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► 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-02064431v1>

Submitted on 19 Oct 2021

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

3 J.M. Ariza^{1,2,a}, N. Bareille^{1,a}, A. Lehebel^{1,a}, K. Oberle^{2,b}, A. Relun^{1,a}, and R. Guatteo^{1,a}

4 ¹ *BIOEPAR, INRA, Oniris, 44307, Université Bretagne Loire, Nantes, France.*

5 ² *Qalian, Neovia group, Segré, 49500, France.*

6

7 ^a *Present address: BIOEPAR, ONIRIS, Atlanpole-Chantrerie*

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

9 ^b *Present address: Qalian, Neovia group, 34 Rue Jean Monnet, 49500 Segré, France.*

10

11 Corresponding author: Raphaël Guatteo. Email: raphael.guatteo@oniris-nantes.fr

12

13 **ABSTRACT**

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

26 in the milking parlor. Nested survival models were used to estimate the relative impact of the
27 footbath regimens and other concomitant risk factors on the time that bDD lesions occurred or
28 healed. No effect of Pink-Step™ was evidenced on the bDD occurrence during the trial. The
29 risk for bDD occurrence was increased by poor feet cleanliness at both the cow (HR 1.69, CI
30 1.21–2.39) and farm level (HR 2.06, CI 1.44–2.94). Otherwise, the use of Pink-Step™ in an
31 IR was effective in improving the healing of bDD lesions (HR 1.79, CI 1.12–2.88). The time
32 to healing was also improved in inactive lesions (HR 2.19, CI 1.42–3.37). Conversely, the
33 time to healing was delayed in feet receiving hoof-trimming (HR 0.41, CI 0.26–0.62), in cows
34 with a contralateral bDD lesion (HR 0.32, CI 0.22–0.46) or in late lactation (HR 0.61, CI
35 0.43–0.85), and finally in farms with more than 100 cows (HR 0.48, CI 0.34–0.67). These
36 findings reinforce the crucial role of hygiene in bDD dynamics and highlight the importance
37 of implementing multiple control measures simultaneously, such as hygiene improvements in
38 the barn, early detection and treatment of bDD lesions and the correct usage of individual and
39 collective treatments. The implementation of Pink-Step™ represents a promising strategy for
40 reducing the persistence of bDD lesions in affected herds.

41

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

43

44 **INTRODUCTION**

45

46 Bovine digital dermatitis (bDD) is currently among the principal cause of infectious lameness
47 in dairy cows (Sogstad et al., 2005; Cramer et al., 2008; Refaai et al., 2013; Solano et al.,
48 2016). bDD raises important economic, public-health and animal-welfare concerns. Indeed,
49 bDD is associated with different challenges such as reduced farmers' incomes, increased use
50 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).

76 Furthermore, the currently available footbath solutions are not adapted to all farming
77 conditions. Indeed, significant differences between farms, for example, in terms of hygiene,
78 housing system, herd size or lameness prevalence, may have an important impact on the
79 implementation and effectiveness of footbathing practices (Relun et al., 2012; Relun et al.,
80 2013), and often this impact is ignored in controlled trials (Ariza et al., 2017). Additionally,
81 the increasing bDD prevalence and the development of non-healing lesions are raising
82 concerns that highly pathogenic or resistant strains are emerging (Evans et al., 2011).
83 Therefore, new solutions for the collective disinfection of feet must consist of efficient and
84 safe practices that can be easily adapted to the complex setting of each farm. Pink-step™
85 (Qalian, France) is a biocide that represents a potential alternative for bDD control. This is a
86 safe and biodegradable disinfectant solution of confirmed bactericidal efficacy under soil
87 conditions designed for the footbath administration (European Commission, 2016; European
88 Chemicals Agency, 2017).

89

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

94

95 **MATERIAL AND METHODS**

96

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

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

102

103 **Trial design**

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

107

108 **Study population**

109 The trial was conducted on 10 dairy farms in western France from October 2016 to June 2017.
110 Farms were selected from a list provided by hoof-trimmers and veterinarians according to the
111 trial protocol (Supplementary material S1). These farms were known to have experienced
112 bDD during the preceding two-year period. However, none of the farms had administered
113 footbaths during the two months preceding the trial. The herds were composed of Holstein
114 cows to reduce the potential effects of breed on bDD. Cows were milked in a rotatory or
115 conventional milking-parlor (location for bDD scoring). Additionally, to minimize possible
116 imbalances between farms, after the pre-study visits, only farms with a herd prevalence \geq
117 15% of active bDD lesions were included. Farmers milked on average 90 cows (range: 45–
118 145) twice a day. Cows were mostly housed in cubicles (9 farms), and only one farm had no
119 access to pasture during the spring and summer seasons.

120

121 **Footbath regimen groups and concomitant treatments**

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

125 separated by a grill which partially avoids contamination of the footbath by cow feces (Intra-
126 Bath™, Intracare). Each bath measured 233 cm long × 32.5 cm wide × 19 cm high. The
127 combined volume of the 2 sides of the footbath was 160 L. No pre-footbath foot wash was
128 used in the study. The disinfectant solution administered, named Pink-step™, was a new
129 biocide with recognized *in-vitro* efficacy (European Commission, 2016; European Chemicals
130 Agency, 2017). Pink-step™ solution is composed of lactic acid (30%; w/w) and glycolic acid
131 (10%; w/w) as active substances, anionic and non-ionic surfactants, and other excipients as a
132 dye. The dose recommended by the manufacturer for footbathing was a 5% (v/v) solution in
133 water. The split footbath made it possible to concurrently administer the Pink-step™ solution
134 in one side of the footbath whilst the other side of the footbath remained empty and was used
135 as a control. The feet of the lactating cows enrolled in the trial thus were allocated to three
136 different groups, consisting of two different regimens of footbath administration frequencies
137 and the empty bath (control group). According to the willingness of the farmers, farms were
138 allocated to the moderate or intensive regimen. The Moderate Regimen (MR) was planned to
139 resemble current field practices and consisted in footbath administration for 2 days (4
140 consecutive milkings) every week for the first month, then every two weeks for the second
141 month, and then once a month until the end of the trial. The Intensive Regimen (IR) aimed to
142 evaluate the possible advantages of increasing the frequency of footbathing over time, and
143 consisted in footbath administration for 2 days every week for the first 2 months, and then
144 every fortnight until the end of the trial. For both regimens studied, the biocide solution was
145 expected to be renewed every 100 cow passages following the guidelines for the use of the
146 solution.

147

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

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

153

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

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

166

167 Digital dermatitis status was assessed during milking using a swiveling mirror based on the
168 methodology described by Relun et al. (2011). The hind feet of all lactating cows were
169 washed using tap water before the examination. The hind feet then were recorded using the M
170 scoring system, modified from Döpfer et al. (1997) and Berry et al. (2012). In this system, the
171 M0 stage corresponds to healthy feet without bDD lesions; M1 is considered as an early-stage
172 ulcerative lesion (0–2 cm diameter); M2 represents painful ulcerative lesions with a diameter
173 >2 cm; M3 is the healing stage with a lesion covered by a scab; M4 is the chronic stage
174 characterized by dyskeratosis or surface proliferation; and M4.1 consists in a chronic lesion

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

188

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

195

196 **Sample size**

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

200 because the detectable differences in the target effect between the treatment and control group
201 were smaller (10%) compared with the healing outcome (20%). Therefore, a larger number of
202 animals was needed to achieve statistical power for the occurrence outcome than the healing
203 outcome. Due to the lack of previous data to account for the within cow correlation, a
204 classical intra-cluster correlation coefficient (ICC) was set at 0.05 (Adams et al., 2004). The
205 number of farms included was calculated based on the average French herd size of 60
206 lactating cows and on the occurrence rates (4 cases for 100 feet-months at risk) reported in a
207 previous study following a similar approach (Relun et al., 2013). Therefore, with a type I error
208 risk of 0.05 ($\alpha=0.1$), at least 264 cows by each footbath regimen were necessary to guarantee
209 80% power ($\beta=0.2$) to detect the target difference between control feet and footbath feet
210 (10%). These calculations led to the recruitment of 10 average French farms (n= 60 lactating
211 cows) for the inclusion of at least 528 cows (2 footbath regimen) and accounting for 15% of
212 cows lost during the follow up of the trial.

213

214 **Treatment group allocation**

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

223

224 **Data analysis**

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

246

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

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

266

267 **RESULTS**

268

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

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

276

277 **Effect on Occurrence**

278

279 At the start of the trial, 852 hind feet (41%) out of 2,072 hind feet (1,036 cows) were free of
280 active bDD lesions during two consecutive visits. Then, 109 feet were excluded from the
281 dataset because the interval between visits was longer than 45 days. Finally, 743 hind feet of
282 468 cows from 10 herds were included in the analysis (Figure 2, total initial feet without
283 lesion). Only 275 cows shared the same bDD-free score in both hind feet at the start of the
284 trial. Significant baseline differences were found between the feet allocation groups for cow
285 feet hygiene, initial prevalence, proportion of heifers, herd size and farm feet hygiene
286 covariates (Table 3).

287

288 Among all of the feet included in the analyses, 161 (21%) experienced a bDD lesion during
289 the trial period. Inactive lesions (142) were more prone to occur than active lesions (19). The
290 median time before the occurrence of a bDD lesion was 80 days (37 to 142 days), and the
291 mean incidence rate was 6 cases for 100 feet-months at risk. After the analyses, only the poor
292 feet cleanliness at the cow level and the farm level were significantly associated with a high
293 risk of bDD occurrence in the multivariable analysis (Figure 3). No effect of the Pink-Step™
294 solution was evidenced on the bDD occurrence during the trial. None of the other covariates
295 included in the multivariable model or their interactions were statistically significant in the
296 multivariable model. Finally, the estimated variance of the cluster effect at the farm level was
297 0.0073 (CI 0.0010 - 0.0132, p value=0.0126), and at the cow level 3.65 (CI 2.29 – 5.00, p
298 value<0.001).

299

300 **Effect on Healing**

301

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

309

310 Among all of the feet included in the analyses, 186 (23%) achieved the healing either of an
311 active lesion (74) or an inactive lesion (112). The median time before the healing of a bDD
312 lesion was 46 days (30 to 140 days), and on average the healing rate was 5% between two
313 visits. According to the group allocation, the mean healing rates by month were 4%, 8% and
314 5% in the control, MR, and IR, respectively. From the multivariable analyses, six covariates
315 were significantly associated with the risk of bDD healing (Figure 4). Thus, the time to
316 healing was improved in inactive lesions and by the use of Pink-Step™ footbaths in IR.
317 Otherwise, in feet trimmed during the trial period, the time to healing was reduced. Lesions in
318 cows which either had a contralateral lesion or were at late lactation were identified to be at a
319 higher risk of persisting. Moreover, in larger herds, the risk of lesion persistency was
320 increased. A single interaction between the initial lesion aspect and the allocation footbath
321 group was detected, indicating that feet with an active lesion in the IR group have a reduced
322 time to healing compared to active lesions on feet allocated to the MR and control group.
323 None of the others covariates included in the multivariable model or their interactions were

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

327

328 **DISCUSSION**

329

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

337

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

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

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

399 must implement standardized footbath dimensions to ensure the optimal performance of the
400 disinfectant products studied and to enable reliable and comparable results (Solano et al.,
401 2017).

402

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

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

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

446

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

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

461

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

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

481

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

492

493 **CONCLUSION**

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

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

502

503 **ACKNOWLEDGMENTS**

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

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

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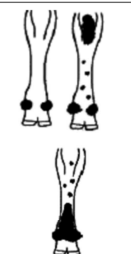


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647 **Figure 1.** Scoring grid to assess feet cleanliness at the cow and farm level (Guatteo et al.,

648 2013).

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

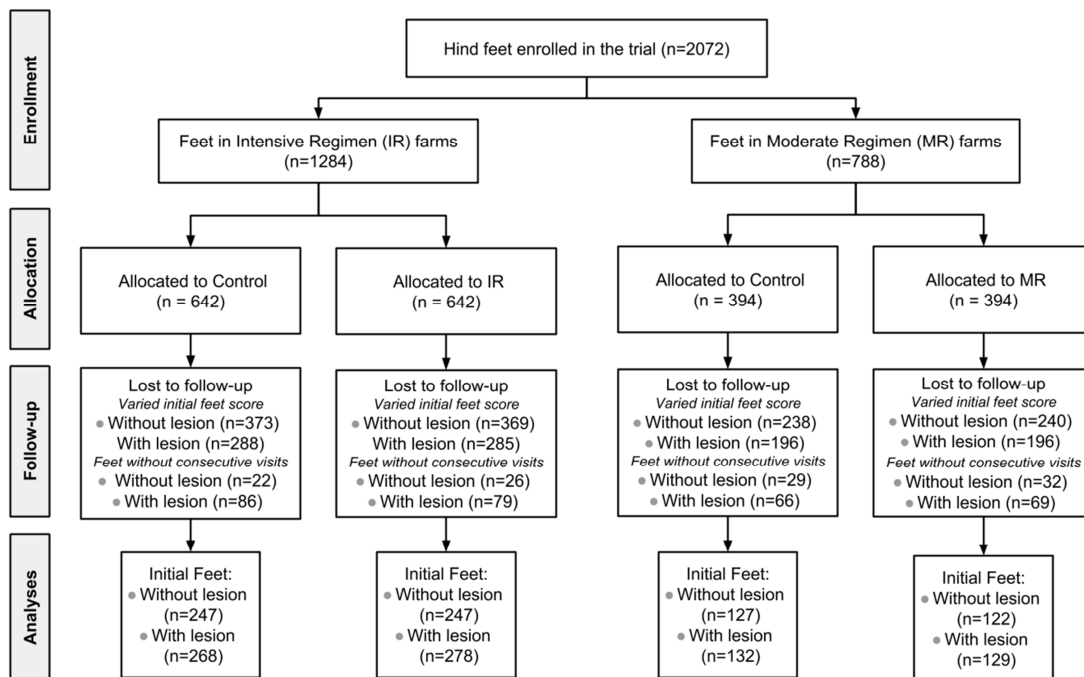
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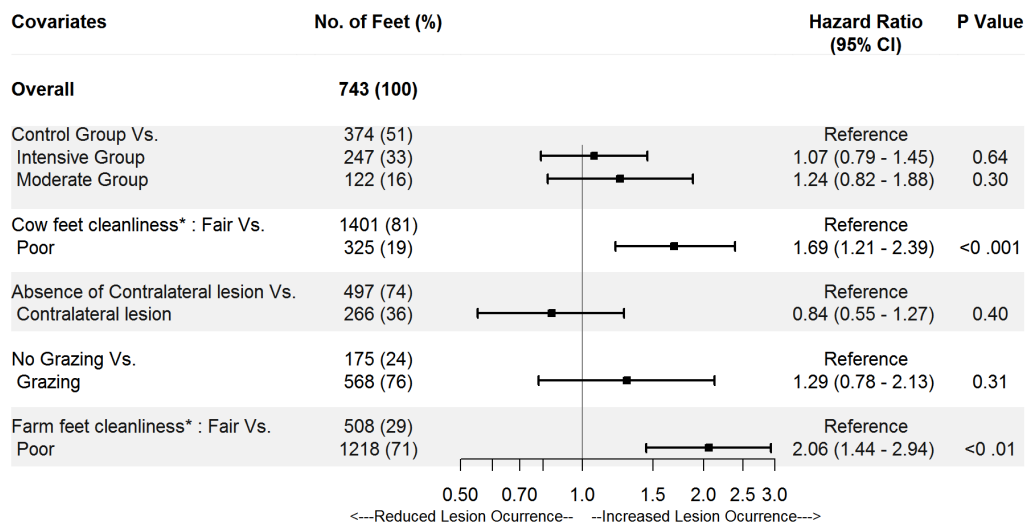
Figure 2. Flow diagram of the feet included in the clinical trial. From enrollment until the final analyses.



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

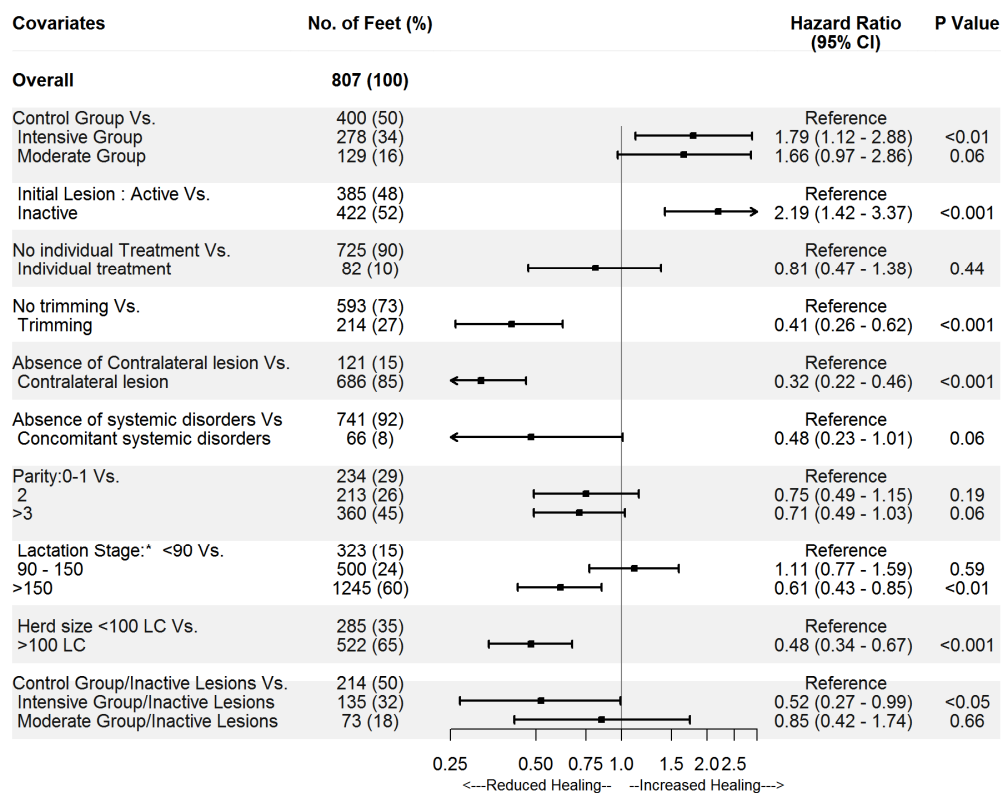


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* Time-dependent covariates; “number of feet” are transitions between categories during the follow-up trial period.

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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.

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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 No	Feet which have received a treatment during the trial period until censoring.
Cow	bDD lesion on the contralateral foot	Yes No	The presence of a bDD lesion in the contralateral foot during the follow-up period until censoring.
	Cow parity at the start of the trial	≥ 3 2 0-1	Number of calvings at the start of the trial follow-up.
	Milk yield potential	Low (<36.4) Moderate (36.4-42.2) High (>42.2)	Based on the milk-production yield recorded during the preceding lactation to the trial start. The values were adjusted by parity.
	Concomitant disease	Yes No	Cows experiencing a concomitant systemic disease during the trial period until censoring.
	Lactation stage ^a	DIM <90 DIM 90–150 DIM ≥ 150	Days in milk across the follow-up visits.
	Cow feet hygiene ^a	Fair (< 2) Poor (≥ 2)	Score of the cow feet hygiene across the follow-up visits.

	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.
Farm	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 ^b	< 100 LC > 100 LC	Average number of lactating cows during the trial period.
	Farm feet hygiene ^a	Good < 1.5 Fair to Poor ≥1.5	Average scoring of the herd feet across the follow-up visits.

^aTime-dependent covariates

^bLC=Lactating cows

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Table 2. Main farms characteristics before side randomization

Allocation	Farm ID	Lactating Cows	Heifers	Initial bDD prevalence (%) ^a	Housing system	Global feet hygiene ^b	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 ^c	10	899 (90)	304 (30)	34 % ^d	Cubicles =9 Loose housing =1	2.17 ^d	Yes=9 No=1	Conventional=8 Rotary =2	Yes=8 No=2	807	743

^aPrevalence of cows with active lesions at the pre-study visit

^bAverage feet hygiene score (1-3) among the animals recorded at the pre-study visit

^cTotal count and mean proportion in parenthesis, unless otherwise specified.

^dTotal average

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

Covariates	Group	<i>Healing outcome</i>				p-value	<i>Occurrence outcome</i>				
		Control (%)	Intensive (%)	Moderate (%)	Total		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	
	Fair (< 2)	315 (78)	215 (77)	103 (80)	633		283 (76)	206 (83)	78 (64)	567	
Cow hygiene	Poor (≥ 2)	85 (22)	63 (23)	26 (20)	174	0.82	91 (24)	41 (17)	44 (36)	176	≤0.001
	Yes	328 (82)	240 (86)	97 (75)	665		318 (85)	200 (81)	110 (90)	628	
Preventive hoof-trimming	No	72 (18)	38 (14)	32 (25)	142	≤0.05	56 (15)	47 (19)	12 (10)	115	0.06
	< 25	93 (23)	38 (14)	47 (36)	178		123 (33)	47 (19)	78 (64)	248	
	25-35	128 (32)	95 (34)	36 (28)	259		154 (41)	127 (51)	23 (19)	304	
Initial prevalence	>35	179 (45)	145 (52)	46 (36)	370	≤0.001	97 (26)	73 (30)	21 (17)	191	≤0.001
	Proportion of < 33%	131 (33)	55 (20)	83 (64)	269		156 (42)	66 (27)	81 (66)	303	

heifers	> 33%	269 (67)	223 (80)	46 (36)	538		218 (58)	181 (73)	41 (34)	440	
Herd size ^a	< 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	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	

^aLC=Lactating cows

757