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Evaluation of selected risk factors for different stages of digital dermatitis in Dutch dairy cows

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ABSTRACT

Digital dermatitis (DD) is a painful infectious disease in dairy cattle that causes ulcerative lesions of the skin just above the coronary band, mainly of the hind legs. Estimates for DD prevalence at cow level in the Netherlands range from 20% to 25%. In this study, risk factors for the various stages of DD were identified and quantified. The hind legs of 6766 cows on 88 farms were scored by trained interns, using the M-scoring system (M0-M4.1). Farms in this study were a convenience sample, based on the prevalence of DD as recorded at the latest herd trim, geographical location and willingness of the farmers to participate. A survey with questions about cow environment and herd management was conducted by the intern at the day of scoring. The data were collected between August 2017 and January 2018. DD was found on 38.6% of the scored legs; 49.8% of the cows had DD on at least one leg and M4 was the most frequent stage (20.9%).

Not removing manure on a regular basis resulted in lower odds for M2, M4 and M4.1 compared to cleaning by automatic scrapers ten times a day or more (odds ratio [OR]= 0.16, 0.49 and 0.18, respectively). The odds for M2 and M4 lesions were higher in cows aged 3–5 years than in first-calved cows (OR> 1.5 and > 1.7, respectively). Rubber flooring in the passageways resulted in lower odds for both M1 and M2 (OR, 0.06 and 0.32, respectively). Prophylactic use of footbaths treatment with an alternative active compound resulted in significant higher odds for M4 lesions than formalin and a combination of formalin and copper sulphate (OR= 1.69 and 2.04 respectively). The odds for an M4.1 lesion were lower in cows from smaller herds ($n = 50–100$) compared to large herds ($n > 100$; OR= 0.67).

1. Introduction

Digital dermatitis (DD) is a painful infectious disease in dairy cattle that causes ulcerative lesions of the skin just above the coronary band, by preferentially in the hind legs. A study in The Netherlands estimated the total economic consequences of clinical DD to be around US$1249/year[1] on a default farm with 65 cows (Bruijnis et al., 2010). Estimates for the prevalence of DD in North and Western Europe range from around 6–25% at cow level (Somers et al., 2003; Capion et al., 2008; Van der Linde et al., 2010; Pirkkalainnen et al., 2021). A scoring system which distinguishes several stages of DD progression was developed by Döpfer et al. (1997) and can be found in the ICAR-atlas (Kofler et al., 2020; see also[2]). This system discriminates between new and chronic lesions; the latter frequently show reactivation. DD is more frequently observed on the hind legs, probably because the environment around the front legs is drier and less dirty than around the hind legs (Zinicola et al., 2015).

DD is multifactorial by origin (Wells et al., 1999). Bacterial species that are thought to play a role in the aetiology of DD are mainly Treponema spp. The microbiome in active (M1, M2 and M4.1) and inactive lesions (M3 and M4) and healthy skin (M0) is very distinctive (Evans et al., 2016). Treponema in DD form cysts in the deeper layers of the digital skin, leading to a chronic infection from which reoccurrence of DD M2-lesions could happen (Döpfer et al., 2012). Since it is thought that moisture enables bacteria to enter the skin, the first logical risk factor for DD is a wet environment at the legs. Lactating cows are at higher odds of DD than cows in the dry period (Somers et al., 2005). The odds of a first case of DD increases during the first lactation (Barker et al., 2009), and in herds that purchase animals and have housed dairy

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[1] US$1 = Approximately £0.82, €0.94 at 14 March 2023.
[2] https://www.icar.org/Documents/ICAR-Claw-Health-Atlas-Appendix-1-DD-stages-M-stages.pdf

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cattle permanently (Rodríguez-Lainz et al., 1996; Somers et al., 2005).

To be able to provide the farmers and their advisors with more customized recommendation regarding DD prevention, the objective of this study was to identify and quantify risk factors related to cow level and herd management for the occurrence of the distinct DD lesions.

2. Materials and methods

2.1. Study design

A cross-sectional study was performed to assess the potential risk factors for the different stages of DD. Farms in this study were a convenience sample based on DD prevalence (<10%, 10–25% and >25%) obtained from the last recordings at preventive herd trimming visit by the hoof trimmer, the geographical location and the willingness of farmers to participate. Out of a total of 15,000 dairy herds in The Netherlands, 1200 participate in the Digiklauw system. In these herds all claw disorders detected during trimming were recorded in the Digiklauw registration system (van der Linde et al., 2010). We attempted to create an extensive database that included farms that differed in DD prevalence to achieve sufficient variation between herds for analysis. The distribution of the three DD prevalence categories scored according ICAR-atlas[2] (Kofler et al., 2020) in the herds studied was before the start of the study 50:30:20, respectively.

2.2. Data collection

A total of 88 farms were included in this study, using only data of lactating dairy cows. Data collection took place between August 2017 and January 2018 on the day of routine herd trimming by a professional hoof trimmer. In order to determine the DD status for each leg, the hind legs of cows were scored and recorded by trained students of Royal GD using the M-scoring system (Döpfer et al., 1997; Berry et al., 2012). A lesion was classified as M1 when it was smaller than 2 cm and showed no sign of proliferative ulceration. The M2 was assigned when the lesion was more than two centimetres and the cow expressed discomfort or pain when the lesion was touched. An M3 was present when the lesion was covered by a scab. An M4 referred to a chronic stage, whereby the cutaneous lesion was hyperkeratotic and could present themselves with a proliferative aspect. An M4.1 was assigned to a new small lesion, as an M1, in the M4 lesion (Berry et al., 2012). The scoring was performed by six trained students (Master students Veterinary Medicine and Agricultural Sciences in their final year) and a claw-health expert (MH). Interns were trained by the expert during the first one or two scoring days, comparing and discussing scores. Next, agreement between observers was assessed using photographs, and estimated in R[3] using R Studio[4] with package irrCAC[5] (Kilem and Gwet, 2019).

All observers scored 23 photographs of hind legs for DD. Cohen's kappa coefficient for the interns varied between 0.44 and 0.71. Five out of six students scored 'moderate' and one scored 'substantial'. The overall kappa value was 0.63 (moderate, 95% CI 0.50–0.78). On the day of scoring, a survey was conducted by means of a questionnaire and personal observations about herd management factors related to claw health about e.g. type of flooring, performing pasturing. The questionnaire and observations in the herd resulted in a list of potential risk factors, which were all included in the analysis.

2.3. Statistical analysis

The data were validated for missing values and (typing) errors. Some variables were then excluded from the analysis, mostly because of missing values or a lack of uniformity in the answers to open questions in the survey. If variables were highly correlated (Spearman r >0.70), the most informative variable was selected, and ordinal, nominal, continuous and biologically most plausible variables were preferred over dichotomous variables. First, a univariable logistic regression analysis was performed using PROC GENMOD (SAS 9.4). This analysis was performed four times with each of the M-stages (present/absent) as the response variable, except for the M3, as only 48 M3 lesions were recorded from 13,532 observations, indicating a low prevalence of this stage of DD in the study population.

Logistic regression analysis requires continuous explanatory variables to be linearly related to the log odds. This assumption was checked using the Box–Tidwell Transformation Test and an interaction term 'X*ln(X)' was added to the model (Fox and Weisberg, 2011). If this interaction was significant (P <0.05), non-linearity was assumed and the continuous variable had to be categorized, which was done by creating biologically plausible groups, ideally with about the same number of observations per category. For the multivariable analysis, a random herd effect was included. Since there were two observations per cow, a cow effect was added to the model, using the PROC GLIMMIX procedure (SAS 9.4). However, two observations per cow were not sufficient for the model to estimate the cow effect, and it was therefore decided not to include this effect in the model. Instead, the variable 'side'(left/right) was tested for significance as an independent variable. Since only one random effect remained, PROC GENMOD (SAS 9.4) was applied, as it provides population-averaged estimates. All variables that showed an association in the univariable models at P <0.25 were included in the multivariable model. From this model, the least significant variable was deleted and the model was rerun. If any of the coefficients (ß) of the remaining variables changed by more than 25% (for ß <-0.4 or >0.4) or by 0.1 (for ß between −0.4 and 0.4), the deleted variable was identified as a confounder and was kept in the model. If not, the variable was permanently deleted from the model. This procedure was repeated until all variables showed significant P-values or were identified as a confounder. The next step was to test all models for significance of biologically relevant interactions. A log likelihood ratio test was performed in order to determine significance. If the interaction improved the model significantly (P <0.05), it remained in the model. In each analysis, the M0 records and records of a specific M stage were used, while records of other stages were excluded from the analysis.

3. Results

3.1. Descriptive statistics

The dataset consisted of 7866 records, of which 916 contained insufficient information due to incomplete surveys. Therefore, records from 6766 individual cows and 88 farms were available for analysis. The distribution of the ages was 2030 < 3 years and 4736 ≥3 years, The number of records collected per farm varied between 47 and 131 (mean, 87.3; standard deviation, 21.4). From these records, 38.55% of animals was diagnosed with DD different from M0 on one or both hind legs. At leg level, M4 and M4.1 were most prevalent (Table 1).

DD lesions (M1, M2, M3, M4 or M4.1) were observed exclusively on the left leg in 744 out of 6766 cows, on the right leg in 783 cows and on

Table 1
Leg level prevalence of all M-lesions of digital dermatitis in hind legs of Dutch dairy cows (n= 13,532 hind legs).

M-stage	Frequency	Prevalence
M0	8315	61.45%
M1	186	1.37%
M2	964	7.12%
M3	48	0.35%
M4	2832	20.93%
M4.1	1187	8.77%

[3] https://www.R-project.org/
[4] http://www.rstudio.com/
[5] https://CRAN.R-project.org/package=irrCAC

both legs in 1845 cows. The prevalence of any DD lesion in the study population was 38.26% and 38.84% for the left and right leg, respectively (Relative Risk is 0.99 (95% CI: 0.94–1.03). DD was more often present than expected by random chance on both legs compared to one leg (observed 1845, expected 1006, ratio 1.84, chi-square $P < 0.0001$). Table 2 shows the variables related to a specific DD in the univariable analyses ($P < 0.25$) which were included in the initial multivariable models.

4. Multivariable analyses

For stage M1, three variables remained in the final multivariable model (P <0.05; Table 3). The odds for M1 were higher in cows that underwent footbath application than in cows that did not (odds ratio [OR] = 2.69 (CI: 1.22–5.99)). The odds for M1 lesions were higher for cows in barns without dead-end passageways compared to barns with dead-end passageways (OR= 3.04 (CI:1.06–8.73)). The odds were lower on farms where manure scraper did not cover the whole width of the walking alley (and in which the remaining area was either cleaned manually or not cleaned at all) compared to herds in which the manure scraper covered the entire area. 'Extra cleaning by hand' (yes/no) was not a significant factor (OR, 0.33 (CI: 0.10–1.14)) in the presence/absence of M1 lesions.

Four variables remained in the multivariable model for M2 lesions (Table 4). Odds were lower in cows on farms where manure was not removed on a regular basis compared to farms that removed manure ten times a day or more (OR= 0.16 (CI:0.08–0.31)). The odds for the variable 'active compound footbath' was significant higher than the components formalin, copper sulphate or a combination of both ($P = 0.02$ (CI: 0.10–0.60)) due to all contrasts with the compound 'other' showing ORs smaller 0.50 being significant ($P < 0.05$), formalin and copper sulphate in the same footbath showed a trend ($P = 0.06$ (CI:0.23–1.02)). The odds were higher for cows in barns without rubber flooring in the passageways compared to rubber flooring (OR= 3.08, CI:1.68–5.66)). The odds were higher in cows aged 3, 4 and 5 years than in cows aged ≤ 2 years (OR=1.41, 1.45 and 1.56, respectively).

For M4, seven variables remained in the multivariable model (Table 5). The odds were higher for cows on farms that used a fixed or robot scraper to remove manure compared to farms that did not remove manure (OR= 2.81 and 2.62, respectively). In addition, the odds were lower for cows on farms that did not remove manure than when manure was removed 10 times a day or more (OR= 0.49 (CI:0.28–0.87)). When the same manure scraper cleaned the alleys in barns where young and adult animals were kept, cows had lower odds for M4 lesions than on farms where no manure was removed (OR= 0.56, (CI:0.32–0.98)). Black and white cows had higher odds for M4 lesions than red and white cows (OR= 1.37 (CI1.18–1.60)). The OR for the variable 'active compound footbath' was significant ($P < 0.0001$) when compared with the use of formalin and the combination formalin and copper sulphate. Regarding M4 lesions, the use of formalin footbaths or no footbath at all was significant compared to the use of 'other' active compounds (OR= 0.48 (CI:0.35–0.66)). Lower stocking density was associated with higher odds for M4 lesions (OR= 1.89 and 2.19 for ≤0.8 cows/bed and 0.9–1 cow/bed, respectively) compared to more than one cow per bed. The odds of M4 lesions were higher in older cows than in younger cows (OR= 1.67, 2.29, 3.16, 3.36 and 3.09 for 3, 4, 5, 6 and ≥7 years old, respectively, compared to ≤2 years old).

Cows in average size herds (50−100) had significantly lower odds of having M4.1 lesions than cows in large herds (>100; OR= 0.67 (CI:0.45–0.99); Table 6). Never removing manure was associated with lower odds of having M4.1 lesions than when manure was removed 10 times a day or more (OR= 0.18 (CI:0.10–0.35)). Cows on farms that used a footbath in lactating cows, but not in dry cows and young stock, had higher odds of M4.1 lesions than cows on farms that did not use footbath treatments (OR= 5.25 CI:1.36–20.23). The overall P-value for the variable 'active compound footbath' was significant for M4.1 lesions ($P < 0.01$). The OR for formalin vs. 'other' was 0.42 (CI:0.24–0.75). The OR for M4.1 lesions was lower for cows in herds with a stocking density of 0.9–1 cow/cubicle than in herds with a stocking density of >1 cow/bed (OR= 2.48 (CI: 1.47–2.18)). The odds for M4.1 lesions were significantly higher in older cows than in young cows (ORs ranging from 2 to 3 compared to ≤ 2 years old).

Table 2
Variables used in the univariable analyses of four of dermatitis digitalis in Dutch dairy cows. Variables marked with '+' showed an association with the respective M- stage at $P<0.25$.

Variable	M1	M2	M4	M4.1	Parameter derived directly (D) or indirectly (I)
Side (left or right)	-	+	-	-	D
Herd size	+	-	+	+	D
Farming system (open or closed)	+	-	+	+	D
Pasture access	+	-	+	-	D
Manure scraping (method)	+	+	+	+	D
Manure scraping frequency	+	+	+	+	I
Extra manure removal by hand (manure scraper can't reach)	+	-	+	+	I
Working clothes provided (for professionals visiting the farm)	-	-	+	-	D
One manure scraper young and adult cows	+	+	+	+	D
Footbath use (lactating cows)	+	+	+	+	D
Active compound footbath	+	+	+	+	I
Coat colour	+	+	+	+	D
Separation stall (in case of lameness)	+	+	+	-	D
Stocking density	+	+	+	+	D
Chalk use	-	+	-	+	I
Rubber in walking path	+	+	+	+	D
Dead ends in stable	+	+	+	+	D
Season	+	+	+	+	D
Age	+	+	+	+	D
Hoof trimming frequency	+	+	+	+	I

5. Discussion

Although this study was performed with an agreement for observation of correct M-lesion of 0.63, which might have influenced the results of the analysis this is in line with a recent study which estimated an overall agreement for the M-score (Vanhoudt et al., 2019). The prevalence of DD in this study (38.6% at cow level) was higher than could be expected based on the selection of farms with low, moderate and high prevalence. This is most likely due to the fact that the selection of farms was based on data from the 'Digiklauw' system (van der Linde et al., 2010) and scoring mainly in the housing season, which might have influenced the strength of the study. Above that, at the moment of the study, Digiklauw recordings did not include chronic lesions (M4 and M4.1), while over 20% of legs in the study showed M4 lesions. Because of our selection criteria, the estimated DD prevalence in this study cannot be regarded as representative for the Netherlands. At leg level, more than half of the DD cases were M4 lesions (54%; n=2832/5217; Table 1) and almost one quarter were M4.1 lesions (i.e., a new small lesion, as in the M1, in an existing M4 lesion; 23%; n=1187/5217), highlighting the importance of the M4. Due to the fact that forceps were not used consistently at all herds the M1 prevalence may have been underestimated. This is in line with a study in The Netherlands that reported that M4 lesions should be prevented to achieve a reproduction ratio below one and eliminate DD from a herd. That might not be easy

Table 3
Multivariable multilevel logistic regression analysis of digital dermatitis (DD) M1 lesions (n=186) vs. absence of DD (n=8015) at leg level. The exchangeable working correlation was 0.032.

Variable	Category	Frequency (n)	Prevalence M1 (%)	OR (95% CI)	P-value	Overall P-value[a]
Footbath use	Lactating cows only	3798	3.48	2.69 (1.22–5.90)	0.01	0.03[b]
	All animals	1182	1.52	0.85 (0.30–2.35)	NS	
	Not (present)	3231	0.96	1.0	Ref	
Rubber in walking path	No	7526	2.38	16.42 (2.81–95.88)	<0.01	
	Yes	685	0.29	1.0 (ref)		
Dead ends in stable	No	4688	3.18	3.04 (1.06–8.73)	0.04	
	Yes	3523	0.91	1.0	Ref	
Extra manure removal by hand (manure scraper can't reach)	Needed, not done	4124	2.18	0.33 (0.10; 1.14)	0.08	NS
	Needed, done	2246	0.93	0.29 (0.07–1.27)	NS	
	Not needed	1841	3.80	1.0	Ref	

OR, Odds ratio; NS, not significant; Ref, reference value; 95% CI, 95% confidence interval.
[a] Overall P-value < 0.05 for variables with three or more categories

Table 4
Multivariable multilevel logistic regression analysis of digital dermatitis stage M2 (n=964) vs. M0 (n=8315) lesions at leg level. The exchangeable working correlation was 0.078.

Variable	Class	Frequency (n)	Prevalence M2 (%)	OR (95% CI)	P-value	Overall P-value
Manure scraping frequency	Rarely	158	2.53	0.16 (0.08; 0.31)	<0.001	<0.001[a]
	1–3 times/day	2848	11.31	1.04 (0.61;1.77)	NS	
	3–10 times/day	2723	7.60	0.54 (0.29; 1.02)	0.06	
	≥10 times/day	3237	12.45	1.0	Ref	
Active compound footbath	Formalin	2240	8.79	0.33 (0.15; 0.74)	<0.01	0.02[a]
	Copper sulphate	170	8.24	0.14 (0.03; 0.66)	0.01	
	Formalin + copper sulphate	2589	12.17	0.49 (0.23; 1.02)	0.06	
	Other	322	20.50	1.0	Ref	
	Footbath not used	3645	9.44	0.36 (0.17; 0.75)	<0.01	
Rubber in walking path	No	8259	11.04	3.08 (1.68; 5.66)	<0.001	
	Yes	707	3.39	1.0	Ref	
Age (years)	≤2	2521	8.57	1.0	Ref	0.008[a]
	3	2156	12.01	1.41 (1.11; 1.79)	<0.01	
	4	1579	12.03	1.45 (1.16; 1.80)	<0.001	
	5	1050	12.10	1.56 (1.12; 2.16)	<0.01	
	6	696	8.48	1.14 (0.85; 1.54)	NS	
	≥7	964	8.82	1.07 (0.74; 1.55)	NS	

OR, Odds ratio; NS, Not significant; Ref, reference value; 95% CI, 95% confidence interval.
[a] Overall P-value<0.05 for variables with four or more categories

because M4 are not painful per definition and therefore the farmers may not recognize these lesions as problematic for the cows and treatment and prevention are not focussed on these M4 lesions. DD on both hind legs was overrepresented, which might be related to a combination of the infectious nature of DD and the selection of the herds with high DD prevalence.

The odds of M4 lesions were higher in cows on farms that used a manure scraper or a manure robot compared to farms that did not clean up manure at all (OR= 2.82 and 2.62, respectively, Table 5). Previous studies on DD prevalence based on the presence/absence of active lesions did not show a significant association with manure removal (Holzhauer et al., 2006), while another study that focussed specifically on M1 and M2 lesions found decreased odds when manure was removed by a manure scraper (Somers et al., 2005). Other environmental factors were not taken into account in the current study, and therefore the association may be indirect also. Manure removal is performed for hygienic reasons. However, it also spreads pathogens throughout the entire floor and does not result in a completely dry and clean floor, thus may increase the odds of development or reactivation of DD lesions (Rodríguez-Lainz et al., 1996).

The odds of M4.1 lesions were 1.49 (1/0.67) times higher in cows in large herds compared to average-sized herds, but there was no difference in odds between large herds and small herds (Table 6). Previous studies have shown an increasing odds for DD with increasing herd size (Wells et al., 1999; Somers et al., 2005; de Jong et al., 2022; Weber et al., 2022). Management differences in larger herds, such as less available time per animal, might be the reason for the higher DD prevalence (Wells et al., 1999). No association with herd size was present for any of the other DD stages, which might indicate that it may be more difficult to keep the environment clean and dry in larger herds, possibly resulting in a higher odds for reactivation of the chronic M4 into M4.1. However, due to our selection criteria, variation in herd size was limited, reducing the power to detect such an association.

The estimated risk factors are the result of presence or absence of different M lesions and potential factors of influence chosen before the start of the study. In a following study it might be advisable to include other factors like lactation stage, selected treatments and frequency of treatments. The odds of M4 lesions were 1.49 (1/0.67) times higher in cows in large herds compared to average sized herds, but there was no differences in odds for M4.1 between large and small sized herds (Table 6). Previous studies have shown an increasing odds for DD with increasing herd size (Wells et al., 1999; Somers et al., 2005; de Jong et al., 2022; Weber et al., 2022). Management differences in larger herds, such as less available time to care for the animals, might be the reason for the higher DD prevalence (Wells et al., 1999). No association with herd size was present for any of the other DD lesions, which might indicate that it may be more difficult to keep the environment clean and dry in larger herds, possibly resulting in a higher odds for reactivation of the chronic M4 lesion. However, due to our selection criteria, variation in herd size was limited, reducing the power to detect such an association.

The frequency of manure removal was also associated with DD

Table 5
Multivariable multilevel logistic regression analysis of digital dermatitis M4 (n=2832) vs. M0 (n=8315) lesions at leg level. The exchangeable working correlation was 0.061.

Variable	Class	Frequency (n)	Prevalence (%)	Odds ratio (95% CI)	P-value	Overall P-value
Manure scraping	Fixed	3444	27.47	2.81 (1.43; 5.49)	<0.01	0.02[a]
	Robot	3577	27.93	2.62 (1.29; 5.36)	<0.01	
	Manual	3082	24.14	1.96 (0.87; 4.43)	NS	
	No manure scraping	715	13.85	1.0	Ref	
Manure scraping frequency	Rarely	197	21.83	0.49 (0.28; 0.87)	0.02	<0.01[a]
	1–2.5 times/day	3493	27.68	1.17 (0.68; 2.03)	0.57	
	3–9 times/day	3506	28.24	1.31 (0.91; 1.87)	0.14	
	≥10 times/day	3622	21.76	1.0	Ref	
One manure scraper young and adult animals	No	8624	25.89	0.67 (0.41; 1.09)	0.11	0.12[a]
	Yes	990	28.48	0.56 (0.32; 0.98)	0.04	
	No manure scraping	1204	22.67	1.0	Ref	
Coat colour	Black and White	8300	26.98	1.37 (1.18; 1.60)	<0.0001	
	Brown and White	2518	21.80	1.0	Ref	
Active compound footbath	Formalin	2806	27.19	0.59 (0.41; 0.87)	0.01	<0.0001[a]
	Copper sulphate	223	30.04	0.96 (0.52; 1.79)	NS	
	Formalin + copper sulphate	3051	25.47	0.48 (0.35; 0.66)	<0.0001	
	Other	402	36.32	1.0	Ref	
	Footbath not used	4336	23.87	0.47 (0.34; 0.64)	<0.0001	
Stocking density	≤0.8 cows/bed	2905	22.07	1.89 (1.10; 3.22)	0.02	<0.01[a]
	0.9–1 cow/bed	6477	28.11	2.19 (1.42; 3.37)	<0.001	
	>1 cow/bed	1436	22.70	1.0	Ref	
Age (years)	≤2	2707	14.85	1.0	Ref	<0.0001[a]
	3	2459	22.85	1.67 (1.41; 1.97)	<0.0001	
	4	1934	28.18	2.29 (1.88; 2.79)	<0.0001	
	5	1427	35.32	3.16 (2.63; 3.79)	<0.0001	
	6	978	34.87	3.36 (2.71; 4.16)	<0.0001	
	≥7	1313	33.05	3.09 (2.50; 3.82)	<0.0001	

OR, Odds ratio; NS, Not significant; Ref, reference value; 95% CI, 95% confidence interval.
[a] Overall P-value<0.05 for variables with three or more categories

Table 6
Multivariable multilevel logistic regression analysis of digital dermatitis M4.1 (n=1187) vs. M0 (n=8315) lesions at leg level. The exchangeable working correlation was 0.056.

Variable	Class	Frequency (n)	Prevalence (%)	OR (95% CI)	P-value	Overall P-value
Herd size	≤50	734	18.66	1.05 (0.49; 2.27)	NS	0.09[a]
	51–100	7468	11.54	0.67 (0.45; 0.99)	0.04	
	>100	995	16.88	1.0	Ref	
Manure scraping frequency	Rarely	160	3.75	0.18 (0.10; 0.35)	<0.001	<0.0001[a]
	1–2.5 times/day	2936	13.96	1.19 (0.74; 1.93)	NS	
	3–9 times/day	2904	13.36	1.19 (0.71; 1.99)	NS	
	≥10 times/day	3197	11.35	1.0	Ref	
Footbath use	Lactating cows only	4361	15.94	5.25 (1.36; 20.23)	0.02	0.02[a]
	All animals	1334	12.74	2.51 (0.66; 9.51)	NS	
	No(t present)	3502	8.62	1.0	Ref	
Active compound footbath	Formalin	2377	14.05	0.42 (0.24; 0.75)	0.01	0.01[a]
	Copper sulphate	188	17.02	1.08 (0.44; 2.68)	NS	
	Formalin + copper sulphate	313	18.21	0.59 (0.32; 1.12)	NS	
	Other	2679	15.12	1.0	Ref	
	Footbath not used	3640	9.31	1.41 (0.35; 5.75)	NS	
Stocking density	≤0.8 cows/bed	2521	10.19	1.86 (0.98; 3.53)	0.06	<0.01[a]
	0.9–1 cow/bed	5448	14.54	2.48 (1.47; 4.18)	<0.001	
	>1 cow/bed	1228	9.61	1.0	Ref	
Age	≤2	2476	6.91	1.0	Ref	<0.0001[a]
	3	2184	13.14	2.02 (1.55; 2.63)	<0.0001	
	4	1656	16.12	2.64 (2.03; 3.44)	<0.0001	
	5	1109	16.77	2.92 (2.21; 3.85)	<0.0001	
	6	752	15.29	2.65 (1.96; 3.58)	<0.0001	
	≥7	1020	13.82	2.36 (1.77; 3.15)	<0.0001	

OR, Odds ratio; NS, Not significant; Ref, reference value; 95% CI, 95% confidence interval.
[a] Overall P-value<0.05 for variables with three or more categories

presence. Manure scraping more than 10 times a day increased the odds of M2, M4 and M4.1 lesions (OR= 6.25, 2.04, 5.56, respectively, Tables 4–6). These results are in contrast with a Danish study that reported a higher incidence of DD when manure was removed less than eight times a day (Oliveira et al., 2017), while in some herds manure removal results in piles of manure being left in odd places where the cows will walk. These piles may pose a larger odds for DD than the scraped clean floor next to it.

'Extra cleaning by hand' (yes/no) showed a trend (P= 0.06) for the presence of M1 lesions (Table 3). This could be related to increased contamination of the environment using a hand scraper, or to other factors that were not considered. Also, farmers might increase the frequency of scraping as a measure to control a relatively high incidence of DD in their herd. An experimental study including different manure removal frequencies and methods may give more insight.

'Footbath used for dry and young cows' showed an association with the presence of M1 and M4.1 lesions in adult cows. The use of footbaths for lactating cows only compared to not using a footbath at all increased the odds of M1 and M4.1 (OR= 3.48 and 15.94, respectively; Tables 3 and 6). A possible explanation for this association is that the footbath may be used because the farmer wants to reduce the incidence of DD (as M2 lesions) and that commercial products in footbaths are frequently used incorrectly (e.g. too low frequency). In addition, the wet conditions that result from cows walking through the footbath may favour bacterial growth. In line with previous research (Solano et al., 2017; Speijers et al., 2010; Teixeria et al., 2010) in our study footbaths showed a preventive effect for M1, M2 and M4-lesions, only a difference in the presence of M4.1 lesions was observed for the active components used in the baths (Tables 3–6). The compound most commonly used in footbaths in this study population was formalin, for which there are few efficacy reports in the literature (Teixera et al., 2010; Holzhauer et al., 2008). Infrequent use of footbaths in summer could also partially explain the current results, where the data were mainly collected in autumn. Both the frequency and concentration of the active compound and footbath design and use are important (Thomsen et al., 2008, Cook et al., 2012), and neither of these was considered in the current study. But serious DD reduction by better footbath design and use is possible in specific herds (MH, personal observation). Another option that was recently was proposed, is benchmarking based on regularly recorded claw health data (Kofler et al., 2022). This may be possible in The Netherlands and in other countries also, where recording on small scale is already done.

Despite the use of copper sulphate is forbidden in the Netherlands since 2013, the farmers still use this in footbaths and despite EU-regulations to discourage the use of formalin due to its potential harm to user's health, this product is still permitted in the Netherlands and most widely used. None of the farms that participated in this study used zinc sulphate and a small number 'other active compounds'. The use of formalin and its combination with copper sulphate reduced the odds for M4 lesions (OR= 0.60) compared to the use of other active compounds (Table 5). Good footbath application is measure of care for hooves and skin at the lower limb and if the compound used in the footbath does not have correct antiseptic properties, or is not used properly, the footbath could potentially spread treponema bacteria, leading to an increased odds of (re-)infection. This may be harmful to the skin, since too high concentrations (of most chemicals, especially $CuSO_4$ can cause chemical burns) and may hamper complete cure of DD also, leading to persistent M4 stages. These results are in line with a Danish study, which showed no DD reduction with modern disinfectants after weekly application (Thomsen et al., 2008).

The odds of an M4 lesion were higher in Holstein Friesian cows than in other breeds (OR= 1.37; Table 5), indicating that genetics contribute to the susceptibility of cows to chronic DD lesions to return to a sound skin (Onyiro et al., 2008; Waaij et al., 2005; Heringstad et al., 2018).

Remarkably was that more than one cow per cubicle was associated with a lower odds for M4 and M4.1 lesions (Tables 5 and 6). It seems rational that increasing the occupancy rate would increase infection pressure and therefore the odds for development of new infections and so higher prevalence of M4 and M4.1 lesions.

The absence of rubber flooring in the passageways increased the odds of M1 lesions 16.42 times compared to barns with rubber flooring (Table 3). For M2 lesions, this OR was 3.08 (Table 4). One possibility is that floors with rubber mats are drier and cleaner than concrete floors. A study in Germany reported a higher incidence of DD when cows were kept on rubber floors compared to concrete floors, which was thought be caused by higher cow activity on rubber (Kremer et al., 2007). In addition, in the German study the floor was flushed with water twice a day, favouring bacterial multiplication.

The odds for an M1 lesion was 4.46 times higher in cows housed in barns without dead-end passageways compared to barns with dead-end passageways (Table 3). The cause of this increase in absence of dead ends in the building is unknown. It might be related to older housing, more staying in such areas and consequently more contact with the infectious manure. Nowadays more attention is paid on prevention of dead-end passageways. Age was significantly associated with stages M2, M4 and M4.1, but not with M1. Cows aged 2–5 years had a significantly higher odds of an M2 lesion compared to cows $\leq$ 2 years (Table 4). Since cows are generally introduced in the lactating herd at around 2 years of age, it is possible that the odds of M2 lesions increases from that time onwards (Holzhauer et al., 2012). The odds for M2 lesions in cows $\geq$ 6 years was not different from young animals ($\leq$ 2 years), potentially because cows especially susceptible to DD were already culled. The odds for M4 lesions gradually increased with age, until approximately 5 years of age. M4 lesions are chronic stage of DD including lesions of various sizes characterized by a non-painful, clearly circumscribed dys- and hyperkeratosis or irregular proliferative overgrowths ('wart-like') of brown-gray color. Proliferative stages of DD may be characterized by filamentous, scab-like or mass proliferations, representing chronic inflammation (active inflammation going on for a long time). Older cows will have had more time to be infected and therefore to develop these M4 lesions. The odds for M4.1 lesions remains constant in cows from 3 years of age onwards and is two to three times higher than in cows $\leq$ 2 years. This indicates that the odds of reinfection or flare-ups remains more or less constant over the course of the life of cows that have had an infection and developed scar tissue (Blowey and Weaver, 2011). Previous research revealed a decreased DD odds with age (Rodríguez-Lainz et al., 1996; Somers et al., 2005; Barker et al., 2009; de Jong et al., 2022). This difference could partly be due to the difference in study design, like the selection of herds with a high prevalence and the influence of heat stress on the prevalence of different M-stages of DD (Gernand et al., 2019) and the fact that most studies look at M2 and not the chronic non-painful M4, that are not always detected and associated with DD or have many different names.

6. Conclusions

Depending on the distribution of stages within the herd, different measures could be taken to reduce the prevalence of DD. Two possible mechanisms of DD transmission were suggested: manure robots or scrapers and footbaths with miscellaneous ('other') active compounds. For both variables, the odds were significantly higher for the M4 stage, but not for the other stages. Rubber mats in the passageways seriously reduce the odds of M1 and M2 lesions. When footbaths are used, it is advisable to do so in line with the legal regulations and to follow only proven strategies with sufficient antiseptic properties to reduce the odds for higher DD prevalence due to new and persistent lesions.

CRediT authorship contribution statement

Menno Holzhauer: Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **Sanne Kalsbeek**: Writing – original draft, Methodology, Investigation, Formal analysis. **Klaas Frankena**: Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Formal analysis, Data curation.

Declaration of Competing Interest

None of the authors has any other financial or personal relationships that could inappropriately influence or bias the content of the paper.

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