



## Farm-level risk factors for digital dermatitis in dairy cows in mountainous regions

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### ABSTRACT

Reduction of risk factors for bovine digital dermatitis (BDD) is crucial in current disease control. However, risk factors that might arise especially in mountainous regions are unknown until now, and an adapted BDD control program is consequently missing. The objective of this observational case-control study was to identify farm-level risk factors for BDD in dairy herds in mountainous regions. To investigate predictors for the occurrence of BDD, 100 farms were visited and information about herd characteristics and management practices, potentially relevant explanatory variables for either introduction or establishment of BDD, were gathered by completing a questionnaire with the farmer or herd manager. Within-herd prevalences of BDD assessed during 3 routine claw trimmings with an interval of 6 mo before the survey were used to define cases (BDD within-herd prevalence of  $\geq 26\%$  during each claw trimming) and controls (no BDD cases in each of the 3 routine claw trimmings before the survey). Data were analyzed using 2 separate binomial generalized linear models according to either establishment or introduction of BDD. After prescreening, 15 of 23 explanatory variables were included in the final analysis, which showed 3 variables related to introduction and establishment, each being significantly associated with the occurrence of BDD within a farm. Results of model 1 (i.e., aspects related to BDD introduction) revealed that access to mountain pastures during the summer season (odds ratio, 95% confidence interval: 0.12, 0.04–0.35), participation in dairy shows (0.32, 0.11–0.94), and the number of new animals introduced into the farm during the last 2 yr (1.28, 1.12–1.52) were significantly associated with the occurrence of BDD. Model 2 (i.e., aspects related to

BDD establishment) showed that cows kept in freestalls were at higher risk for BDD compared with those kept in tiestalls (20.65, 1.59–649.37). Furthermore, number of days between diagnosis and treatment of a BDD lesion (10.31, 3.55–81.21) and the amount of concentrate feeding (median 5 kg) per cow and day (7.72, 2.46–6.47) were positively associated with BDD occurrence. In conclusion, the findings of this study provide a set of risk factors that are associated with BDD status within herds in mountainous regions. These results may help in development of adapted control programs for BDD in dairy cows.

**Key words:** biosecurity, cattle, lameness, treponemes

### INTRODUCTION

Bovine digital dermatitis (BDD) is one of the most important infectious diseases in dairy cows worldwide, responsible for substantial economic losses and detrimental effects on ruminant welfare (Losinger, 2006; Gomez et al., 2015; Evans et al., 2016; Plummer and Krull, 2017). It is characterized by ulcerative or proliferative skin lesions that are typically located at the plantar aspect of the interdigital cleft and can result in severe lameness (Evans et al., 2016; Orsel et al., 2018; Plummer and Krull, 2017). The contagious nature and unsatisfactory treatment responses of BDD (Ariza et al., 2017; Capion et al., 2018; Orsel et al., 2018) result in prevalences of up to 91% at the herd level and 41% at the cow level (Rodriguez-Lainz et al., 1998; Cruz et al., 2001; Relun et al., 2013; Jury et al., 2021; Kofler et al., 2022). In the United States, up to 75% of dairy farms and approximately 19% of the dairy cows are affected with BDD (UW-Extension Dairy Team, 2016; USDA, 2018). Diagnosis of BDD is usually based on visual inspection of the feet using the Mortellaro (M) scoring system by Döpfer et al. (1997) and modified by Berry et al. (2012), where active (M1/M2/M4.1), healing (M3), chronic (M4), and healed stages without signs of pre-existing lesions (M5; now more commonly

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referred to as M0 lesions) are differentiated. Bovine digital dermatitis is considered a multifactorial disease with the exact pathogenesis remaining unclear (Wilson-Welder et al., 2015; Evans et al., 2016). *Treponema* spp. are generally considered to be the central etiological agents (Evans et al., 2008; Alsaad et al., 2019; Kuhnert et al., 2020).

In recent years, numerous studies have been conducted to identify risk factors for the occurrence of BDD at both the farm and cow levels (Palmer and O'Connell, 2015). At the cow level, the main risk factors are breed, parity, lactation stage, and interindividual differences in immune and inflammatory response (Somers et al., 2005; Holzhauser et al., 2006; Relun et al., 2013; Schöpke et al., 2015). At the farm level, identified risk factors include, among others, housing and flooring type, nutrition, and aspects of external and internal biosecurity (Somers et al., 2005; Becker et al., 2014; Gomez et al., 2014; Oliveira et al., 2017; Yang et al., 2019). However, further potential risk factors that might arise when dairy farming is practiced under unconventional conditions have not yet been investigated. In mountainous regions, small-scale farms are more frequent, and cows are commonly kept on communal mountainous pastures during the summer season (Gordon et al., 2013; van den Borne et al., 2017). Seasonal movement of livestock to mountain pastures in response to the variability of environmental resources is practiced in various European countries such as France, Italy, Austria, and southern Germany (Santini et al., 2013; Sturaro et al., 2013; Fürstl-Waltl et al., 2019). Farms with small herd sizes and lower-yielding breeds can also be found in many regions of Central and South America (e.g., Peru, Mexico, and Brazil), where those small-scale producers contribute to the vast majority of milk production (Aubron et al., 2009; Bartl et al., 2009; Avendaño-Reyes et al., 2020).

Tiestall systems are also prevalent in other countries outside mountainous regions, accounting for ~73% and ~41% of primary housing forms in Canadian and American dairy operations, respectively (USDA, 2018; CDIC, 2020). Moreover, there is a greater extent of animal traffic (i.e., sharing or exchange of genetically valuable animals with other farms, rearing of youngstock in other farms with subsequent reintroduction into the herd, frequent participation in dairy cow shows) compared with other countries (Gordon et al., 2013; van den Borne et al., 2017).

The objective of our study was to identify farm-level risk factors for BDD in Swiss dairy herds as an example of small-scale farms located in mountainous regions. We hypothesized that specific aspects of herd characteristics, herd management, and claw health manage-

ment are associated with high BDD prevalences within these herds.

## MATERIALS AND METHODS

Reporting of this study is conducted according to the “Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)” guidelines (von Elm et al., 2007). Ethical approval was not needed for this study because no animal procedures were performed.

### Study Design and Farm Selection

As part of a government-initiated project to improve claw health of cattle in Switzerland, information about the occurrence of foot and claw disorders is continuously collected by professional claw trimmers using a digital recording software (KLAUE, dsp-Agrosoft GmbH). To minimize interobserver bias, only data collected by specially trained claw trimmers were included. As described by Strauss et al. (2021), training consisted of individual education sessions to classify and digitally record foot and claw disorders according to the ICAR Claw Health Atlas. The ICAR Claw Health Atlas was developed by a consortium of international claw health experts (Egger-Danner et al., 2020; available online at [https://www.icar.org/ICAR\\_Claw\\_Health\\_Atlas.pdf](https://www.icar.org/ICAR_Claw_Health_Atlas.pdf)). At the end of the training course, claw trimmers' ability to allocate the correct diagnoses to certain claw disorders was evaluated using pictures from the ICAR Claw Health Atlas or by examination of affected animals in the trimming chute. Only data of claw trimmers that reached sufficient agreement ( $\kappa$ -values  $\geq 0.61$ ) with the veterinarian who conducted the training (and who in turn had been trained by a member of the ICAR consortium) were included. Within-herd prevalences of BDD assessed during routine claw trimmings were available from 689 Swiss dairy herds during an 18-month period (January 2020 to June 2021). A subset of these farms was enrolled in a multicenter, observational, unmatched case-control study. Sample size was calculated using a web-based calculator (<https://riskcalc.org/samplesize/>). To detect a practically relevant odds ratio (OR) of  $\geq 3.2$  with a statistical power of 0.8,  $\alpha = 0.05$ , and assuming that at least 30% of the control farms are exposed to the factor of interest, a minimum of 49 analyzable case and 49 analyzable control farms was necessary. This approach was based on Thrusfield et al. (2001) and Houe et al. (2004).

Inclusion criteria were a minimum herd size of 20 lactating cows (to include farms representing typical regional conditions) and biannual preventive claw trimming of the entire herd (i.e.,  $\geq 95\%$  of the cows)

adapted to Toussaint Raven (1989). Occurrence of claw disorders other than BDD did not exclude a farm from the study.

### Case and Control Definitions

To ensure objective definitions for cases and controls, we performed a 3-step selection process. After implementing the general inclusion criteria mentioned above (step 1), we preselected herds showing the highest BDD within-herd prevalences and those showing the lowest BDD within-herd prevalences (estimated at the herd level for each farm separately) during the most recent routine claw trimming using the third and first quartile, respectively (step 2). Finally, 50 herds each were randomly selected from those above the third quartile and below the first quartile, respectively (step 3). For randomization, a random selection function in Excel was used (Microsoft Corp.). With regard to assessment of BDD within-herd prevalences, cows were deemed affected by BDD if they showed BDD lesions in  $\geq 1$  foot, regardless of the lesion stage. Cows without lesions were considered BDD-negative. On the basis of this approach, herds revealing a BDD within-herd prevalence of  $\geq 26\%$  (range 26–63%) during each of the most recent 3 routine claw trimmings were considered case farms. Farms without a BDD problem (defined as no BDD cases within the previous 18 mo) served as control farms.

### Data Collection

To obtain information on potential risk factors, farms were visited and a questionnaire was completed by the first author together with the farmer or herd manager. Farm visits took place with a maximum time lag of 2 mo after the most recent biannual routine claw trimming of the entire herd. In this context, BDD within-herd prevalences were digitally recorded by the claw trimmers as described above. Informed written consent was given by the farmer. The questionnaire was created based on 23 items dealing with herd characteristics and management practices for either introduction (aspects of external biosecurity) or establishment (aspects of internal biosecurity) of BDD at the farm-level (Supplemental Table S1; [https://figshare.com/articles/dataset/Suppl\\_Tab1\\_weber\\_docx/21603738](https://figshare.com/articles/dataset/Suppl_Tab1_weber_docx/21603738); Weber, 2022). Items were thereby determined using the results of previous studies dealing with herd-level risk factors for BDD and based on the knowledge and experience of claw health experts. Questions referred to the previous 2 yr to gather current information and to reduce the risk for recall bias. As this study was part of an overall project, information regarding 8 of the 23 items collect-

ed via the questionnaire was also recorded by the claw trimmers twice a year during routine claw trimmings (Supplemental Table S1). We rechecked that there were no inconsistencies between this data set and information provided by the farmers during the farm visits. Additionally, information about herd size, predominant breeds, and average 305-d milk yields of the herds were extracted from a centralized national livestock register. Data were transferred to Excel (Microsoft Corp.).

### Statistical Analyses

For statistical analyses, SPSS Statistics 25 (IBM Corp.) and R (<https://www.r-project.org/>) software were used. Categorical variables were described by frequency distributions, and continuous data were summarized by the mean  $\pm$  standard deviation (SD) or median and range. Normal distribution was checked using histograms and the Shapiro-Wilk test.

Associations between the BDD status of the herds as binary outcome (case vs. control) and each explanatory variable were analyzed in an univariable logistic regression model. Only variables significant at  $P \leq 0.05$  and that showed no collinearity were considered for further analyses. If a correlation was present between 2 potential explanatory variables, we discarded the variable with lower biological relevance. Variables were regarded as correlated when  $\varphi$  (mean square contingency coefficient  $\varphi$ ) was  $> 0.6$ . Furthermore, variables revealing  $> 10\%$  of missing observations were excluded. After this prescreening, we decided to build 2 separate models for potential risk factors for either establishment or introduction of BDD using the lme4 package in R. In both models, the binary outcome was being a case farm (BDD within-herd prevalence of  $\geq 26\%$ ) or a control farm (no BDD cases within the previous 18 mo) using the following formula:

$$\text{Ln} \left( \frac{P_i}{1 - P_i} \right) = \beta_0 + \beta_1 (\text{var}1_i) + \beta_2 (\text{var}2_i) + \dots + \beta_x (\text{var}x_i),$$

where  $P_i$  is the probability of being a case farm,  $\beta_0$  is the intercept from the linear regression equation, and  $\beta_1$  to  $\beta_x$  are the regression coefficients corresponding to the independent explanatory variables  $\text{var}1_i$  to  $\text{var}x_i$ . The experimental unit of the analysis was the farm, as data were collected at the farm level. Correspondingly, binomial generalized linear models were fitted to the data to identify associations between BDD herd status as dependent variable and potential predictors (Dohoo et al., 2009). Associations were expressed as OR and their respective 95% confidence intervals (95% CI), which were calculated from the parameter estimates

of the model. To optimize model fit, we applied stepwise backward elimination using the stepAIC() function from the MASS package (<https://cran.r-project.org/web/packages/MASS/MASS.pdf>). Model fit was assessed, looking for the lowest value of Akaike information criterion (Akaike, 1969), by calculating Tjur's  $R^2$  (Tjur, 2009) and by visual assessment of residuals. Significance level for models was set at  $P \leq 0.05$ .

## RESULTS

### Descriptive Statistics

In total, 50 case and control farms each were included in the final analysis. Holstein Friesian was the predominant breed in 51 farms (51%) and Brown Swiss in 34 farms (34%). Breeds found in the remaining farms were Swiss Fleckvieh, Simmental, Montbéliarde, and Jersey. The average 305-d milk yield of the herds was  $8,258 \pm 1,331$  kg. Of the 23 explanatory variables, 6 variables revealed missing values of  $>10\%$  (i.e., commingling during mountain pasturing, flooring type, control of newly introduced animals, floor hygiene, bandage application). Furthermore, the variable "305-d milk yield" was discarded as it correlated with the variable "concentrate feeding." Similarly, the number of farms cows were recruited from correlated with the number of animals that had been purchased during the previous 2 yr and was removed. After this prescreening, 15 variables remained. Table 1 provides an overview of descriptive results, showing all variables screened by univariable analysis and their distribution over the study population.

### Regression Analysis with Explanatory Variables

Univariable analysis resulted in 8 variables that were significantly associated with being a case or a control farm (Table 1). Regarding items related to the introduction of BDD, mountain pasturing, participation in dairy shows, and the number of purchased cattle during the previous 2 yr were significantly associated with the occurrence of BDD within the farm. Considering factors responsible for the establishment of BDD, herd size, housing type, preventive measures against BDD, average time between diagnosis and treatment, and amount of concentrate fed showed significant associations with the occurrence of BDD.

Results of the final multivariable regression models are presented in Table 2. Based on the outcome of the model for BDD introduction (model 1), access to mountainous pastures during the summer season was negatively associated with the odds of a herd being affected with BDD (OR, 95% CI: 0.12, 0.04–0.35;  $P$

$< 0.001$ ). Likewise, attendance at dairy shows was negatively associated with the odds for the occurrence of BDD within the herd (0.32, 0.11–0.94;  $P = 0.039$ ). In contrast, the odds for occurrence of BDD showed a positive association with the number of new animals brought to the farm during the previous 2 yr (1.28, 1.12–1.52;  $P = 0.001$ ). Tjur's  $R^2$  value of 0.65 indicated a moderate separation between the predicted values for cases and controls. Among the predictors including items of BDD establishment within the farm (model 2), 3 of 5 variables were significantly associated with the occurrence of BDD in the final model. Farms where cows were kept in freestalls had higher odds of being case farms (i.e., BDD within-herd prevalence of  $\geq 26\%$ ) compared with farms using tiestall housing (20.65, 1.59–649.37;  $P = 0.039$ ). Furthermore, the occurrence of BDD was significantly associated with an increasing number of days between diagnosis and treatment of a BDD lesion (10.31, 3.55–81.21;  $P = 0.002$ ), as well as an increasing amount of concentrate feeding that was delivered per cow and day (7.72, 2.46–6.47;  $P = 0.010$ ). Tjur's  $R^2$  value of 0.88 indicated good separation between the predicted values for cases and controls.

## DISCUSSION

Even though ample research in the area of etio-pathogenesis has been conducted over the last decades, BDD is still a problem in almost all countries with intensive dairy production. The main reason for this might be that some aspects of pathogenesis such as immunological mechanisms or pathogen–host interactions in BDD-affected animals are still not fully understood (Wilson-Welder et al., 2015; Evans et al., 2016; Weber et al., 2019). Thus, identification and consequent reduction of risk factors remain mainstays in current disease control. We could identify 6 of 23 potential predictors at farm-level that were significantly associated with the occurrence of BDD. To our knowledge, this is the first study describing potential risk factors for BDD in small-scale dairy farms located in mountainous regions.

### Farm-Level Predictors for BDD Related to Disease Introduction

Although previous studies investigating seasonal mountain pasturing as a potential risk factor for BDD are lacking, several authors have described associations between pasturing and the occurrence of BDD with inconsistent results. While our finding that access to pastures resulted in a protective effect for BDD is in line with observations of studies where cows were grazed both restricted and over the entire year (Wells et al., 1999; Somers et al., 2005; Holzhauer et al., 2012; Berg-

**Table 1.** Summary of explanatory variables related to either introduction or establishment (i.e., external or internal biosecurity) of bovine digital dermatitis (BDD) screened by univariable analysis for their association with BDD herd status (case vs. control),<sup>1</sup> based on data collected on 100 small-scale dairy farms located in mountainous regions

Variable <sup>2</sup>	Farms <sup>3</sup> (n/N; %)		P-value
	Case	Control	
<b>BDD introduction</b>			
Mountain pasturing			<0.001
No	34/50; 68	11/50; 22	
Yes	16/50; 32	39/50; 78	
Participation in dairy shows			<0.001
No	40/50; 80	18/50; 36	
Yes	10/50; 20	32/50; 64	
Rearing on other farms			0.067
No	26/50; 52	35/50; 70	
Yes	24/50; 48	15/50; 30	
No. of purchased cattle during the previous 2 yr <sup>4</sup>			<0.001
Median (Q1–Q3)	6 (3–11)	2 (0–4)	
Range	0–24	0–15	
Disinfection of claw trimming equipment			0.126
No	39/50; 78	32/50; 64	
Yes	11/50; 22	18/50; 36	
<b>BDD establishment</b>			
Herd size <sup>4</sup>			<0.001
Median (Q1–Q3)	45 (34–60)	28 (22–34)	
Range	20–108	20–61	
Breed			0.058
Holstein Friesian	38/50; 76	13/50; 26	
Braunvieh	7/50; 14	27/50; 54	
Swiss Fleckvieh	2/50; 4	6/50; 12	
Other	3/50; 6	4/50; 8	
Participation in the RAUS program <sup>5</sup>			0.423
No	8/50; 16	10/50; 20	
Yes	42/50; 84	40/50; 80	
Housing type			0.001
Tiestall	3/50; 6	22/50; 44	
Freestall	41/50; 82	25/50; 50	
Both	6/50; 12	3/50; 6	
Frequency of claw trimming per year			0.980
<1×	2/50; 4	0/50; 0	
1–2×	43/50; 86	49/50; 98	
≥3×	5/50; 10	1/50; 2	
Age at first claw trimming (mo)			0.764
<12	2/50; 4	3/50; 6	
12–24	17/50; 34	34/50; 68	
>24	31/50; 62	13/50; 26	
Preventive measures against BDD			0.002
No	32/50; 64	49/50; 98	
Yes	18/50; 36	1/50; 2	
Medical treatment of acute BDD lesions			0.245
Oxytetracycline/chlortetracycline	13/50; 26	7/40; 17.5	
Keratinolytic paste <sup>6</sup>	12/50; 24	20/40; 50	
Other	25/50; 50	13/40; 32.5	
Time between diagnosis and treatment <sup>4</sup> (d)			<0.001
Median (Q1–Q3)	7 (5–7)	3 (1–3)	
Range	3–14	1–7	
Concentrate feeding <sup>4</sup> (kg per cow and d)			<0.001
Median (Q1–Q3)	5.0 (4.0–5.0)	2.25 (1.0–3.5)	
Range	0.5–7.5	0.0–6.5	

<sup>1</sup>Case farms (classified as “1”) revealed BDD within-herd prevalences ≥26% and control farms (classified as “0”) revealed no BDD cases during the previous 18 mo.

<sup>2</sup>Q1 = 25th percentile; Q3 = 75th percentile; RAUS = regular access to outdoors.

<sup>3</sup>Applies only to categorical variables.

<sup>4</sup>Metric variable.

<sup>5</sup>Regular access to pastures (>26 d/mo) from May 1 to October 31; regular access to pastures or regular exercise in an outdoor pen (>13 d/mo) from November 1 to April 30.

<sup>6</sup>Containing 660 mg/g salicylic acid and 7.7 mg/g methylsalicylate (Novaderma, Streuli).

**Table 2.** Results of final binomial generalized linear regression models on the bovine digital dermatitis (BDD) herd status (case vs. control)<sup>1</sup> dependent on risk factors for either introduction or establishment of the disease, based on data collected on 100 small-scale dairy farms located in mountainous regions

Item	$\beta^2 \pm SE$	Odds ratio (95% CI)	<i>P</i> -value
Introduction of BDD (model 1)			
Mountain pasturing			
No	Referent	1	
Yes	$-2.10 \pm 0.56$	0.12 (0.04–0.35)	<0.001
Participation in dairy shows			
No	Referent	1	
Yes	$-1.13 \pm 0.55$	0.32 (0.11–0.94)	0.039
No. of purchased cattle during the previous 2 yr	$0.25 \pm 0.08$	1.28 (1.12–1.52)	0.001
Establishment of BDD (model 2)			
Housing type			
Tiestall	Referent	1	
Freestall	$3.03 \pm 1.47$	20.65 (1.59–649.37)	0.039
Both	$0.85 \pm 2.08$	2.34 (0.04–192.75)	0.681
Time between diagnosis and treatment (d)	$2.33 \pm 0.75$	10.31 (3.55–81.21)	0.002
Concentrate feeding (kg per cow and d)	$2.04 \pm 0.79$	7.72 (2.46–6.47)	0.010

<sup>1</sup>Case farms (classified as “1”) revealed BDD within-herd prevalences  $\geq 26\%$  and control farms (classified as “0”) revealed no BDD cases during the previous 18 mo.

<sup>2</sup> $\beta^2$  = parameter estimate.

sten et al., 2015), others found higher odds for cows to suffer from BDD when they had access to pasture (Holzhauer et al., 2006; Oliveira et al., 2017). Caution is warranted when comparing these results, because comparability is impaired among the different studies. Various durations during which animals were pastured (e.g., seasonal vs. year-round with different numbers of hours per day) or the character of pastures (i.e., ground condition and stocking density) as well as walking paths to reach them are possible sources of inter-study variation (Burow et al., 2014; Oliveira et al., 2017). Furthermore, different herd characteristics and management practices between these studies enhance heterogeneity. Because mountain pastures are extensive areas with low stocking rates to protect the local biodiversity, infection pressure is probably low; therefore, the risk for either introduction or establishment of BDD within the farm is likely limited (Dumont et al., 2007). As BDD-associated treponemes are primarily transmitted via direct skin-to-skin contact and via feces between the animals, it can be assumed that there is a reduced risk for disease transmission on pastures with decreased stocking densities (Evans et al., 2016).

The association of attending dairy shows with a lower likelihood of occurrence of BDD, as observed in the present study, could be a reverse effect. Hence, farmers who exhibit their animals at such shows may put more emphasis on animal health and cow cleanliness, and exhibition of cows suffering from active BDD lesions is, therefore, unlikely. Consequently, the risk for disease transmission will also be small. Participation in dairy shows as a potential risk factor was only investigated

in the study by Oliveira et al. (2017); however, they could not find an association between this variable and high within-herd BDD prevalence. It would be useful to include information concerning show type (regional vs. national vs. international), duration, and frequency of attendance in further studies.

The results of our study were in line with previously published literature that recent animal purchase increases the odds for the occurrence of BDD (Rodriguez-Lainz et al., 1999; Wells et al., 1999; Oliveira et al., 2017; Yang et al., 2019). The study of New Zealand differentiated between purchased heifers and adult cows and found only an association between the purchase of heifers and the probability of a farm being BDD-positive for this result (Yang et al., 2019). They mentioned much lower numbers of purchased cows compared with heifers as the main reason for this finding. However, this cannot be extrapolated to dairy farming practices in mountainous regions, where the purchase of both youngstock and genetically valuable adult animals is common. Animal purchase is of particular importance on small-scale farms and may be difficult to restrict. Implementing quarantine of appropriate duration as well as strict inspection of new animals brought onto the farm would likely reduce the risk for BDD introduction, and the present results stress their application (Bergsten et al., 2016). Even if rearing on other farms only revealed a tendency to be a risk factor for BDD in the present study, it should be considered a potential source of disease introduction; it is recommended to inspect and quarantine animals when they are reintroduced into the farm of origin (Oliveira et al., 2017).

### **Farm-Level Predictors for BDD Related to Disease Establishment**

Interestingly, the literature lacks studies investigating associations between tiestall housing and the occurrence of BDD, despite their prevalence outside mountainous regions as mentioned above. Other authors described a higher risk for the occurrence of BDD in cows housed in cubicles than in animals kept in straw yards or that had access to pasture (Laven, 1999; Somers et al., 2005; Onyiro et al., 2008). One possible explanation for our finding is a conceivably lower exposure to manure of cows in tiestalls that is in accordance with the results published by Ostojić-Andrić et al. (2011), who found improved lower-leg hygiene scores in dairy cows kept in tiestalls. In contrast, animals that can move freely tend to have more slurry on their legs and their feet are exposed to a moist environment, both of which are known factors for a higher risk of occurrence of BDD (Relun et al., 2013; Palmer and O'Connell, 2015). However, because other studies found that freestalls were associated with improved stall hygiene compared with tiestalls, this aspect remains speculative (Herlin et al., 1994). Furthermore, transmission of BDD between cows might be lower if crossing of walking paths is limited, as it can be assumed to apply for tiestall systems.

As far as we know, average time between diagnosis and treatment of a BDD lesion was not previously described as a farm-level risk factor, although its relevance is not restricted to dairy farming in mountainous regions. Our results indicate that for each additional day between diagnosis and treatment, the odds for having a BDD problem increases by a factor of 9. Since treponemes spread from BDD lesions as mentioned before, it appears biologically plausible that immediate treatment of the lesions could significantly reduce infection pressure (Evans et al., 2016). It has to be emphasized that in addition to active M2 lesions, chronic M4 stages contribute to disease transmission, as they are present for most of the animal's infection time and can revert to M4.1 stages (Berry et al., 2012; Biemans et al., 2018). Therefore, immediate treatment of both active and chronic BDD lesions represents an important target in disease control. Although it would have been desirable to include information about bandage application (bandage should consist of sterile gauze swab, cotton wool, elastic bandage, and waterproof outer layer; Klawitter et al., 2019; Alsaad et al., 2022) and the animal selection procedure for BDD treatment in our analysis, these items had to be excluded based on missing data or because the validity of the data could not be ensured. Even though there is a definite risk of recall bias when asking for this time period, our result might help to develop concepts for BDD control.

Only one study, conducted by Somers et al. (2005), has examined concentrate feeding as a potential risk factor for BDD. The authors found that a rapid increase in concentrate supplementation of cows within less than 2 wk postpartum was associated with the occurrence of BDD compared with animals where the maximum amount of concentrate feed was delivered after 2 to 3 wk postpartum. This is, in principle, in accordance with our results, although we did not only focus on concentrate supplementation during the postpartum period. One reason for this could be due to increased metabolic stress arising from SARA, which triggers chronic inflammatory responses with subsequent immune suppression in affected cattle (Oetzel, 2017). Furthermore, mineral absorption and biotin production can be impaired in cows suffering from SARA (Oetzel, 2017; Goff, 2018). This could also explain the results of our study, because supplementation of minerals and biotin has been shown to improve immune response and healing of BDD lesions (Lean and Rabiee, 2011; Gomez et al., 2014; Zhao et al., 2015). However, its pathogenesis is not fully characterized and a potential association with BDD onset remains unclear. Therefore, further studies are warranted to clarify the effect of metabolic imbalances on the cow's susceptibility to BDD. Another explanation might be that supplementation of concentrate feeding may lead to a more fluid fecal consistency due to dysfermentation and subsequent environmental contamination with slurry and impaired leg cleanliness (Nordlund et al., 2004).

### **Methodological Strengths and Limitations**

Implementation of stringent inclusion criteria to select case and control farms in a multistage process resulted in a limited number of eligible farms. As a consequence, only strong associations may have been detected, whilst smaller effects might remain undetected. This approach was necessary to ensure typical dairy farming conditions in mountainous regions and to produce robust results, because valid prevalence data can only be obtained from farms where the vast majority (or all cows) are subjected to regular claw trimmings. However, we can assume that BDD within-herd prevalence changed slightly between biannual functional claw trimmings, even though some authors suggest that there are no significant alterations in the occurrence of BDD over the year (Bruijnjs et al., 2010; Oliveira et al., 2017). Therefore, farmers of control herds were also able to provide information regarding aspects of BDD management. It is common practice that individual cows showing BDD lesions are treated by the farmer (and documented in writing but not recorded digitally) between the routine claw trimmings of the entire herd. To

minimize the risk for changes in BDD status and management practices during the period between the most recent prevalence assessment and the farm visit, this time lag was limited to a maximum of 2 mo. Furthermore, as there was no possibility per se for blinding of the participants regarding their BDD herd status, this could have resulted in preferable answers or changed attitudes when completing the questionnaire. This may have led to an underestimation of true effects, although farmers were informed that these data would be treated anonymously and that there would be no negative consequences regardless of their answers. In general, the quality of data collected via a questionnaire might be impaired in some cases because individual questions remain subjective (Hassenstein and Vanella, 2022).

The use of percentiles based on the distribution of BDD within-herd prevalences over prescreened farms to identify cases and controls allowed for objective and robust definitions of both groups. Furthermore, it reduced the risk for misclassification bias. It is relevant that such cross-sectional studies are able to identify associations between exposure factors and the occurrence of the disease, but it is not possible to assign direct causality to the associations found without following-up on disease occurrence and without exposure to the respective factor. Finally, in this study, we focused primarily on risk factors that were deemed specific for mountainous regions, whereas other risk factors known to be associated with the occurrence of BDD from previous studies could not be investigated. Therefore, results of this study represent a subset of risk factors for BDD only. Nevertheless, our findings provide a set of aspects related to either introduction or establishment of BDD that can contribute to developing adapted control programs for BDD in dairy cows under those conditions, which can be found in various countries worldwide.

## CONCLUSIONS

This is the first study investigating risk factors for BDD in dairy cows kept in small-scale farms and pastured in mountainous regions, where some risk factors determined in previous studies cannot be applied. We found various risk factors to consider in a control program, some of which are easy to implement. Although some aspects were already known for animals in conventional housing and management conditions, this study reveals novel findings; namely, that mountain pasturing, time between diagnosis and treatment, tie-stall housing, and the amount of concentrate fed are associated with the occurrence of BDD. More research is needed, because we cannot exclude the possibility that additional risk factors exist for BDD. In this context, studies investigating aspects of treatment such as ban-

dage application, pharmaceuticals used for treatment, or animal selection for treatment seem worthwhile. Furthermore, the negative effect of concentrate supplementation on disease development has to be confirmed and its pathomechanism needs to be clarified.

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