Dairy Science* 98, 2539–2544.

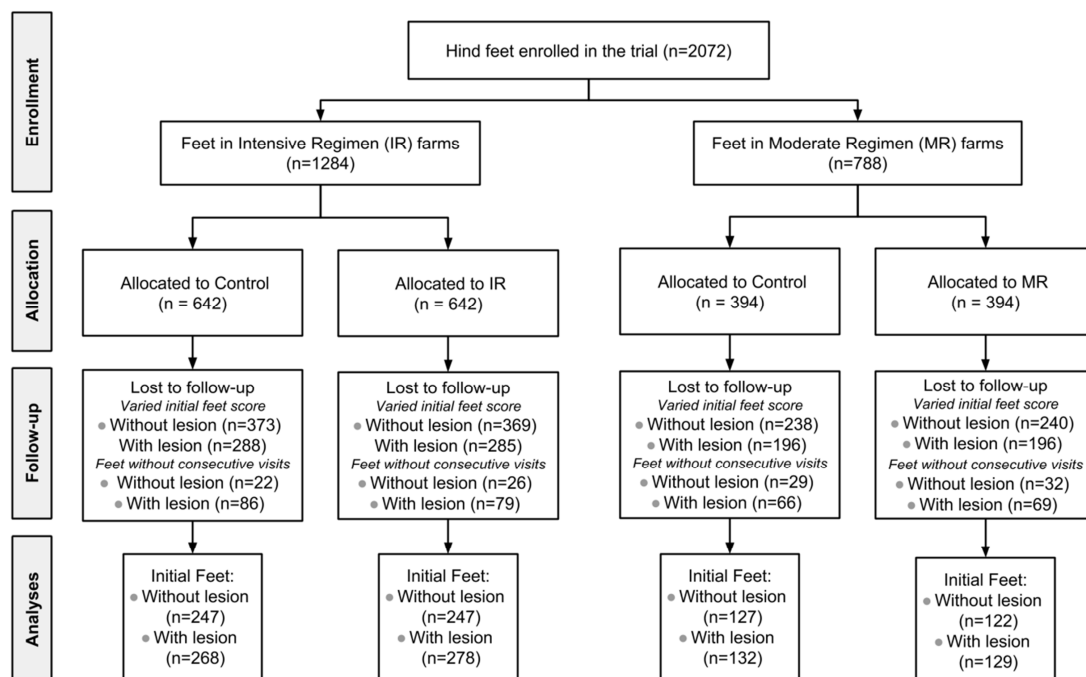
Thomsen PT, Ersbøll AK and Sørensen JT 2012. Short communication: Automatic washing of hooves can help control digital dermatitis in dairy cows. *Journal of dairy science* 95, 7195–9.

Xie T and Waksman J 2003. Design and sample size estimation in clinical trials with clustered survival times as the primary endpoint. *Statistics in Medicine* 22, 2835–2846.

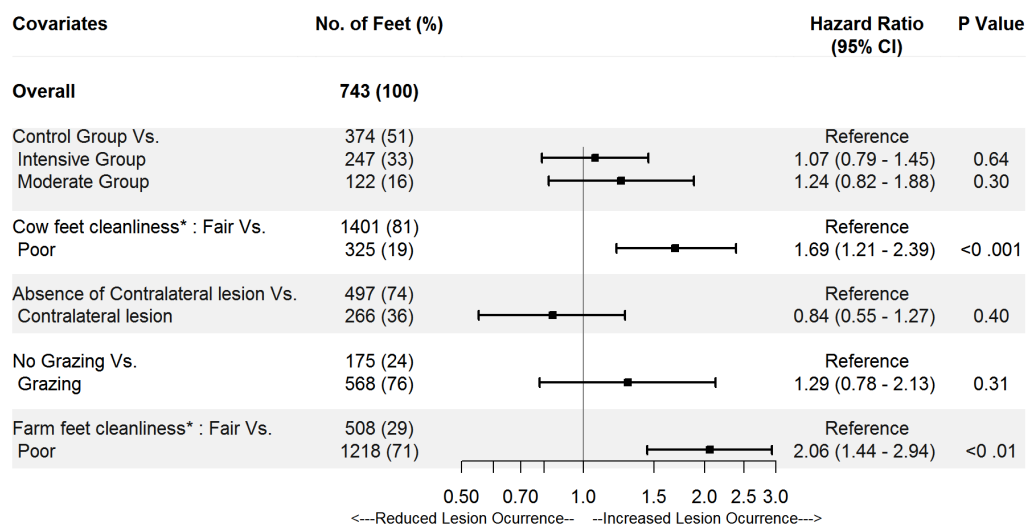
**Figure 1.** Scoring grid to assess feet cleanliness at the cow and farm level (Guatteo et al., 2013).

Hygiene Score	Feet Hygiene Assessment		Cleanliness Category
1			Clean
2			Moderate
3			Dirty

**Figure 2.** Flow diagram of the feet included in the clinical trial. From enrollment until the final analyses.

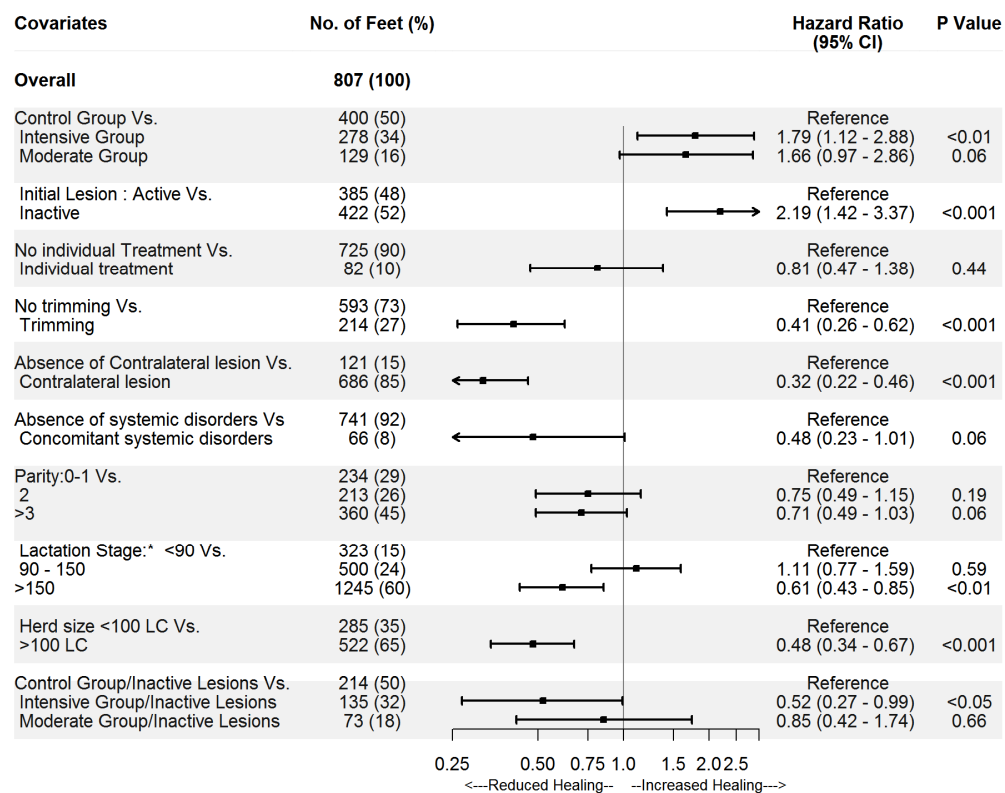


**Figure 3.** The effect of footbath regimen on delaying the first occurrence of digital dermatitis (bDD) lesion adjusted for herd, feet, and cow characteristics in the nested survival model including observations on 743 hind feet from 468 cows from 10 French dairy herds involved in a clinical trial.



\* Time-dependent covariates; “number of feet” are transitions between categories during the follow-up trial period.

**Figure 4.** The healing effect of footbath regimen adjusted for herd, cow, and feet characteristics on the healing of digital dermatitis (bDD) lesions in the nested survival model of the observations of 807 hind feet from 508 cows from 10 French dairy herds involved in a clinical trial.



\* Time-dependent covariates; “number of feet” are transitions between categories during the follow-up trial period.

**Table 1.** A detailed description of the covariates investigated during the clinical trial.

Level	Covariates	Group	Definition
Feet	Footbath regimen group allocation	Control Moderate Intensive	Allocation group.
	Initial score	M4 – 3 M1-M2-M4.1	Foot score recorded at the analyses inclusion according to Döpfer et al. (1997) and Berry et al. (2012).
	Hoof-trimming	Yes No	Feet which have received a hoof-trimming during the follow-up period until censoring.
	Individual treatment	Yes	Feet which have received a treatment during the trial period until censoring.
		No	
Cow	bDD lesion on the contralateral foot	Yes	The presence of a bDD lesion in the contralateral foot during the follow-up period until censoring.
		No	
	Cow parity at the start of the trial	$\geq 3$	Number of calvings at the start of the trial follow-up.
		2	
		0-1	
	Milk yield potential	Low (<36.4)	Based on the milk-production yield recorded during the preceding lactation to the trial start. The values were adjusted by parity.
		Moderate (36.4-42.2)	
		High (>42.2)	
	Concomitant disease	Yes No	Cows experiencing a concomitant systemic disease during the trial period until censoring.
	Lactation stage <sup>a</sup>	DIM <90 DIM 90–150 DIM $\geq 150$	Days in milk across the follow-up visits.
	Cow feet hygiene <sup>a</sup>	Fair (< 2) Poor ( $\geq 2$ )	Score of the cow feet hygiene across the follow-up visits.

Farm	Grazing	Yes No	Farms which have practiced grazing during the trial.
	Preventive hoof-trimming	Yes No	Farms which have practiced a hoof-trimming for a large part of the herd at least once during the 2 previous months or during the trial.
	Initial prevalence	< 25 25-35 >35	Prevalence of cows with active lesions at the pre-study visit.
	Heifer proportion	< 10% > 10%	Heifer proportion introduced in the herd during the trial period.
	Herd size <sup>b</sup>	< 100 LC > 100 LC	Average number of lactating cows during the trial period.
	Farm feet hygiene <sup>a</sup>	Good < 1.5 Fair to Poor ≥1.5	Average scoring of the herd feet across the follow-up visits.

<sup>a</sup>Time-dependent covariates

<sup>b</sup>LC=Lactating cows

737

738

739

740

**Table 2.** Main farms characteristics before side randomization

Allocation	Farm ID	Lactating Cows	Heifers	Initial bDD prevalence (%) <sup>a</sup>	Housing system	Global feet hygiene <sup>b</sup>	Grazing period	Milking system	Preventive hoof trimming practices	Number of feet included in the healing outcome	Number of feet included in the occurrence outcome
Moderate Regimen	1	45	14	36	Cubicles	2.00	Yes	Conventional	Yes	59	26
	2	115	37	18	Cubicles	2.42	No	Conventional	Yes	75	118
	3	47	13	47	Cubicles	2.00	Yes	Conventional	Yes	32	21
	4	78	26	33	Cubicles	2.38	Yes	Conventional	Yes	75	43
	5	57	22	21	Loose housing	2.30	Yes	Conventional	No	20	41
Intensive Regimen	6	85	33	40	Cubicles	2.31	Yes	Conventional	Yes	99	45
	7	145	49	59	Cubicles	2.18	Yes	Rotary	Yes	180	99
	8	105	35	30	Cubicles	2.11	Yes	Conventional	Yes	81	123
	9	123	38	31	Cubicles	2.02	Yes	Rotary	Yes	103	138
	10	99	37	20	Cubicles	2.03	Yes	Conventional	No	83	89
Summary <sup>c</sup>	10	899 (90)	304 (30)	34 % <sup>d</sup>	Cubicles =9 Loose housing =1	2.17 <sup>d</sup>	Yes=9 No=1	Conventional=8 Rotary =2	Yes=8 No=2	807	743

<sup>a</sup>Prevalence of cows with active lesions at the pre-study visit

<sup>b</sup>Average feet hygiene score (1-3) among the animals recorded at the pre-study visit

<sup>c</sup>Total count and mean proportion in parenthesis, unless otherwise specified.

<sup>d</sup>Total average

741  
742  
743  
744  
745  
746  
747  
748  
749  
750  
751  
752  
753  
754  
755  
756

**Table 3.** Feet baseline characteristics according to the allocation group, the covariates recorded and the outcomes of interest.

Covariates	Group	<i>Healing outcome</i>					<i>Occurrence outcome</i>				
		Control (%)	Intensive (%)	Moderate (%)	Total	p-value	Control (%)	Intensive (%)	Moderate (%)	Total	p-value
Initial score	M4 – 3	214 (53)	135 (49)	73 (57)	422	0.25	-	-	-	-	-
	M1-M2-M4.1	186 (47)	143 (51)	56 (43)	385						
	0-1	115 (28)	83 (30)	36 (28)	234		189 (50)	124 (50)	60 (49)	373	
Cow parity	2	103 (29)	68 (25)	42 (33)	213	0.50	83 (22)	49 (20)	29 (24)	161	0.88
	≥ 3	182 (45)	127 (45)	51 (39)	360		102 (27)	74 (30)	33 (27)	209	
	Low (<36.4)	101 (26)	63 (23)	42 (34)	206		132 (35)	77 (31)	46 (38)	255	
Milk yield potential	Moderate (36.4-42.2)	157 (41)	120 (45)	49 (40)	326	0.21	112 (30)	78 (32)	30 (25)	220	0.56
	High (>42.2)	126 (33)	86 (32)	32 (26)	244		130 (35)	92 (37)	46 (37)	268	
	DIM <90	109 (27)	77 (28)	37 (29)	223		143 (39)	93 (38)	43 (35)	279	
Lactation stage	DIM 90–150	85 (21)	58 (21)	20 (15)	163	0.70	61 (16)	45 (18)	20 (16)	126	0.90
	DIM ≥150	206 (52)	143 (51)	72 (56)	421		168 (45)	107 (44)	59 (48)	334	
Cow feet hygiene	Fair (< 2)	315 (78)	215 (77)	103 (80)	633	0.82	283 (76)	206 (83)	78 (64)	567	≤0.001
	Poor (≥ 2)	85 (22)	63 (23)	26 (20)	174		91 (24)	41 (17)	44 (36)	176	
Preventive hoof-trimming	Yes	328 (82)	240 (86)	97 (75)	665	≤0.05	318 (85)	200 (81)	110 (90)	628	0.06
	No	72 (18)	38 (14)	32 (25)	142		56 (15)	47 (19)	12 (10)	115	
	< 25	93 (23)	38 (14)	47 (36)	178		123 (33)	47 (19)	78 (64)	248	
Initial prevalence	25-35	128 (32)	95 (34)	36 (28)	259	≤0.001	154 (41)	127 (51)	23 (19)	304	≤0.001
	>35	179 (45)	145 (52)	46 (36)	370		97 (26)	73 (30)	21 (17)	191	
Proportion of < 33%		131 (33)	55 (20)	83 (64)	269	≤0.001	156 (42)	66 (27)	81 (66)	303	≤0.001



heifers	> 33%	269 (67)	223 (80)	46 (36)	538		218 (58)	181 (73)	41 (34)	440	
Herd size <sup>a</sup>	< 100 LC	144 (36)	49 (18)	92 (71)	285	≤0.001	90 (24)	24 (10)	62 (51)	176	≤0.001
	> 100 LC	256 (64)	229 (82)	37 (28)	522		284 (76)	223 (90)	60 (49)	567	
Farm hygiene	Good to Fair N feet < 2	181 (45)	127 (46)	46 (36)	354	0.38	151 (40)	132 (53)	21 (17)	304	≤0.001
	Poor ≥2	219 (54)	151 (54)	83 (64)	453		223 (60)	115 (47)	101 (83)	439	

<sup>a</sup>LC=Lactating cows

757