Heifer Mastitis Risk Post-Parturition: Management factors associated with a low rate of Subclinical Heifer Mastitis in a Herd-Level Study

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Abstract

This study focused on assessing risk factors for heifer mastitis post-parturition, with a particular emphasis on understanding the associated management factors at herd level. Through examination of on-farm practices, milking routine observation and heat-stress consideration of 77 German dairy herds, the study set out to unravel the complexities contributing to a high / low rate of subclinical mastitis in heifers. For this purpose, the annual heifer mastitis rate (HMR) was provided by the dairy herd improvement (DHI) test and farms were categorized into two groups based on their HMR: herds with a low HMR (LHMR) and herds with a high HMR (HHMR). In the final multivariable model, two variables differed significantly among the herd categories. Herds with a higher proportion of heifers with a BCS < 3 were more likely to fall into the LHMR category. Herds with a higher proportion of heifers with a lameness score of 3 were more likely to be HHMR herds. These findings offer practical implications for dairy farmers in optimizing udder health and productivity in heifers at herd level. These associations from our cross-sectional study should be verified in a prospective study.

Keywords: mastitis, heifer mastitis, risk factors

Introduction

Mastitis in dairy cows, an inflammatory disease of the mammary gland, remains a critical condition in the dairy industry worldwide [1]. While the impact of mastitis in lactating, multiparous cows is well studied, heifer mastitis has gained recognition as a distinct challenge deserving focused attention.

Mastitis in heifers, whether appearing clinically or subclinically, leads to substantial economic losses for dairy farmers [2, 3]. These losses result from expenditure for diagnostics and therapeutics, nonsaleable milk, reduced milk yield in the subsequent lactation [3, 4] as well as future reproductive losses [5], consequently contributing to premature culling [6, 7].

In various studies examining subclinical mastitis (SCM) in heifers, diverse findings regarding prevalence have been reported. De Vliegher et al. (2004) [8] found SCM in 27.5% of postpartum heifers using a threshold of >200,000 cells/mL for Somatic Cell Count (SCC). In contrast, Bareille et al. (2000) [9] reported a lower rate of 17.7% using the same threshold. De Vlieghers's earlier study in 2001 found a prevalence of 35% [10], but with a lower threshold of 150,000 cells/mL. Bludau et al. (2014) [11] observed SCM at a rate of 20.6% with a threshold of 100,000 cells/mL.

Intramammary infections (IMI) in heifers may already manifest prior to calving, with a reported prevalence ranging from 29% to 75% of quarters as reviewed by De Vliegher et al. (2012) [12]. Mostly non-aureus staphylococci (NAS), but also *Staphylococcus (S.) aureus*, and environmental mastitis pathogens are commonly identified as significant causative agents for heifer mastitis [13].

Identifying and understanding the risk factors associated with heifer mastitis is crucial for effective prevention and management strategies in dairy farming. Various factors contribute to the susceptibility of heifers to mastitis during the pre- and post-partum periods. They can either be teat-related, such as the loss of the keratin plug [14], animal-related, including an increased age at first calving [15], or herd-related, like season and climate [10, 16]. In addition, farm management influences the udder health of both lactating cows and heifers. Specifically, the milking routine contains many hazard points, since the udders of lactating cows may serve as reservoirs for transmissible mastitis pathogens, posing a risk of infecting other animals throughout the milking process. Additionally, higher vacuum levels at the teat end may lead to increased teat end thickness and the occurance of hyperkeratosis [17]. Nitz et al. (2020) [15] found detaching of milking cups because of kicking off during milking to be a risk factor for new IMI between day 3 and 17 postpartum in their study, while in a previous study of ours we found insufficient milking hygiene practices prior to the milking process, teat disinfection and treatment of mastitis in heifers to be risk factors associated with the HMR [18].

Another factor contributing to a higher risk for mastitis and other diseases is heat stress. Previous studies have established an association between elevated temperature-humidity-index (THI) values and an upswing in SCC among cows [19-21]. There are no studies on the effect of heat stress specifically on heifers.

When assessing udder health parameters in heifers at herd level, such as in this study, the heifer mastitis rate (HMR) serves as a useful tool for comparison. It is defined as the percentage of heifers with a SCC exceeding 100,000 cells/mL in the first DHI test conducted after parturition. It refers to the composite milk sample, wherein milk from all four quarters is collected during DHI. Monitoring the HMR in the first DHI provides valuable insights into the prevalence of SCM within a specific population, which makes it a useful tool for developing management and prevention strategies, as already proven in recent studies [4, 8, 11, 18, 22].

This study pursued two different objectives. The first step focused on accessing management parameters directly on the farm or on the animals. The aim was to conduct a comparative analysis between farms exhibiting a lower HMR and those with a higher HMR. The second step involved collecting climatic parameters (temperature and humidity) from the preceding year leading up to the farm visit.

This study focused on assessing the post-partum risk factors influencing early lactation SCC in heifers. We aimed to contribute to a better understanding of the challenges by heifer mastitis during this critical period. By studying risk factors at herd level, we intended to develop management strategies aimed at prevention and reduction.

Materials and Methods

All applicable guidelines for the care and use of animals were followed. The study complied with the International Guiding Principles for Biomedical Research Involving Animals (1985).

Farms: A convenient sample of 77 dairy farms was randomly selected based on their willingness to take part in the study, representing a diverse range of geographic location, herd sizes, and management systems. Information regarding the mastitis status or udder pathogen status of the herd was not available beforehand, and thus, the selection of the herds was not affected by this factor. The herds were required to have a conventional milking system (herringbone n = 40; side-by-side n = 18; tandem n = 4; rotary n = 11; swingover n = 3; milking in tie stall n = 1), and to participate in the local DHI testing. The mean herd size was 177 (21-872) cows in lactation, mostly black and red German Holsteins with a very small proportion of other breeds, such as Jersey. The mean 305-d milk production was 9801 kg (4.07% fat, 3.44% protein). One herd was housed in a tie stall, the remainder were housed in freestalls in cubicles and were milked either two or three times a day. A total of 73 farms had a conventional production and 4 were organic farms. The majority of the farms (n = 66) were located in the German states of North Rhine-Westphalia, five farms in Rhineland-Palatinate, four in Lower Saxony, and two in Hesse. Each farm was visited once between August 2019 and September 2020.

Table 1: Overview of management practices collected through observation.

Milking process	
	Teat cleaning procedure, number of cows the cloth is used on, wearing of disposable gloves, disinfection of hands/gloves, liner slips, detaching of milking cups because of kicking off, defecation in the milking parlor, intermediate disinfection of the milking cups, active ingredient of disinfection, teat dip: yes or no, type of teat dip, staff in the milking parlor
Animal health scores at herd level	
	Body condition score (BCS), lameness, udder edema, udder hygiene

Data collection: The aim of this study was to perform a comparative analysis between farms with a lower HMR and those with a higher HMR. Two categories were created and each farm was assigned to one of them: farms with a low HMR (LHMR) and farms with a high HMR (HHMR). The threshold of 30.3% HMR was used for categorization, since this benchmark represented the average HMR in herds in North Rhine-Westphalia for the year 2019 [23].

Given that a few risk factors associated with heifer mastitis were already identified in previous studies, the aim of this study was to assess risk factors that might be significantly associated with the outcome variable in the surveyed population.

In addition, we examined risk factors associated with mastitis in multiparous cows, along with risk factors that appeared biologically plausible to us.

Table 2: Overview of questions asked in the interview.

General management	
	Number of lactating cows, type of production, milking times per day, water source, calving season, access to pasture, time access to pasture, duration of pasture season, age at first access to pasture, start of the pasture season, end of the pasture season, consolidation of livestock trails, fly control strategy
Management around calving	
	Housing of heifers with dry cows, type of cubicle for pregnant heifers, cleaning of the cubicles, bedding material, reapplying bedding material, frequency of raking the cubicles, cover layer on top of bedding material, shortage of bedding material, cleansing of the drinking trough, calving pen shared with cows, moving heifers to calving pen, mucking out the calving pen, disinfection of the calving pen after mucking out, intersucking among heifers, pre-fresh diet, mineral supplementation, vitamin E/selenium supplementation, udder clipping, use of teat sealants, and/or antibiotic treatment prior to calving
Milking process	
	Active ingredient of the teat dip, active ingredient of the disinfection of the milking cups, staff in milking parlor, mastitis therapy in heifers, moving fresh heifers to the milking herd/group, treatment of udder edema

Based on these findings, we developed an observation sheet targeting management-associated risk factors for heifers at herd level (Table 1 and Appendix 1). Simultaneously, we created an interview protocol primarily focusing on risk factors on management of the herd in general and heifers specifically, which was directed to the farmer or the herd manager on the day of the farm visit (Table 2 and Appendix 2). To gain an impression of the overall farm management practices and to get meaningful and truthful statements, the farmer or the person responsible for herd management was interviewed. In the face-to-face questionnaire interview, the interviewer asked specific questions from the standardized questionnaire and recorded the responses based on the provided answer options. The same interviewer was used throughout the study (first author) and the same person was also responsible for assessing potential risk factors by observation.

The questionnaire included open-ended and closed-ended questions. Closed-ended questions were dichotomous (and generally "yes/no" questions), nominal-polytomous, or ordinal-polytomous.

In addition, the milking routine was observed once to assess the

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procedures and hygiene practices related to milking (Table 1). Specifically, the teat cleaning procedure, pre- and post-milking teat disinfection, and stress indicators such as the percentage of cows kicking off the milking cups in one milking or liner slips were assessed. These parameters were examined not only for heifers, but also for at least 50% of the milking herd.
In addition, the HMR of the month of the farm visit was provided by the DHI.
Lastly, to evaluate heat stress, the THI was calculated based on data gathered from the nearest weather station of each farm [24].

Management factors:
Heifer Mastitis Rate (HMR) Assessment
As already proven in recent studies [4, 18, 22], the HMR served as a key parameter in this study, aiming to assess the prevalence of SCM in freshly calved heifers. This describes the proportion of heifers >100,000 cells/mL in the initial DHI test post-partum, including heifers from day 5 to day 30. It was used as the dependent variable in our study. The HMR of the month of the farm visit was conducted, representing the annual HMR leading up to the visit.

Categorization into LHMR- and HHMR herds
Given the regional benchmark of 30.3%, the average HMR in herds in North Rhine-Westphalia for the year 2019 [23], each herd was allocated to one of two categories, based on their HMR of the month of the farm visit: herds falling below the threshold of HMR 30.3% were categorized as low HMR (LHMR), while farms exceeding the threshold of 30.3% HMR were categorized as high HMR (HHMR).

Animal health scores at herd level
Heifers up to 30 days in lactation were individually assessed for BCS, lameness, udder edema, and udder hygiene. The BCS developed by Edmonson et al. (1989) [25] was used for evaluation, dividing heifers into a 1–5 scale ranging from 1 (severe under-conditioning) to 5 (severe over-conditioning) with 0.25 increments. For the statistical analysis, we categorized heifers into three groups: Category 1 with a BCS < 3, Category 2 with a BCS of 3-3.5, and Category 3 with a BCS >3.5.
For lameness evaluation, the system developed by Sprecher et al. (1997) [26] was used. Heifers were allocated to five categories: Category 1 (normal): the heifer stands and walks with a straight back posture and a normal gait. Category 2 (mildly lame): the heifer develops an arched-back posture while walking. Her gait remains normal. Category 3 (moderately lame): the heifer exhibits an arched-back posture, noticeable in both standing and walking. Her gait is impacted. Category 4 (lame): the heifer consistently displays an arched-back posture and she takes one deliberate step at a time. Category 5 (severely lame): the heifer additionally is unable to bear weight on one or more of her limbs. A similar scale was used to assess the severity of udder edema: 1 (no edema) to 5 (extremely severe) [27].
In regard to the heifers' udder hygiene, each udder was assigned to a category depending on its degree of contamination: Category 1 (free of dirt), Category 2 (slightly dirty: 2–10% of surface area), Category 3 (moderately covered with dirt: 10–30% of surface area), and Category 4 (covered with caked on dirt: >30% of surface area) [28].
The individual animal scores were recorded in a way that assigned a percentage per category to each farm in the data table to obtain a herd-level perspective. No clinical examinations on heifer level were conducted.

Temperature-Humidity-Index: The THI was used to evaluate the degree of heat stress experienced by the heifers. Previous research has demonstrated an association between elevated THI values and an increase in SCC in cows [19-21]. Monthly data on temperature (°C) and relative humidity (%) were gathered from the Deutscher Wetterdienst weather station nearest to the farm, and the monthly average THI was calculated using the following function [24]:

$$THI = (1.8 \times T + 32) – [(0.55 – 0.0055 \times RH) \times (1.8 \times T – 26.8)]$$

T = Temperature in degrees Celsius
RH = relative humidity in % as a fraction of the unit.

Contrary to the other model, which utilized the annual HMR, this model employed the monthly HMR of the past 12 months. The aim was to examine the relationship between variations in THI throughout different seasons of the year and its impact on the HMR.

Statistical analysis: Data were gathered and analyzed using the programs Excel 2013 (Microsoft Corporation, Redmond, WA, USA), and SPSS (IBM SPSS 28.0, Chicago, IL, USA). The dairy herd was the statistical unit. The heifer mastitis rate category of the farm was used as outcome variable. Explanatory variables were housing, feeding, milking, and animal health-associated management variables. Associations between the heifer mastitis category and risk factors (independent variables) were examined with generalized linear models with logit link and binomial response (below/above the mean HMR in North-Rhine Westfalia (logistic regression)) after pre-screening for variable selection in univariable analysis. First, all variables were assessed in univariable models, and all those with a P-value <0.10 in relevant tests (Chi-Square, T-Test, Man-Whitney U-Test) were offered to a multivariable model. The relation between dependent and independent variables was tested first by appropriate univariable tests. Multicollinearity was checked with Spearman/Kendall's tau, which indicated a correlation of r > 0.70 with one another.

Table 3: Distribution of risk factors linked to the HMR[1] in LHMR[2] herds and HHMR[3] herds.

HMR[1] category	LHMR[2]			HHMR[3]			Total		
	Mean	N	Std. Deviation	Mean	N	Std. Deviation	Mean	N	Std. Deviation
Proportion of heifers (%) with a lameness score of 3	1.830	43	6.3344	4.889	28	8.0247	3.037	71	7.1540
Proportion of heifers (%) with BCS[4] < 3	35.744	43	30.5792	22.971	28	26.3866	30.707	71	29.4810

[1] heifer mastitis rate, [2] low heifer mastitis rate, [3] high heifer mastitis rate, [4] body condition score.

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For this reason, no variables were excluded. A backward stepwise procedure was used to select the final multivariable regression model. Potential risk factors were excluded if $p > 0.05$. Meaningful biological interactions between the fixed effects were also used in the final model if significant ($p < 0.05$) and if they did not increase the Akaike information criterion (AIC). Non-significant effects or interactions that increased the AIC were not included in the final model. Model fit was evaluated by checking normality of the residuals. Statistical significance was assumed at $p \leq 0.05$.
In addition, THI data were gathered and analyzed using the programs Excel 2013 (Microsoft Corporation, Redmond, WA, USA), and SPSS (IBM SPSS 28.0, Chicago, IL, USA). The statistical unit was the monthly HMR data for each individual herd for 12 months, resulting in 924 records in total. With generalized linear mixed models associations between several predictor variables and the binary outcome variable (HMR) were assessed. The model's goodness of fit was evaluated using the Akaike Corrected and Bayesian Information Criteria.

Results

Management factors: Of the initial 77 farms, 71 were included in the analysis, accounting for 92.2% of the sample. The exclusion of six farms (7.8%) resulted from missing or invalid data. The dependent variable, HMR, exhibited a distribution of 60.6% (n=43) for category LHMR (HMR < 30.3%) and 39.4% (n=28) for category HHMR (HMR ≥ 30.3%).
A comparison between high and low HMR herds and logistic regression analysis identified relationships between HMR categories and potential risk factors as independent variables. Two herd characteristics were associated with herd category at $p < 0.05$ in the multivariable logistic regression analysis: the proportion of heifers with a lameness score of 3 and the proportion of heifers with BCS < 3. The distribution of risk factors associated with the HMR in LHMR herds and HHMR herds are presented in Table 3.
Table 4 provides the final logistic regression model for the two categories of farms based on their HMR. The goodness-of-fit measure provided no grounds to question the model's validity. The Omnibus test confirmed the model's significance ($p<0.001$). According to the results of the final multivariable model, two variables differed significantly among herds categorized as LHMR and HHMR: a BCS < 3 and lameness score 3. Farms with a higher proportion of heifers with a BCS < 3 were more likely to fall into the LHMR category, while herds with a higher proportion of heifers with a lameness score of 3 were more likely to be HHMR herds.
THI: The model investigating the influence of THI on HMR did not produce any significant association.

Discussion

Management factors:
Heifer Mastitis Rate (HMR) Assessment
The HMR serves as a useful tool for evaluating udder health in heifers at herd level. In this study, the HMR derived from the DHI, employing a threshold of >100,000 cells/mL for SCM, aligning with the approachtaken by Bludau et al. (2014) [11], Gösling et al. (2018) [22], tho Seeth & Krömker (2021) [4], and Rueben et al. (2023) [18]. Other studies proposed higher thresholds for SCM, such as 200,000 cells/mL [9] and 150,000 cells/mL [10]. SCC > 100,000 cells/mL at quarter level can be assumed to indicate inflammation [29] and economic losses are already present at single animal level at the threshold of 100,000 cells/mL [4]. Internationally, the threshold of 200,000 cells/mL is used because it provides the highest probability of detecting IMI [30].
The HMR refers to the composite SCC collected during DHI and is commonly used in standard veterinary practice for detection of IMI in cows with SCM [31]. However, composite sampling is affected by dilution. This means, healthy quarters can mask the presence of an infection of one quarter when the samples are combined [32]. Nonetheless, this sampling technique is economical and valuable when assessing management strategies at both the cow and herd levels [31]. It is essential to note that the HMR used in our study was defined as the annual HMR. As a consequence, this parameter may not include current or acute high prevalence of mastitis in heifers. Averaging the data over time may make the HMR less sensitive to recent changes in udder health. Thus, it is important to keep this limitation in mind when interpreting results. Nonetheless, it provides a good representation of the general management situation regarding heifer mastitis on a farm. Also, cases of clinical mastitis were not assessed and microbiological tests were not performed

Categorization into LHMR- and HHMR herds
The threshold value of 30.3% HMR, deriving from the regional benchmark of average HMR in herds in the state of North Rhine-Westphalia for the year 2019 [23], was chosen in our study as the threshold for distinguishing the surveyed herds into LHMR and HHMR groups. The threshold of choice is justified by the fact that 66 of the total 77 surveyed farms were situated within the state of North Rhine-Westphalia. By aligning with the average HMR observed in the specific geographic area, the categorization becomes logical within the context, and our findings in this study may be applicable to other herds in the same region. On the other hand, potential limitations associated with the chosen threshold should be taken into account: the threshold derived from historical data and its applicability to different settings and regions should be made with caution. Changes in management practices, technological advances, and evolving herd health dynamics should also be considered.

Animal Health Scores at Herd Level
Heifers in the first month of parturition (up to 30 days in lactation on the day of the farm visit) were chosen for this study, since during this critical period heifers are particularly susceptible to mastitis and other diseases. Therefore, risk factors contributing to mastitis can exert a pronounced effect on their health. In the multivariable logistic regression analysis, two herd characteristics showed a significant association with herd category: the proportion of heifers with a

Table 4: Multivariable logistic regression analysis to identify risk factors associated with herds having an HMR[1] below (LHMR[2]) or above (HHMR[3]) the average HMR[1] of herds in North Rhine-Westphalia for the year 2019 (30.3%).

	B[4]	SE[5]	95% CI[6]	Wald Chi-Square	Df[7]	Sig.[8]
Proportion of heifers (%) with a lameness score of 3	-.100	0.0481	-0.195- -0.006	4.333	1	0.037
Proportion of heifers (%) with BCS[9] < 3	0.032	0.0127	0.007- 0.057	6.428	1	0.011

[1] heifer mastitis rate, [2] low heifer mastitis rate, [3] high heifer mastitis rate, [4] regression coefficient, [5] standard error, [6] confidence interval, [7] degree of freedom, [8] significance, [9] body condition score.

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lameness score of 3 and the proportion of heifers with BCS < 3.

Herds with a higher proportion of heifers with a lameness score of 3 were more likely to be HHMR herds. Lame cows are at greater risk of developing mastitis, metabolic disorders, and reduced fertility [33]. Hisira et al. (2020) [34] found a high prevalence of mastitis in lame cows suffering from claw diseases. Lameness, regardless of the severity, is a painful condition [35]. Pain influences the heifers' behavior in order to reduce discomfort [36]. Decreased movement and shorter standing periods leading to an increased lying time [37-39] may favor the risk of bacterial contamination of the teat and lead to the animals developing IMI. However, if the heifers' immune system were intact, this condition might not be as problematic. A further reason could be the metabolic imbalance typicially occurring in the post partum period. It is known that subclinical ketosis is associated with the occurance and severity of mastitis [40]. Hillreiner et al. (2016) [41] suggested that an elevated BHBA concentration during negative energy balance could contribute to increased risk for mastitis in early lactation due to impaired immune function in the udder.

The reason why lameness Categories 4 and 5 were not significantly associated with HMR categories may be due to the overall lower occurrence of heifers in these categories. They include more severe cases and as our variable only included primiparous cows, their prevalence of lameness was expected to be relatively low [42, 43]. Additionally, lameness is often noticed when the condition is already severe [44], demanding urgent and costly intervention in the form of hoof trimming or separating the animal from the herd. Tranter & Morris (1991) [45] found the mean time from the onset of lameness to clinical recognition by the farmer to be 27 days, which may be a reason why heifers with lameness score 3 are more likely to be overlooked and this category contains more heifers.

Herds were more likely to be classified as LHMR when they had a higher proportion of fresh heifers with a BCS below 3.

Heifers with an increased age at calving tend to have a higher BCS, increasing the risk for IMI with non-*aureus* staphylococci (NAS) and coryneforms 17 days postpartum [15, 46]. Moreover, heifers calving at older age face an increased risk of IMI caused by *S. aureus* and environmental pathogens [47]. On the other hand, according to Piepers et al. (2010) [48], heifers infected with NAS at the beginning of lactation have fewer cases of CM and produce more milk in their first lactation. Farms with intensive heifer rearing practices, emphasizing high quality and quantity of feed intake and favorable environmental conditions aimed at high daily weight gains and therefore a high milk yield, may also favor IMI. Nitz et al. (2020) [15] showed that a high milk yield contributes to the development of udder edema, increasing the risk of IMI with NAS and coryneforms. Our findings indicate that heifers in early lactation, similar to multiparous cows, experience issues such as lameness and high body condition scores, which can impact udder health due to metabolic stress.

Interview

Response errors can arise from various factors such as the design of the questionnaire, the approach used in interviews, the characteristics, attitudes, or knowledge of the respondent, among many other causes [49]. To exclude interviewer errors, the interview was always performed by the same person (first author). Instead of just handing over the questionnaire to the farmer, questions were asked in person. This gave the farmer the opportunity to ask questions for understanding immediately (if necessary), to exclude errors in answers and to check the answers for plausibility.

THI: No significant association between THI and the SCC was produced, in contrast to the findings of a study by Bouraoui et al. (2002) [19].That study reported a significant decrease in milk, fat, and protein yields, accompanied by an increase in SCC in cows during the summer season (THI = 78) compared with the spring period (THI = 68). In studies on THI and its impact on SCC, THI is typically considered as a snapshot of the current climatic conditions in a specific geographic region, often assessed on a daily or even hourly basis [21, 50]. Due to the availability of only monthly HMR data and the absence of more detailed SCC data, our approach was limited to using monthly THI for variable comparison.

Conclusions

When comparing LHMR herds and HHMR herds selected based on the SCC of heifers in their first DHI, two variables differed significantly among herds categorized as LHMR and HHMR: the proportion of heifers with a low BCS (< 3) and the the proportion of heifers that were moderately lame. This means that the likelihood of a herd having favorable udder health in heifers increased, when the proportion of heifers maintaining a low BCS after parturition was high and the proportion of heifers exhibiting moderate lameness was low. This confirms the importance of BCS and lameness in influencing udder health in heifers. Maintaining optimal body condition, especially heifers not being fat, and reducing lameness in heifers may contribute to a better udder health. Therefore, our results emphasize the need for good management strategies that address both nutritional aspects, reflected in BCS and lameness control to enhance udder health in heifers. In conclusion, the identified associations between BCS, lameness, and the HMR provide valuable insights for dairy farmers and herd managers. Implementing practices that promote optimal body condition and effectively manage lameness can contribute to reducing the risk for mastitis in heifers, thereby enhancing overall udder health outcomes at herd level.

While our study contributes valuable insights into the associations between BCS and lameness and HMR in the context of our study design, transferring these findings to different geographic areas needs further investigation.

Disclosure of conflicts of interest

The authors declare no potential conflicts of interest.

Compliance with Ethical Standards

This study has been conducted in compliance with ethical standards. The protocol was approved by the animal welfare commitee of the University of Veterinary Medicine Hanover, Foundation (TVO-2019-V-67; date of approval: 15 July 2021).

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Appendix 1: Detailed presentation of management practices related to milking and risk factors at heifer level assessed by observation.

Independent Variable	Description / Classification	Break-down Categories Final Model
Teat cleaning	Whether or not teats get cleaned before the milking unit is attached	Yes vs. no
Teat cleaning device	The kind of implement used for teat cleaning	Paper vs. reusable cloth vs. udder shower vs. teat scrubber
Type of teat cleaning	Whether the cloth is dry or moist	Dry vs. moist
Number of cows the cloth is used on	Number of cows one cloth is used on	
Disposable gloves	Whether or not the milker uses disposable gloves	Yes vs. no
Disinfecting the hands/gloves	Whether or not the hands/gloves are disinfected during and between the milking process	Yes vs. no
Air infiltration	Proportion of air infiltration in percent	
Detaching of milking cups because of kicking off	Proportion of detached cups in percent	
Defecation	Proportion of defecating cows in percent	
Intermediate disinfection	Whether or not the milking cups are disinfected between cows	Yes vs. no
Active ingredient	Active ingredient of the intermediate disinfection	
Type of intermediate disinfection	Whether the milking cups are dunked in disinfection or the disinfection is sprayed on	Dunked vs. sprayed on
Teat dip	Whether or not a teat dip is used	Yes vs. no
Type of teat dip	Time of teat dipping	Pre vs. post vs. both
Body Condition Score	Assessment of the Body Condition Score on heifers post-partum	<3; 3.0-3.5; >3.5
Lameness	Assessment of lameness of the heifers post-partum	1(normal) vs. 2(mildly lame) vs. 3(moderately lame) vs. 4(lame) vs. 5(severely lame)
Udder edema	Assessment of udder edema of the heifers post-partum	1(no edema) vs. 2(slight edema) vs. 3(moderate edema) vs. 4(severe edema) vs. 5(extremely severe)
Udder hygiene	Assessment of udder hygiene of the heifers post-partum	1(free of dirt) vs. 2(slightly dirty) vs. 3(moderately covered with dirt) vs. 4(covered with caked on dirt)

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Appendix 2: Detailed presentation of questions asked in the interview.

Independent Variable	Description / Classification	Break-down Categories Final Model
Active ingredient	Active ingredient of the intermediate disinfection of the milking cups	
Active ingredient teat dip	Active ingredient of teat dip	
Staff in milking parlor	Number of persons present in milking parlor	
Mastitis therapy in heifers	Whether heifer mastitis is treated with antibiotics, homeopathy, or with both	Antibiotics vs. homeopathy vs. both
Mastitis therapy in heifers	Whether heifer mastitis is treated systemically, locally, or both	Systemic vs. local vs. both
Housing of heifers in milk		Together with cows vs. in their own group
Moving fresh heifers to milking herd/group	Time period the fresh heifers stay in a separated group after calving before being moved to the milking herd expressed in days	
Udder edema	Whether or not udder edema is treated in any way	Yes vs. no
Type of cubicle for fresh heifers	What kind of cubicle is provided for the fresh heifers?	Deep-bedding cubicles vs. cubicles with rubber mats vs. deep litter barn vs. slatted flooring
Raking the cubicles clean where the fresh heifers are housed	How often are the cubicles raked clean?	Daily vs. weekly vs. if required vs. never
Bedding material	What kind of bedding material is used?	Manure solids vs. horse manure vs. straw vs. sawdust vs. none
Adding new bedding material	Interval of adding new bedding material expressed in days	≤3 vs. >3 ≤6 vs. >6 ≤9 vs. >9
Cover layer on top of bedding material	Whether or not a layer is added on top of the bedding material	Yes vs. no
Shortage of bedding material	Whether or not there was a shortage of bedding material	Yes vs. no
Cleaning of drinking trough	Interval of cleaning expressed in days	Zero vs. 1 vs. 2 vs. ≥3
Number of lactating cows	Number of lactating cows on day of farm visit	
Type of production	Whether the farms practice conventional or organic farming	Conventional vs. organic
Times cows are milked per day	Two–three times	Two vs. three
Water source	Drinking water source in the stall	Well water vs. drinking water vs. sea level
Type of milking parlor		
Calving season	Whether or not cows only freshen in specific seasons	Yes vs. no
Access to pasture	Whether or not the heifers and/or cows have access to pasture	Yes vs. no
Access to pasture	When do the cows/heifers gain access to pasture?	During the day vs. during the night vs. day and night
Access time to pasture per day	Expressed in hours per day	
Duration of the pasture season	Expressed in months per year	
Age at first access to pasture	Age at first access to pasture expressed in months	
Start of pasture season	Expressed in name of month the pasture season starts	
End of pasture season	Expressed in name of month the pasture season ends	
Water source	Water source on pasture	Well water vs. drinking water vs. sea level
Consolidation of livestock trials	Whether or not livestock trials are consolidated	Yes vs. no
Consolidation material of livestock trials		Attached vs. loose vs. perforated
Fly control strategy	Whether or not a fly control strategy is practiced on the farm	Yes vs. no
Fly control strategy	Type of fly control strategy	Pour on vs. ear tags vs. both
Housing of pregnant heifers	Location of pregnant heifers prior to calving	Together with dry cows vs. own group vs. on pasture vs. on a rearing farm
yTpe of cubicle for pregnant heifers	What kind of cubicle is provided for the pregnant heifers?	Deep-bedding cubicles vs. cubicles with rubber mats vs. deep litter barn vs. slatted flooring
Raking the cubicles clean where pregnant heifers are housed	How often are the cubicles raked clean?	Daily vs. weekly vs. if required vs. never
Bedding material	What kind of bedding material is used?	Manure solids vs. horse manure vs. straw vs. sawdust vs. none
Adding new bedding material	Interval between adding new bedding material expressed in days	≤3 vs. >3 ≤6 vs. >6 ≤9 vs. >9

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