J. Dairy Sci. 100:8398–8408
https://doi.org/10.3168/jds.2017-12815

Associations between biosecurity practices and bovine digital dermatitis in Danish dairy herds

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ABSTRACT

The relationship between biosecurity and digital dermatitis (DD) was evaluated in 8,269 cows from a convenience sample of 39 freestall dairy herds. The hypothesis was that poor implementation of biosecurity was associated with higher within-herd prevalence of DD. All lactating cows were scored as negative or positive for DD at the hind legs during milking in the milking parlor. Information about biosecurity was obtained through questionnaires addressed to farmers, on-farm observations, and information from the Danish Cattle Database (www.seges.dk). These assessment tools covered potential infection sources of DD pathogens to susceptible cows (e.g., via animals, humans, manure, vehicles, equipment, and facilities). External and internal biosecurity measures were explanatory variables in 2 separate logistic regression models, whereas within-herd DD prevalence was the outcome. Overall DD prevalence among cows and herds were 24 and 97%, respectively; the within-herd DD prevalence ranged from 0 to 56%. Poor external biosecurity measures associated with higher prevalence of DD were recent animal purchase, access to pasture, lack of boots available for visitors, farm staff working at other dairy farms as well, hoof trimming without a professional attending, and animal transporters having access to cattle area. For internal biosecurity, higher DD prevalence were associated with infrequent hoof bathing, manure scraping less than 8 times a day, manure removal direction from cows to heifers, animal pens' exit without water hoses, manure-handling vehicle used in other activities, and water troughs contaminated with manure. These findings showed that improvements on biosecurity may be beneficial for controlling DD in dairy herds. The study is relevant for farmers facing problems with DD, as well as hoof trimmers, advisors, and veterinarians, who can use the results for optimized recommendations regarding biosecurity in relation to DD. Furthermore, our results might be considered by future studies investigating DD pathogen reservoirs and transmission routes.
Key words: hoof disorder, dairy cow, biosecurity, digital dermatitis

INTRODUCTION

Digital dermatitis (**DD**) is a major infectious hoof disease occurring worldwide in cattle herds (Laven and Logue, 2006). In Denmark, DD prevalence among dairy cows exceeded 20% (Thomsen et al., 2012b), and DD was present in 85% of dairy herds (Capion et al., 2008). Spirochetes of the genus *Treponema* are most commonly associated with DD lesions (Evans et al., 2012; Zinicola et al., 2015); these painful lesions can cause lameness, reduced milk yield, reproductive problems, and early culling (Bruijnis et al., 2012). Costs associated with DD were approximately $133 per case (Cha et al., 2010); moreover, annual losses attributed to DD in a dairy herd may be the greatest among hoof disorders, as the incidence of clinical cases is high (Bruijnis et al., 2010).

Preventive and curative measures against DD involve antibiotics and other chemicals applied in hoof baths or topically that are not entirely successful (Laven and Logue, 2006; Thomsen et al., 2008b; Berry et al., 2012; Döpfer et al., 2012; Thomsen, 2015). These strategies may cause environmental contamination and expenses associated with treatments and extra labor (Laven and Logue, 2006; Relun et al., 2013a). Furthermore, excessive use of antibiotics may have major negative effects on human and animal health due to the possible association with the development of antimicrobial resistance (Prescott, 2014). The focus on biosecurity to control infections in dairy production has increased including recommendations in general (Villarroel et al., 2007; Brennan and Christley, 2012; Sarrazin et al., 2014), and for specific diseases (Lindberg and Houe, 2005; Ellis-Iversen et al., 2008). The biosecurity concept comprises (1) external biosecurity to avoid introduction and minimize reintroductions of pathogens into a herd, and (2) internal biosecurity to reduce dissemination of pathogens between animals within a herd. Improved biosecurity implementation seems to improve animal

Received March 1, 2017.
Accepted July 1, 2017.
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health, welfare, and productivity, and reduce antibiotic use (Brennan and Christley, 2012; Laanen et al., 2013).

The influences that biosecurity, per se, may have for controlling DD is unclear; however, some conclusions can be drawn from studies of risk factors and potential infection reservoirs to susceptible cows. Regarding external biosecurity, cows in herds that purchased heifers had nearly 3 times higher odds of DD compared with cows in closed herds (Rodriguez-Lainz et al., 1999). Quarantine and hoof examinations before introducing new animals into herds are recommended, as DD was observed in 12.1% of cows presenting at auctions (Hulek et al., 2010). Herds visited by hoof trimmers who also attended other farms had 2.8 times higher odds of increased DD incidence (Wells et al., 1999). The contribution of these visitors' equipment is probably relevant, as DD *Treponema* were detected in 100% of trimming equipment used on cattle affected by DD (Sullivan et al., 2014). Because hoof lesions in other animal species (i.e., sheep, goats, and elk) have etiological, clinical, and pathological similarities to DD (Knappe-Poindecker et al., 2014; Clegg et al., 2015; Knappe-Poindecker, 2015; Sullivan et al., 2015a,b); the participation of these animals in DD circulation has become a concern.

With respect to internal biosecurity, farm hygiene and hoof health management improvements were previously highlighted to mitigate DD risks. They included adequate floor scraping (Somers et al., 2005), access of cows to pasture (Rodriguez-Lainz et al., 1999; Wells et al., 1999; Somers et al., 2005), prophylactic hoof washing and bathing (Rodriguez-Lainz et al., 1999; Thomsen et al., 2012a), and routine trimming with disinfection procedure (Wells et al., 1999; Somers et al., 2005; Relun et al., 2013b). However, many of these measures were not always effective (Holzhauer et al., 2006; Cramer et al., 2009). Given a hypothesis that poor biosecurity implementation in dairy herds is associated with higher prevalence of DD, our objective was to evaluate the relationship between external as well as internal biosecurity measures and within-herd DD prevalence in dairy cattle herds.

MATERIALS AND METHODS

Study Design and Population

This cross-sectional study involved Danish commercial dairy herds visited from January 2015 until July 2016. Selection of herds for recruitment was performed using a list of herds being members of the milk control association (RYK Registrering og YdelsesKontrol, 2015), which comprises over 90% of Danish dairy herds. For practical reasons, only herds located less than a 3-h drive from the Foulum campus of Aarhus University were considered; the majority of Danish dairy herds are located within this area. In addition to having more than 80 lactating cows per year, other inclusion criteria were a freestall housing system with a conventional or carousel milking parlor due to practicalities regarding the method for diagnosing DD (see next section). Our final list for recruitment comprised 310 herds that met the aforementioned inclusion criteria. Farmers were approached in a random order through a letter containing explanations about the study followed by a phone call approximately 1 wk later. We contacted farmers until 50 herd visits were scheduled to achieve the minimum estimated sample size. Written approvals granting access to herd data were acquired from farmers before data collection.

A sample size calculation used the 5% significance level and power of 80% in a 1-sided test. It was based on the comparison of the relative risk between farms with low and high level of biosecurity (i.e., exposed to a different range of biosecurity practices). The prevalence of DD in the reference group was assumed to be 0.25 considering findings from previous reports (Capion et al., 2008; Thomsen et al., 2012b), and the relative risk to be detected was set at 2.5. We referred to Houe et al. (2004) for the formula and assumptions used in calculations. A necessary sample size of approximately 40 herds was estimated.

DD Recordings

Clinical evaluations of hind feet were conducted on all lactating cows during milking in the milking parlor (Thomsen et al., 2008a) using a flashlight and a manual counter as supporting tools. Prior to the herd visits, a single observer (first author) was trained to score DD by an experienced researcher (last author) in 2 dairy herds that were not included in the study.

Washing procedures were performed before evaluating the feet. The observer washed the hind legs of cows after the milking equipment had been attached to the udder to avoid splashing of water contaminated with manure onto the teats during the procedure. Approximately 90% of DD lesions in dairy cows are found in the hind feet (Murray et al., 2002; Relun et al., 2011; Solano et al., 2016), so only the hind feet skin were evaluated. We used the M-stages classification to define DD cases (Döpfer et al., 1997; Berry et al., 2012). Cows were recorded as DD-positive depending on the presence of DD manifestations (M1–M4.1) in at least 1 of their hind feet or DD-negative if cows had normal skin of the hind feet (M0). Cows with unsuitable conditions for examination of hind feet were excluded from the study; that is, inappropriate hoof angles with low height between their accessory digits and the floor or under treatment with hoof bandages. The latter was minimized by scheduling our visits with an interval greater than 2 wk from the last hoof trimming in the herd. The number of lactating cows per herd with missing recordings did not exceed 5% in any herd.

Biosecurity Data Collection

Information about biosecurity was obtained through a questionnaire addressed to farmers and on-farm observations conducted by a single observer (first author). Data regarding breed, herd size, and cattle movements were obtained from the Danish Cattle Database (www.seges.dk).

Farmers received the questionnaire by letter or email 1 wk before the herd visit. Farmers not having completed the questionnaire at the day of the herd visit had an option of sending the completed questionnaire through a free postage envelope. A pilot study in 5 herds assessed the ease of interpretation of the questionnaire, and minor adjustments were made. Data from those 5 herds were included in the main study, as the responses considered for analysis were acquired from questions not modified for the final version of the questionnaire. The questionnaire contained 43 questions, mainly closed and semiclosed, and farmers took from 10 to 15 min to fill it out. The observer used a checklist with 25 items for the on-farm biosecurity measurements, which lasted approximately 30 min per herd. The questionnaire and the checklist are available upon request from the corresponding author.

Both questionnaire and checklist were designed after a review of existing literature as well as discussions among the authors regarding biosecurity in dairy production and strategies for controlling DD. Questionnaire and checklist comprised external and internal biosecurity as main themes. Questions about external biosecurity focused on potential transmission routes of pathogens from one herd to another by contact with an infected host (e.g., via cattle purchased, attendance at shows, and cattle from a neighbor farm) and by indirect contact (e.g., via other animal species, visitors, equipment, vehicles, feed, water, and manure). In relation to internal biosecurity, we asked farmers about adopted management practices that could have an effect on the spread of infectious diseases among cows. This consisted of cleaning and disinfection procedures of equipment and facilities, personal hygiene of farm staff, and strategies for improved hoof health of cows. In addition, the observer made sketches of the facilities, registered housing characteristics, and identified evidence of applied biosecurity that included manure handling, distribution of groups of animals (heifers, cows, and sick cows) among the pens, and routes followed by animals and employees during farm routines.

Statistical Analysis

A database was created in the software Microsoft Access (Microsoft Corp., Redmond, WA) for data entry. Handling of data and statistical analysis were performed in R statistical software version 3.1.2 (R Core Team, 2014). Descriptive statistics included frequency distributions, medians, and interquartile ranges of the outcome variable (within-herd DD prevalence) over the categories of the explanatory variables (biosecurity measures). The within-herd DD prevalence was obtained for each herd by dividing the number of DD-positive cows by the number of evaluated cows for DD in the herd.

A large number of biosecurity explanatory variables (n = 55) were collected through our assessment tools (questionnaire, checklist, and Danish Cattle Database). Thus, the model-building process involved techniques to acquire a lower number of explanatory variables, as proposed by Dohoo et al., (2009). First, we screened variables and grouped categories of some of these variables based on biological plausibility. Second, variables with a nonresponse rate above 20% and with less than 10% of observations in one of its categories were excluded. In total, 10 (out of 21) external biosecurity measures and 13 (out of 34) internal biosecurity measures passed these initial screening steps. Thereafter, we tested unconditional associations between each explanatory variable (one at a time) and the outcome variable (within-herd DD prevalence). This was performed fitting univariable regression models considering a significance level of 0.2. Lastly, possible associations between explanatory variables were inspected using Fisher's exact tests to check for multicollinearity. If a pair of variables showed a significant relationship ($P < 0.05$), we considered for multivariable modeling the variable that resulted in the lower P-value in the univariable analysis.

To facilitate interpretation of the results, explanatory variables were coded based on our hypothesis regarding the implementation of appropriate biosecurity to control DD. Reference categories were assumed to be good biosecurity practices, coded as 0; other categories were assumed to be poorer biosecurity practices, coded as 1 or 2. Explanatory variables maintained for building multivariable logistic regression models for external and internal biosecurity are presented in Table 1 and 2, respectively; the number of observed herds and cows in each category is also shown in Table 1 and 2. Herd size was included as an explanatory variable in both models

Table 1. External biosecurity explanatory variables used for multivariable modeling in a study to evaluate the relationship between biosecurity and digital dermatitis among 8,269 cows in 39 Danish dairy herds

Variable	Category	No. of herds[1] (%)	No. of cows[2]
Last animal purchase	0: >1 yr	29 (76.3)	6,414
	1: ≤1 yr[3]	9 (26.7)	1,709
Access to pasture	0: never	13 (35.1)	3,053
	1: seasonal	24 (64.9)	4,979
Heifer facilities	0: unique herd	19 (50.0)	3,244
	1: separate herds[4]	19 (50.0)	4,879
Farm show	0: not attended	34 (89.5)	7,208
	1: attended	4 (10.5)	915
Boots for visitors	0: available	20 (54.1)	4,357
	1: not available	17 (45.9)	3,410
Farm staff	0: working exclusively	33 (86.8)	7,338
	1: working in another herd	5 (13.2)	785
Hoof trimming performer	0: trained farm person	4 (10.6)	1,145
	1: professional trimmer	17 (44.7)	3,902
	2: both	17 (44.7)	3,076
Vehicle from another farm	0: not used	33 (86.8)	7,247
	1: used	5 (13.2)	876
Pick up of carcasses	0: area distant from the barn	22 (57.9)	5,244
	1: area nearby the barn	16 (42.1)	2,879
Pick up of animals for slaughter	0: no access to barn	14 (36.8)	3,269
	1: access to barn	24 (63.2)	4,854

[1]Not always equal to 39 herds per variable due to missing data.

[2]Not always equal to 8,269 cows per variable due to missing data.

[3]None of the herds adopted quarantine.

[4]Heifers were kept in a rearing unit. One herd commingled cattle from different sources in the rearing unit.

(external and internal biosecurity) due to its assumed relevance for biosecurity, in general, and to account for its potential effect on model outcome. Because of a lack of linearity in the size of herds sampled, tercile (T) ranges (minimum to T1, T1–T2, and T2 to maximum) were used as categories for this variable.

Table 2. Internal biosecurity explanatory variables used for multivariable modeling in a study to evaluate the relationship between biosecurity and digital dermatitis among 8,269 cows in 39 Danish dairy herds

Variable	Analyzed category	No. of herds[1] (%)	No. of cows[2]
Hoof bathing	0: ≥1 time per week	21 (55.3)	3,844
	1: never or <1 time per week	17 (44.7)	4,344
Cows selected for digital dermatitis treatment	0: all cases	21 (55.3)	4,363
	1: only lame cows	17 (44.7)	3,760
Manure scraping frequency	0: ≤4 times per day	16 (43.2)	3,446
	1: >4 and ≤8 times per day	11 (29.7)	2,601
	2: >8 times per day	10 (27.0)	1,948
Manure scraping direction between groups	0: never or from heifers to cows	28 (71.8)	6,042
	1: from cows to heifers	11 (28.2)	2,227
Water hoses at pen exits	0: present	11 (28.2)	2,069
	1: not present	28 (71.8)	6,200
Walking path of animals to milking parlor	0: not crossing feed alley	22 (56.4)	4,170
	1: crossing feed alley	17 (43.6)	4,099
Manure scraping vehicle in other activities	0: not used	11 (29.7)	2,605
	1: used	26 (70.3)	5,390
Manure application	0: on the grazing areas	5 (13.2)	6,762
	1: not on the grazing areas	33 (86.8)	1,361
Manure in water troughs	0: clean	23 (59.0)	4,718
	1: small or large amount	16 (41.0)	3,551
Equipment for handling animals	0: not shared between groups	18 (50.0)	4,003
	1: shared between groups	18 (50.0)	3,588
Hygiene of farm staff between handling different animal groups	0: boots and hands washed	26 (72.2)	6,198
	1: boots and hands not washed	10 (27.8)	1,625

[1]Not always equal to 39 herds per variable due to missing data.

[2]Not always equal to 8,269 cows per variable due to missing data.

A manual backward elimination procedure was adopted to define the final models, which retained variables significantly associated with the outcome ($P < 0.05$). Confounding was assessed by excluding the possible confounding variables from the final models individually with a subsequent odds ratio check. A variable was considered a confounder if its exclusion resulted in a change of more than 20% in at least 1 of the estimated odds ratios of the final model. Interactions were not tested because of the small sample size in relation to the number of explanatory variables. The goodness of fit of the models was tested using Hosmer-Lemeshow statistics (Dohoo et al., 2009).

RESULTS

Descriptive Results

A total of 50 of 99 contacted farmers agreed to take part in the study. The main reasons for declining were lack of interest and concerns about a delayed milking of cows caused by DD recordings. One farm was excluded from analysis because a filled questionnaire was not received. Data from another 10 farms were also not considered because the farmers asked the observer not to wash the feet of cows during the visit.

Our study population included 39 herds from which 8,269 cows were evaluated for DD (mean 212 cows per herd); among these herds, 35 were conventional and 4 were organic. Twenty-two of the herds had Danish Holstein and 17 had other breeds (Jersey or Danish Red) or more than 1 breed. Size of herds ranged from 105 to 673 cows (lactating and dry cows), with a median of 218, mean of 252, and standard deviation ±133. The mean energy corrected milk yield per cow per year was 10,480 kg and standard deviation ±1,020.

Digital dermatitis was present in 97.4% (n = 38) of the herds. The overall prevalence of DD among lactating cows was 24.1% (n = 1,993). Within-herd prevalence of DD ranged from 0% to 56.2% (Figure 1). Descriptive statistics with the distribution of the within-herd DD prevalence across categories of external and internal biosecurity variables maintained in the final multivariable models are shown in Table 3.

Biosecurity Regression Models

Results from the final external and internal biosecurity multivariable logistic regression models are presented in Tables 4 and 5, respectively. Among external biosecurity variables tested, 8 had significant associations with higher within-herd prevalence of DD,

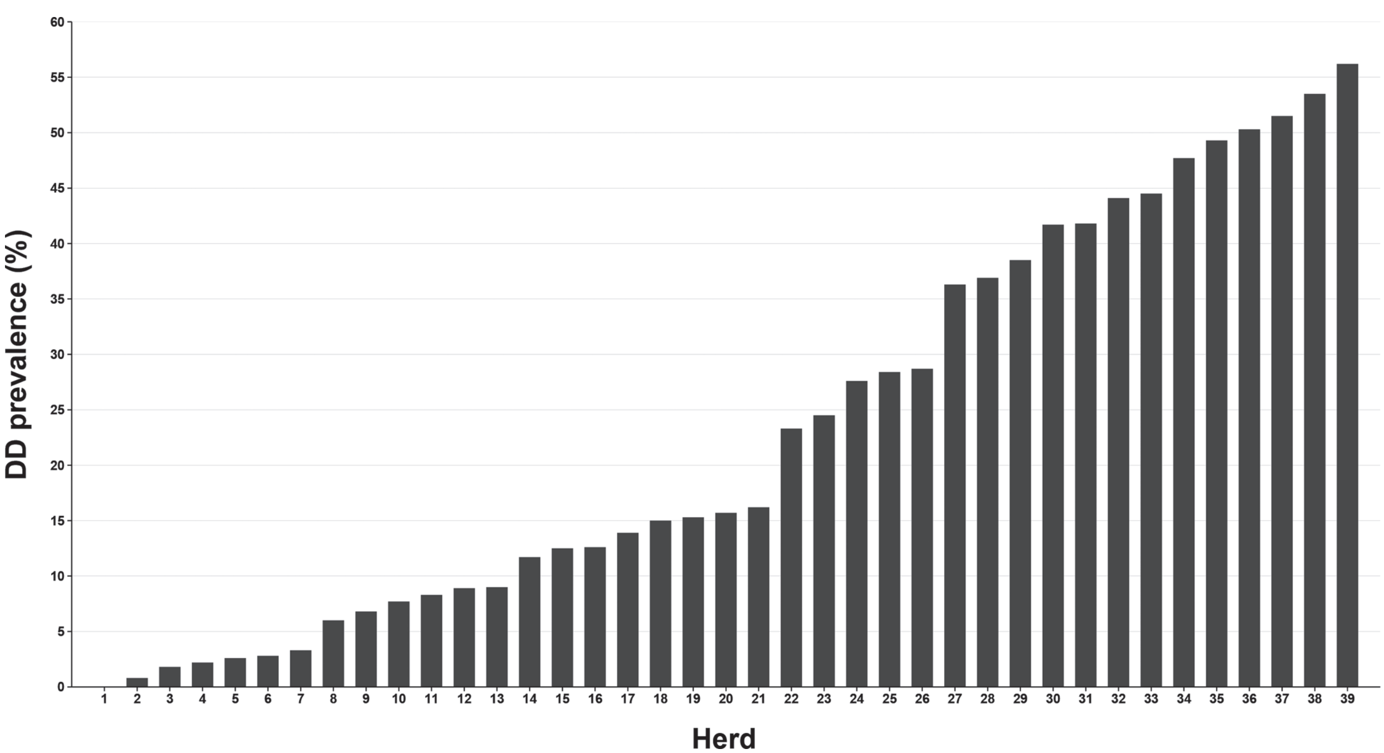

Figure 1. Distribution of the within-herd prevalence of digital dermatitis (DD) in 39 Danish dairy herds included in a study evaluating the associations between biosecurity and DD.

including purchase of cattle in the previous year, access to pasture, housing of heifers in different herds, boots not available for visitors, farm staff working in other dairy farm as well, hoof trimming performed solely by farm personal or professional hoof trimmer (as opposed to both), and truck for transportation of animals to slaughter accessing the barn. In herds that used vehicles from other farms in their facilities, an association with a lower DD prevalence was found.

The final internal biosecurity model had 7 significant explanatory variables. Higher DD prevalence was related to absence or sporadic hoof bathing of cows, low frequency of floor scraping, direction of manure removal in the alleys from cows to heifers, lack of water hoses close to pen exits, use of manure scraping vehicle for other activities, and presence of manure in water troughs. Walking paths of animals to milking parlor crossing the feed alley was associated with a lower DD prevalence.

Associations between herd size and within-herd DD prevalence were detected in both models. Herd size had confounding effects only in the external biosecurity model with the practices farm staff working in another herd as well, last animal purchase ≤1 yr ago, and hoof trimming performed by a trained person from the farm. Comparisons between crude and adjusted odds ratio of these explanatory variables did not change the biological interpretation of their associations with the outcome. The final regression models were well fitted according to the Hosmer-Lemeshow statistics, as results were not significant for the external biosecurity model ($P = 1$) or for the internal biosecurity model ($P = 0.998$).

Table 3. Descriptive statistics of final external and internal biosecurity variables used in 2 separate multivariable regression models in a study to evaluate the relationship between biosecurity and digital dermatitis among 8,269 cows in 39 Danish dairy herds

		Within-herd digital dermatitis prevalence (%)	
Variable	Category	Median	IQR[1]
External biosecurity			
Last animal purchase	>1 yr	12.5	6.0–27.6
	≤1 yr	41.7	36.3–49.3
Access to pasture	Never	15.0	8.3–28.7
	Seasonal	15.5	7.5–37.3
Heifer facilities	Unique herd	12.6	4.6–31.9
	Separate herds[2]	23.3	10.7–41.3
Boots for visitors	Available	9.0	5.3–36.5
	Not available	27.6	13.9–41.7
Farm staff	Working exclusively	15.3	8.3–38.5
	Working in another herd	27.6	6.0–36.3
Hoof trimming performer	Trained farm person	25.5	18.3–29.9
	Professional trimmer	16.2	8.9–38.5
	Both	12.5	7.7–41.8
Pick up of animals for slaughter	No access to barn	15.1	6.7–33.8
	Access to barn	16.0	8.2–41.7
Vehicle from another farm	Not used	23.3	9.0–41.7
	Used	6.8	2.6–8.3
Internal biosecurity			
Hoof bathing	≥1 time per week	16.2	6.8–41.7
	Never or <1 time per week	15.7	11.7–38.5
Manure scraping frequency	≤4 times per day	23.9	10.7–36.9
	>4 and ≤8 times per day	27.6	12.4–45.5
	>8 times per day	7.6	4.0–14.7
Manure scraping direction between groups	Never or from heifers to cows	14.4	7.5–38.1
	From cows to heifers	27.6	14.4–41.3
Water hoses at pen exits	Present	8.9	3.1–21.7
	Not present	23.9	11.0–42.3
Walking path of animals to milking parlor	Not crossing feed alley	15.6	4.0–41.7
	Crossing feed alley	15.7	8.9–28.7
Manure scraping vehicle in other activities	Not used	27.6	13.2–35.2
	Used	15.1	6.2–38.1
Manure in water troughs	Clean	12.6	3.1–32.3
	Small or large amount	28.1	14.3–43.2
Cows selection for digital dermatitis treatment	All cases	23.3	11.7–41.7
	Only lame cows	12.5	3.3–28.4

[1]IQR = interquartile range.

[2]Heifers were kept in a rearing unit. One herd commingled heifers from different sources in the rearing unit.

Table 4. Results of the final multivariable logistic regression model for external biosecurity showing significant variables ($P < 0.05$) associated with digital dermatitis in 39 Danish dairy herds

Variable	Category	OR[1]	95% CI	P-value
Last animal purchase	>1 yr	1	—	<0.001
	≤1 yr	2.55	2.21–2.96	
Access to pasture	Never	1	—	<0.001
	Seasonal	1.43	1.24–1.66	
Heifer facilities	Unique herd	1	—	<0.001
	Separate herds[2]	1.48	1.29–1.71	
Boots for visitors	Available	1	—	0.003
	Not available	1.20	1.06–1.36	
Farm staff	Working exclusively on study herd	1	—	<0.001
	Also working on other herds	1.67	1.33–2.09	
Hoof trimming performer	Both	1	—	<0.001
	Trained farm person	1.44	1.19–1.74	
	Professional trimmer	1.20	1.04–1.38	
Pick up of animals for slaughter	No access to barn	1	—	<0.001
	Access to barn	1.35	1.17–1.56	
Vehicle from another farm	Not used	1	—	<0.001
	Used	0.39	0.29–0.51	
Herd size[3]	Small	1	—	<0.001
	Medium	0.86	0.72–1.04	
	Large	1.61	1.35–1.93	

[1]OR = odds ratio.

[2]Heifers were kept in a rearing unit. One herd commingled heifers from different sources in the rearing unit.

[3]Number of cattle: small (<338), medium (338–520), and large (≥521).

DISCUSSION

Our study evaluated the relationship between biosecurity and within-herd DD prevalence in dairy herds. Our hypothesis was supported by the findings that higher odds of DD was associated with several of the considered external and internal biosecurity measures not applied in dairy herds.

Our chosen method for recording DD in the milking parlor was reliable and less stressful to cows (Thomsen et al., 2008a; Relun et al., 2011; Stokes et al., 2012; Solano et al., 2017) when the intention was to determine DD prevalence; also, it required less cost, time, and labor from the observer compared with the gold standard method of scoring cows in the trimming chute. However, one-fifth of visited herds were excluded from

Table 5. Results of the final multivariable logistic regression model for internal biosecurity showing significant variables ($P < 0.05$) associated with digital dermatitis in 39 Danish dairy herds

Variable	Category	OR[1]	95% CI	P-value
Hoof bathing	≥1 time per week	1	—	0.001
	Never or <1 time per week	1.24	1.09–1.41	
Manure scraping frequency	>8 times per day	1	—	<0.001
	>4 and ≤8 times per day	1.90	1.58–2.27	
	≤4 times per day	2.01	1.68–2.42	
Manure scraping direction between groups	Never or from heifers to cows	1	—	<0.001
	From cows to heifers	1.44	1.26–1.66	
Water hoses at pen exits	Present	1	—	<0.001
	Not present	1.38	1.18–1.62	
Animal walking path	Not crossing feed alley	1	—	0.005
	Crossing feed alley	0.84	0.75–0.95	
Vehicle for manure scraping in other activities	Not used	1	—	0.03
	Used	1.17	1.01–1.35	
Manure in water troughs	Clean	1	—	<0.001
	Small or large amount	1.72	1.52–1.96	
Herd size[2]	Small	1	—	<0.001
	Medium	0.88	0.72–1.08	
	Large	1.22	1.04–1.43	

[1]OR = odds ratio.

[2]Number of cattle: small (<338), medium (338–520) and large (≥521).

our analysis due to impossibility of recording the cows after washing their feet, as requested by milkers. As washing of feet does not stimulate cows' defecation, the concern of milkers was probably related to the novelty (Robichaud et al., 2013).

Recent animal purchases having the highest odds of DD compared with other assessed external biosecurity measures was not surprising. Its confounding effect with herd size was also expected, as herds potentially expanding or recently expanded (that could be the medium-sized and large herds, respectively), are more likely to be open herds. Quarantine procedures were generally not performed by farmers in our study, and the introduction of cattle into herds has been shown to be associated with high odds of DD (Rodriguez-Lainz et al., 1999; Wells et al., 1999). Our result, that access of cows to pasture was associated with higher odds of DD, agrees with others who evaluated cows with restricted pasture access (i.e., for a certain length of time per day; Somers et al., 2005; Holzhauer et al., 2006). Restricted pasture access is a common practice in Denmark, which could explain our findings. Poor walking path for the cows between the pasture and barn, with long distances and unprepared tracks (e.g., without concrete flooring), could be present in the herds (Burow et al., 2014). This might have contributed to a greater exposure of cows to muddy conditions, possibly being related to the observed association. Potential cattle contact with neighboring herds may have affected the odds of DD, though this should be interpreted with caution, as others found protective effects for DD related to full access of cows to pasture (Wells et al., 1999; Bergsten et al., 2015). Furthermore, Holzhauer et al., (2012) found lower odds of DD associated with cows grazing during summer months.

Higher odds of DD were detected when heifers were reared in separate herds. The practice of commingling young stock from different sources in rearing units was not common, and defining its effect on DD prevalence was not possible. Also, biosecurity practices undertaken on these rearing units (not visited) and on the lactating herds were possibly different, which might be relevant for DD status of the herds. With regard to visitors, having boots available is extensively recommended to prevent infectious diseases in cattle herds (Villarroel et al., 2007; Kuster et al., 2015), and this should not be neglected for DD based on our findings. In our study, farm staff also working at other dairy herds appeared as humans possibly contributing to DD cross-contamination between herds. To our knowledge, only hoof trimmers were previously reported as contributing to DD cases in dairy herds (Wells et al., 1999). As a minimum of 2 hoof trimmings per year in dairy herds are recommended with protective effects against DD (Somers et al., 2005; Relun et al., 2013b) and other hoof disorders (Manske et al., 2002; Bruijnis et al., 2010), this practice cannot be overlooked. However, disinfection of equipment should be part of the trimming procedure. This is reinforced by findings of a study that detected DD treponemes in 100% of trimming equipment used in DD-affected cattle; this rate could be reduced to 41% by performing disinfection of the equipment (Sullivan et al., 2014). Our results showed that the greater odds of DD occurred without the participation of hoof trimmers, what could be due to the lack of follow up of DD cases on the herds. Hence, the trimming of cows assisted by the professionals seems to be essential despite the previous report of increased odds of DD associated with them (Wells et al., 1999). The negative effect of visitors responsible for animal transportation is pertinent considering their contact with cattle from several herds that may occur on the same day. Other studies with dairy herds showed that visitors responsible for picking up animals mostly have access to cattle areas, as in our results; moreover, these visitors poorly washed and disinfected their vehicles in addition to inadequate use of protective clothing (Brennan et al., 2008; Nöremark et al., 2010; Sarrazin et al., 2014).

No biologically plausible explanation for the protective effect of using vehicles from other herds could be found. A variable not assessed in our study could have been a confounder influencing this apparently protective association. We assume the few number of farms sharing vehicles (n = 5) might be another reason for the unexpected result.

Among internal biosecurity practices, lack of routine hoof bathing associated with higher odds of DD might be a consequence of high infection pressure in the herds. Hoof bathing was pointed out as a factor reducing the odds of DD previously (Rodriguez-Lainz et al., 1999); likewise, DD treponemes are mainly isolated from hoof lesions (Evans et al., 2012; Klitgaard et al., 2014; Zinicola et al., 2015). An evaluation of hoof bathing strategies was beyond the scope of this study; however, this should be further investigated, as many treatment regimens failed or were based on clinical trials without control groups (Laven and Logue, 2006; Thomsen et al., 2008b; Thomsen, 2015). Furthermore, chemicals commonly used (e.g., copper sulfate, formalin, glutaraldehyde, and acids) have undesirable side effects, such as irritation to both humans and cows when applied, environmental contamination, and carcinogenic effects (Laven and Logue, 2006; Relun et al., 2013a).

Contamination with manure was an important aspect for most of the internal biosecurity variables. Confined dairy cows are constantly exposed to manure, where DD bacteria can be detected (Klitgaard et al., 2014). In the present study, more frequent floor scraping was associated with lower odds of DD. The benefits of having slatted floors compared with solid floors was previously described (Somers et al., 2005). Others considered the scraping frequency and found a negative effect on DD if it is more frequent ($\geq$3 times per day), suggesting that cows may be more exposed to manure (Cramer et al., 2009). Our results showed the lowest odds of DD in herds scraping alley's floor more than 8 times per day. A Danish study failed to find a relationship between scraping frequency and dirty legs on cows (Nielsen et al., 2011). The influence of floor type was not assessed in the current study, as it was not included in the biosecurity models. The manure scraping system was evaluated and might be relevant. This variable had a significant unconditional association with DD, but it was excluded from the analysis to avoid multicollinearity in the multivariable model. We assume manure was removed more frequently when the scraping system in the farm consisted of automatic alley scrapers or robots compared with manual scraping. Manure may also be related to the spreading of DD between cattle housed in different pens (heifers and cows), as the odds of DD varied depending on the direction of manure transport in the barn. It has been documented that cows were more likely to have DD when affected before first calving (Laven and Logue, 2007; Holzhauer et al., 2012). Hence, the transport of manure from cows to heifers may have caused more cases among heifers and, in turn, had an effect on DD prevalence. The interpretation of a shared environment between cows and heifers being bad in terms of biosecurity might be extended to other possible types of indirect contact of cows with DD pathogens. This includes farm workers carrying manure through unwashed boots when water hoses were not present close to each pen exit, or hoses being present and not being used.

We found conflicting results related to practices that might have contributed to contamination of feed with manure; that is, cows crossing the feed alley and sharing of vehicles for handling manure and feed. The latter showed an association with higher odds of DD, whereas the former had a protective effect that could be a consequence of a routine cleaning of manure shed by the animals. Interestingly, the presence of manure in the water troughs showed a stronger association with higher odds of DD compared with those feed variables. To our knowledge, other studies that focused on detection of DD treponemes in the dairy cows' environment (Evans et al., 2012; Klitgaard et al., 2014) did not include water samples. Well-managed herds with hygienic conditions in the facilities may also adopt adequate control measures against DD; clean water may thus be a proxy measure for good hygiene overall. However, the gastrointestinal tract as potential host reservoir of DD and its potential role on DD transmission besides direct contact have been considered. This was based on the identification of DD bacteria in oral cavity, rumen, and rectum of the host (Evans et al., 2012; Nascimento et al., 2015; Zinicola et al., 2015). Therefore, the influences that biosecurity practices might have on diminishing the spread of DD pathogens from those potential infectious sources should not be ignored.

With the cross-sectional study design used, we were unable to imply causality; however, the associations found indicate the importance of biosecurity practices to have a lower within-herd prevalence of DD and serve as valuable information for intervention studies. In the current study, we recruited farmers participating in another study, which could have resulted in a selection bias. The low acceptance rate of recruited farmers (nearly 50%) and the exclusion of 10 herds in which the observer was requested not to wash cows' feet to record DD may also be limitations to our study population. Reasons for farmers' unwillingness could be requirements of extra labor and a procedure causing discomfort to cows (Relun et al., 2013a). Besides this, concerns about udder hygiene caused by washing hind legs before DD recordings during milking might have influenced their decision. Despite the 1.5-year study period, a potential seasonal effect on DD prevalence was not considered. In the study of Bruijnis et al. (2010), DD prevalence did not seem to have substantial changes throughout the year; however, we cannot rule out that month of observation might have had an effect on the prevalence of DD in the herds. Another constraint is a potential bias related to biosecurity recordings; the moment in which data were collected during herd visits may not fully indicate an applied biosecurity measure, as some of these measures could have been undertaken in a specific period of the day and not during the visit by the researcher. Furthermore, attitudes of farmers might change when enrolled in a study. Cardwell et al., (2016) showed decreased risks of diseases for both control and intervention group of farms participating in a longitudinal study about biosecurity. Lastly, farmers that were usually adopting proper control measures against DD in their herds (e.g., treating DD-affected cows) could also be more likely to implement both external and internal biosecurity, which might have influenced the presented findings.

CONCLUSIONS

Improved biosecurity appears to be a relevant strategy for controlling DD in dairy herds, as the lack of implementation of various external and internal biosecurity practices were associated with higher within-herd DD prevalence. Important external biosecurity measures that potentially prevent introduction and intermittent reintroductions of DD into a herd were related to maintenance of a closed herd status and avoidance of possible DD pathogens entries carried by visitors (i.e., farm staff, hoof trimmers, and animal transporters) through boots, vehicles, and equipment. In relation to internal biosecurity, measures that could have restrained dissemination of DD between animals in herds involved periodic group hoof bath treatments, farm hygiene improvements, and manure removal without exposing animals of different groups (cows and heifers) to the same potentially contaminated housing conditions.

ACKNOWLEDGMENTS

The authors are grateful to Mette Holme and Heidi Voss (Aarhus University) for the assistance in the recruitment of farmers. We acknowledge Mari Reiten (Aarhus University) and Nina Otten (University of Copenhagen, Copenhagen, Denmark) for their help with the communication with famers during some farm visits. We thank participating farmers as well as their farm staff. Funding for this study was provided by Aarhus University and Science without Borders scholarship program from CNPq (National Council for Scientific and Technological Development, Brasilia, DF, Brazil).

REFERENCES

Bergsten, C., J. Carlsson, and M. J. Mörk. 2015. Influence of grazing management on claw disorders in Swedish freestall dairies with mandatory grazing. J. Dairy Sci. 98:6151–6162. https://doi.org/10.3168/jds.2014-9237.

Berry, S. L., D. H. Read, T. R. Famula, A. Mongini, and D. Döpfer. 2012. Long-term observations on the dynamics of bovine digital dermatitis lesions on a California dairy after topical treatment with lincomycin HCl. Vet. J. 193:654–658. https://doi.org/10.1016/j.tvjl.2012.06.048.

Brennan, M. L., and R. M. Christley. 2012. Biosecurity on cattle farms: A study in north-west England. PLoS One 7:e28139. https://doi.org/10.1371/journal.pone.0028139.

Brennan, M. L., R. Kemp, and R. M. Christley. 2008. Direct and indirect contacts between cattle farms in north-west England. Prev. Vet. Med. 84:242–260. https://doi.org/10.1016/j.prevetmed.2007.12.009.

Bruijnis, M. R. N., B. Beerda, H. Hogeveen, and E. N. Stassen. 2012. Assessing the welfare impact of foot disorders in dairy cattle by a modeling approach. Animal 6:962–970. https://doi.org/10.1017/S1751731111002606.

Bruijnis, M. R. N., H. Hogeveen, and E. N. Stassen. 2010. Assessing economic consequences of foot disorders in dairy cattle using a dynamic stochastic simulation model. J. Dairy Sci. 93:2419–2432. https://doi.org/10.3168/jds.2009-2721.

Burow, E., P. T. Thomsen, T. Rousing, and J. T. Sørensen. 2014. Track way distance and cover as risk factors for lameness in Danish dairy cows. Prev. Vet. Med. 113:625–628. /https://doi.org/10.1016/j.prevetmed.2013.11.018.

Capion, N., S. M. Thamsborg, and C. Enevoldsen. 2008. Prevalence of foot lesions in Danish Holstein cows. Vet. Rec. 163:80–85. https://doi.org/10.1136/vr.163.3.80.

Cardwell, J. M., S. Van Winden, W. Beauvais, A. Mastin, W. A. De Glanville, J. Hardstaff, R. E. Booth, J. Fishwick, and D. U. Pfeiffer. 2016. Assessing the impact of tailored biosecurity advice on farmer behaviour and pathogen presence in beef herds in England and Wales. Prev. Vet. Med. 135:9–16. https://doi.org/10.1016/j.prevetmed.2016.10.018.

Cha, E., J. A. Hertl, D. Bar, and Y. T. Gröhn. 2010. The cost of different types of lameness in dairy cows calculated by dynamic programming. Prev. Vet. Med. 97:1–8. /https://doi.org/10.1016/j.prevetmed.2010.07.011.

Clegg, S. R., K. G. Mansfield, K. Newbrook, L. E. Sullivan, R. W. Blowey, S. D. Carter, and N. J. Evans. 2015. Isolation of digital dermatitis treponemes from hoof lesions in wild North American elk (*Cervus elaphus*) in Washington State, USA. J. Clin. Microbiol. 53:88–94. https://doi.org/10.1128/JCM.02276-14.

Cramer, G., K. D. Lissemore, C. L. Guard, K. E. Leslie, and D. F. Kelton. 2009. Herd-level risk factors for seven different foot lesions in Ontario Holstein cattle housed in tie stalls or free stalls. J. Dairy Sci. 92:1404–1411. https://doi.org/10.3168/jds.2008-1134.

Dohoo, I., W. Martin, and H. Stryhn. 2009. Veterinary Epidemiologic Research. 2nd ed. VER Inc., Charlottetown, Prince Edward Island, Canada.

Döpfer, D., M. Holzhauer, and M. Van Boven. 2012. The dynamics of digital dermatitis in populations of dairy cattle: Model-based estimates of transition rates and implications for control. Vet. J. 193:648–653. https://doi.org/10.1016/j.tvjl.2012.06.047.

Döpfer, D., A. Koopmans, F. A. Meijer, I. Szakáll, Y. H. Schukken, W. Klee, R. B. Bosma, J. L. Cornelisse, A. J. A. M. van Asten, and A. A. H. M. ter Huurne. 1997. Histological and bacteriological evaluation of digital dermatitis in cattle, with special reference to spirochaetes and *Campylobacter faecalis*. Vet. Rec. 140:620–623. https://doi.org/10.1136/vr.140.24.620.

Ellis-Iversen, J., R. P. Smith, S. Van Winden, G. A. Paiba, E. Watson, L. C. Snow, and A. J. C. Cook. 2008. Farm practices to control *E. coli* O157 in young cattle—A randomised controlled trial. Vet. Res. 39:03. https://doi.org/10.1051/vetres:2007041.

Evans, N. J., D. Timofte, D. R. Isherwood, J. M. Brown, J. M. Williams, K. Sherlock, M. J. Lehane, R. D. Murray, R. J. Birtles, C. Anthony Hart, and S. D. Carter. 2012. Host and environmental reservoirs of infection for bovine digital dermatitis treponemes. Vet. Microbiol. 156:102–109. https://doi.org/10.1016/j.vetmic.2011.09.029.

Holzhauer, M., B. Brummelman, K. Frankena, and T. J. G. M. Lam. 2012. A longitudinal study into the effect of grazing on claw disorders in female calves and young dairy cows. Vet. J. 193:633–638. https://doi.org/10.1016/j.tvjl.2012.06.044.

Holzhauer, M., C. Hardenberg, C. J. M. Bartels, and K. Frankena. 2006. Herd- and cow-level prevalence of digital dermatitis in The Netherlands and associated risk factors. J. Dairy Sci. 89:580–588. https://doi.org/10.3168/jds.S0022-0302(06)72121-X.

Houe, H., A. K. Ersbøll, and N. Toft. 2004. Introduction to Veterinary Epidemiology. 1st ed. Biofolia, Frederiksberg C, Denmark.

Hulek, M., I. Sommerfeld-Stur, and J. Kofler. 2010. Prevalence of digital dermatitis in first lactation cows assessed at breeding cattle auctions. Vet. J. 183:161–165. https://doi.org/10.1016/j.tvjl.2008.11.001.

Klitgaard, K., M. W. Nielsen, H.-C. Ingerslev, M. Boye, and T. K. Jensen. 2014. Discovery of novine digital dermatitis-associated *Treponema* spp. in the dairy herd environment by a targeted deep-sequencing approach. Appl. Environ. Microbiol. 80:4427–4432. https://doi.org/10.1128/AEM.00873-14.

Knappe-Poindecker, M. 2015. Goats, lameness and treponemes—Time for preventive measures? Vet. Rec. 176:281–282. https://doi.org/10.1136/vr.h1224.

Knappe-Poindecker, M., M. Gilhuus, T. K. Jensen, S. Vatn, H. J. Jørgensen, and T. Fjeldaas. 2014. Cross-infection of virulent *Dichelobacter nodosus* between sheep and co-grazing cattle. Vet. Microbiol. 170:375–382. /https://doi.org/10.1016/j.vetmic.2014.02.044.

Kuster, K., M.-E. Cousin, T. Jemmi, G. Schüpbach-Regula, and I. Magouras. 2015. Expert opinion on the perceived effectiveness and importance of on-farm biosecurity measures for cattle and swine

farms in Switzerland. PLoS One 10:e0144533. https://doi.org/10.1371/journal.pone.0144533.
Laanen, M., D. Persoons, S. Ribbens, E. de Jong, B. Callens, M. Strubbe, D. Maes, and J. Dewulf. 2013. Relationship between biosecurity and production/antimicrobial treatment characteristics in pig herds. Vet. J. 198:508–512. https://doi.org/10.1016/j.tvjl.2013.08.029.
Laven, R. A., and D. N. Logue. 2006. Treatment strategies for digital dermatitis for the UK. Vet. J. 171:79–88. https://doi.org/10.1016/j.tvjl.2004.08.009.
Laven, R. A., and D. N. Logue. 2007. The effect of pre-calving environment on the development of digital dermatitis in first lactation heifers. Vet. J. 174:310–315. https://doi.org/10.1016/j.tvjl.2006.07.008.
Lindberg, A., and H. Houe. 2005. Characteristics in the epidemiology of bovine viral diarrhea virus (BVDV) of relevance to control. Prev. Vet. Med. 72:55–73. https://doi.org/10.1016/j.prevetmed.2005.07.018.
Manske, T., J. Hultgren, and C. Bergsten. 2002. The effect of claw trimming on the hoof health of Swedish dairy cattle. Prev. Vet. Med. 54:113–129. https://doi.org/10.1016/S0167-5877(02)00020-X.
Murray, R. D., D. Y. Downham, I. Demirkan, and S. D. Carter. 2002. Some relationships between spirochaete infections and digital dermatitis in four UK dairy herds. Res. Vet. Sci. 73:223–230. https://doi.org/10.1016/S0034-5288(02)00027-9.
Nascimento, L. V., M. T. Mauerwerk, C. L. dos Santos, I. B. Filho, E. H. Birgel Júnior, C. S. Sotomaior, H. M. F. Madeira, and R. D. Ollhoff. 2015. Treponemes detected in digital dermatitis lesions in Brazilian dairy cattle and possible host reservoirs of infection. J. Clin. Microbiol. 53:1935–1937. https://doi.org/10.1128/JCM.03586-14.
Nielsen, B. H., P. T. Thomsen, and J. T. Sørensen. 2011. Identifying risk factors for poor hind limb cleanliness in Danish loose-housed dairy cows. Animal 5:1613–1619. https://doi.org/10.1017/S1751731111000905.
Nöremark, M., J. Frössling, and S. S. Lewerin. 2010. Application of routines that contribute to on-farm biosecurity as reported by Swedish livestock farmers. Transbound. Emerg. Dis. 57:225–236. https://doi.org/10.1111/j.1865-1682.2010.01140.x.
Prescott, J. F. 2014. The resistance tsunami, antimicrobial stewardship, and the golden age of microbiology. Vet. Microbiol. 171:273–278. https://doi.org/10.1016/j.vetmic.2014.02.035.
R Core Team. 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.
Relun, A., R. Guatteo, M. M. Auzanneau, and N. Bareille. 2013a. Farmers' practices, motivators and barriers for adoption of treatments of digital dermatitis in dairy farms. Animal 7:1542–1550. https://doi.org/10.1017/S1751731113000803.
Relun, A., R. Guatteo, P. Roussel, and N. Bareille. 2011. A simple method to score digital dermatitis in dairy cows in the milking parlor. J. Dairy Sci. 94:5424–5434. https://doi.org/10.3168/jds.2010-4054.
Relun, A., A. Lehebel, M. Bruggink, N. Bareille, and R. Guatteo. 2013b. Estimation of the relative impact of treatment and herd management practices on prevention of digital dermatitis in French dairy herds. Prev. Vet. Med. 110:558–562. https://doi.org/10.1016/j.prevetmed.2012.12.015.
Robichaud, M. V., A. M. de Passillé, and J. Rushen. 2013. Does water or blowing air stimulate cows to defecate? Appl. Anim. Behav. Sci. 144:98–101. https://doi.org/10.1016/j.applanim.2013.01.002.
Rodriguez-Lainz, A., P. Melendez-Retamal, D. W. Hird, D. H. Read, and R. L. Walker. 1999. Farm- and host-level risk factors for papillomatous digital dermatitis in Chilean dairy cattle. Prev. Vet. Med. 42:87–97. https://doi.org/10.1016/S0167-5877(99)00067-7.
RYK Registrering og YdelsesKontrol. 2015. RYK Årsberetining. Accessed Jan. 17, 2017. http://epages.nord-ad.dk/nordad/3454/.
Sarrazin, S., A. B. Cay, J. Laureyns, and J. Dewulf. 2014. A survey on biosecurity and management practices in selected Belgian cattle farms. Prev. Vet. Med. 117:129–139. https://doi.org/10.1016/j.prevetmed.2014.07.014.
Solano, L., H. W. Barkema, C. Jacobs, and K. Orsel. 2017. Validation of the M-stage scoring system for digital dermatitis on dairy cows in the milking parlor. J. Dairy Sci. 100:1592–1603. https://doi.org/10.3168/jds.2016-11365.
Solano, L., H. W. Barkema, S. Mason, E. A. Pajor, S. J. LeBlanc, and K. Orsel. 2016. Prevalence and distribution of foot lesions in dairy cattle in Alberta, Canada. J. Dairy Sci. 99:6828–6841. https://doi.org/10.3168/jds.2016-10941.
Somers, J. G. C. J., K. Frankena, E. N. Noordhuizen-Stassen, and J. H. M. Metz. 2005. Risk factors for digital dermatitis in dairy cows kept in cubicle houses in The Netherlands. Prev. Vet. Med. 71:11–21. https://doi.org/10.1016/j.prevetmed.2005.05.002.
Stokes, J. E., K. A. Leach, D. C. J. Main, and H. R. Whay. 2012. The reliability of detecting digital dermatitis in the milking parlour. Vet. J. 193:679–684. https://doi.org/10.1016/j.tvjl.2012.06.053.
Sullivan, L. E., R. W. Blowey, S. D. Carter, J. S. Duncan, D. H. Grove-White, P. Page, T. Iveson, J. W. Angell, and N. J. Evans. 2014. Presence of digital dermatitis treponemes on cattle and sheep hoof trimming equipment. Vet. Rec. 175:201. https://doi.org/10.1136/vr.102269.
Sullivan, L. E., N. J. Evans, R. W. Blowey, D. H. Grove-White, S. R. Clegg, J. S. Duncan, and S. D. Carter. 2015a. A molecular epidemiology of treponemes in beef cattle digital dermatitis lesions and comparative analyses with sheep contagious ovine digital dermatitis and dairy cattle digital dermatitis lesions. Vet. Microbiol. 178:77–87. https://doi.org/10.1016/j.vetmic.2015.04.011.
Sullivan, L. E., N. J. Evans, S. R. Clegg, S. D. Carter, J. E. Horsfield, D. Grove-White, and J. S. Duncan. 2015b. Digital dermatitis treponemes associated with a severe foot disease in dairy goats. Vet. Rec. 176:283. https://doi.org/10.1136/vr.102858.
Thomsen, P. T. 2015. Efficacy of copper sulfate hoof baths against digital dermatitis—Where is the evidence? J. Dairy Sci. 98:2539–2544. https://doi.org/10.3168/jds.2014-9135.
Thomsen, P. T., A. K. Ersbøll, and J. T. Sørensen. 2012a. Automatic washing of hooves can help control digital dermatitis in dairy cows. J. Dairy Sci. 95:7195–7199. https://doi.org/10.3168/jds.2011-5171.
Thomsen, P. T., I. C. Klaas, and K. Bach. 2008a. Scoring of digital dermatitis during milking as an alternative to scoring in a hoof trimming chute. J. Dairy Sci. 91:4679–4682. https://doi.org/10.3168/jds.2008-1342.
Thomsen, P. T., L. Munksgaard, and J. T. Sørensen. 2012b. Locomotion scores and lying behaviour are indicators of hoof lesions in dairy cows. Vet. J. 193:644–647. https://doi.org/10.1016/j.tvjl.2012.06.046.
Thomsen, P. T., J. T. Sørensen, and A. K. Ersbøll. 2008b. Evaluation of three commercial hoof-care products used in footbaths in Danish dairy herds. J. Dairy Sci. 91:1361–1365. https://doi.org/10.3168/jds.2007-0820.
Villarroel, A., D. A. Dargatz, V. M. Lane, B. J. McCluskey, and M. D. Salman. 2007. Suggested outline of potential critical control points for biosecurity and biocontainment on large dairy farms. J. Am. Vet. Med. Assoc. 230:808–819. https://doi.org/10.2460/javma.230.6.808.
Wells, S. J., L. P. Garber, and B. A. Wagner. 1999. Papillomatous digital dermatitis and associated risk factors in US dairy herds. Prev. Vet. Med. 38:11–24. https://doi.org/10.1016/S0167-5877(98)00132-9.
Zinicola, M., F. Lima, S. Lima, V. Machado, M. Gomez, D. Döpfer, C. Guard, and R. Bicalho. 2015. Altered microbiomes in bovine digital dermatitis lesions, and the gut as a pathogen reservoir. PLoS One 10:e0120504. https://doi.org/10.1371/journal.pone.0120504.