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Blood in milk in horned dairy cows–Exploration of incidences and prevention opportunities

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ABSTRACT

Husbandry of horned cows is one alternative to disbudding that respects the animals' integrity. Concurrently, housing and management should be aligned with the animals' needs to minimize social interactions that can result in integument damage (ID) and udder injuries associated with blood in milk (BM). No information is available on incidences of BM, nor on specific preventive measures. Thus, this study aimed at investigating BM incidences in horned herds and testing whether compliance with guideline recommendations on the prevention of ID also contributes to mitigate BM. Twenty-one farms documented BM, cows' parity, social rank, and udder damage over seven months covering part of summer with pasture and winter in the barn. A total of 52 factors relating to the guideline were recorded and grouped into 'milking', 'feeding', 'lying', 'walking/activity' and 'herd management'. Each factor was categorised into fulfilled or not, and for each group percentages of fulfilment were calculated. Monthly BM incidences varied from 0.3 to 7.8% with 38% being associated with visible udder damage (BMvD). Most cows affected were in the 2nd to 4th lactation (44%) and middle-ranking (58%). A mixed model regarding 'monthly binomial BMvD' per farm revealed a higher BMvD risk during the barn season (OR = 2.39), highlighting the importance of pasture to alleviate social conflicts. Higher fulfilment of recommendations concerning 'feeding' and 'walking/activity' was associated with a lower risk (OR = 0.94 and 0.95), indicating that the conflict potential in these areas also plays a role for BMvD and can be lowered by a combination of improvement measures.

1. Introduction

More than 80% of dairy farms in Europe keep hornless cows, mostly to reduce the risk of injuries among pen mates and for the stockmen under intensive production conditions (Cozzi et al., 2015). However, the procedure of disbudding (i.e., destruction or excision of the horn-producing cells) or dehorning (i.e., removal of grown horns) is criticized for several reasons, including inflicting pain and not respecting the integrity of the animals (Kling-Eveillard et al., 2015). The two alternatives to disbudding or dehorning are breeding for polledness (e.g., Windig et al. 2015) or the husbandry of horned cattle. In a European project on this issue, Mirabito et al. (2009) concluded that both alternatives should be pursued, and that the husbandry of horned cattle should be supported not only by financial means, but also by providing information and advice. However, only a few scientific studies have been conducted so far that have focused on possible measures to reduce horn-related integument damage in dairy cows in loose housing (Johns & Knierim, 2019; Menke et al., 1999; Schneider, 2010). A further possible problem is blood in milk (BM) when horn thrusts result in udder injuries with burst blood vessels. To our knowledge, the occurrence of BM has not yet been scientifically investigated specifically in horned herds. Blood milk, though, also occurs in hornless dairy herds. The clinical picture on the milk of affected cows can vary from a few blood clots to almost pure blood. One or several udder quarters can be affected at the same time (Blowey & Edmondson, 2010; Moroni et al., 2018; Stampa et al., 2006). Prolonged bleeding can be associated with an increased risk of mastitis (Moroni et al., 2018; Stampa et al., 2006). This and the inflicted pain are welfare relevant, but also economic losses for the farm are associated, as the milk of affected animals is not marketable.

It is suspected that trauma, even without external involvement, is usually the ultimate cause of BM (Moroni et al., 2018). Although no comparative data are available, incidences of externally caused BM are likely higher in horned dairy herds as a consequence of horn thrusts. Drawing from studies on agonistic interactions and horn-induced integument damage, it can be expected that incidences of BM vary

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Table 1
Descriptive data on herd, management, and housing characteristics of the investigated farms (n=21).

Herd	Median (Min-Max)	Mean±SD
Herd size	45 (20–83)	46.0 ±16.7
% Horned cows in the herd	95 (50–100)	91.1 ±12.5
		Number
Main breed	Holstein Friesian	4
	Fleckvieh	10
	Brown Swiss	5
	Grey cattle	1
	German black pied	1
Management		**Number**
General farm management	organic	20
	conventional	1[a]
Status of husbandry of horned cows	established herd[b]	4
	transition: hornless to horned	11
	transition: tethering to loose housing	5
Location of concentrate provision	feeding table	10
	milking parlour	6
	concentrate station	2
	no concentrate feeding	3
	Median (Min-Max)	**Mean ±SD**
Concentrates (max. kg/cow*d)	3.0 (0.0–6.0)	2.7±1.9
Housing	**Median (min-max)**	**Mean ±SD**
Number of feeding places per cow	1.0 (0.9–1.5)	1.1±0.1
Feed alley width (m)	3.2 (2.2–5.1)	3.5±0.8
Number of cows per drinking place	7.7 (4.0–12.7)	8.4±2.6
Number of lying places per cow	1.1 (0.9–1.6)	1.1±0.2
Outdoor loafing area (m^2/cow)	2.6 (0.0–9.0)	2.8±2.8
		Number
Cubicle type	deep-bedded cubicles	13
	raised cubicles	6
	deep bedded and raised cubicles	2
Milking parlour	fishbone	13
	tandem	6
	side-by-side	1
	swing-over	1

[a] this farm had changed from tethered to loose housing with a fully horned herd
[b] herds had consisted of at least 95% horned cows in loose housing for at least five years

between farms and that this is related to factors affecting social conflicts and the opportunity to avoid physical encounters. Related to housing conditions, these comprise the total space allowance per cow (Menke et al., 1999), stocking density in terms of number of cows per cubicle or feeding place (Collings et al., 2011; de Vries et al., 2015; Krawczel et al., 2012; Lobeck-Luchterhand et al., 2015; Talebi et al., 2014), barn design affecting the cows' overview, distribution and optimal height of water troughs, the width of alleys (Schneider, 2010), space allowance in the waiting area of the milking parlour (Irrgang et al., 2015; Schneider, 2010), dimensions of the lying area (Schneider, 2010), access to and space allowance in the outdoor loafing area (Lutz et al., 2019; Schneider, 2010), and access to pasture providing extensive space for the avoidance of conflicts within the herd (Irrgang, 2012; Waiblinger et al., 2000; Wierenga, 1984).

With regard to management, relevant factors include feeding (Collings et al., 2011; Val-Laillet et al., 2008), separation of cows in heat, regrouping, the way of integration of new animals (de Vries et al., 2015; Johns & Knierim, 2019; Menke, 1996; von Keyserlingk et al., 2008), and the human-animal relationship (Menke et al., 1999) or the number of stockpersons (Schneider, 2010). Also breed differences, with Holstein-Friesian herds being more affected by horn-induced damage, have been reported (Johns & Knierim, 2019; Schneider, 2010).

Based on these research results and the exchange of knowledge between farmers, advisors and scientists, a guideline to prevent social conflicts and horn-induced integument damage (Johns et al., 2019) was developed within the German project 'horns in loose housing'. Results of this project suggested that the interplay between housing, herd management, stockperson, and animals is more important than the influence of individual factors. At the same time, it was also found that the more recommendations covered in the guideline were implemented on the farms, the less horn-related integument damage was recorded (Knierim et al., 2020).

In addition, on the individual level, lower-ranking cows may be more frequently recipients of agonistic interactions, and most primiparous cows may be lower ranking due to lower body size, and experience (review in Bouissou et al., 2001; Lindberg, 2001). They may also be involved in agonistic interactions more frequently (Hasegawa et al., 1997). Accordingly, Waiblinger et al. (2000) found a negative correlation between horn-related integument damage and the age of the cows.

Regarding incidences of BM in horned herds, there is hardly any scientific information to date, nor on specific preventive measures at farm level. Therefore, the objectives of the present investigation were to explore in a sample of horned dairy herds in loose housing (1) incidences of BM with and without visible damage to the udder (BMvD) and associations between BMvD and horn-induced integument damage, (2) whether primiparous or low-ranking cows are more frequently affected than multiparous or high-ranking cows, and (3) whether compliance with the guideline recommendations for the prevention of horn-related integument damage also contributes to the mitigation of BMvD.

2. Material and methods

2.1. Farms and animals

The present investigation was part of the German project 'Horns in loose housing: monitoring and assisting transition from dehorned to horned dairy herds or from tethered to loose housing systems with horned cows involving demonstration farms as a basis for qualified advisory services for dairy cattle farming'. Farms were acquired via advisory organizations, organic farming associations, internet platforms, research organizations, and agricultural press. Out of 90 responders, a total of 40 farms participated in the project. They comprised five farms that already kept an established horned dairy herd in loose housing and 35 farms changing from hornless to horned dairy cows in loose housing, or from tethered to loose housing with completely horned herds. Of the 40 project farms, 21 farms also agreed to participate in the present investigation and documented blood milk cases. Table 1 provides descriptive data on herd, management, and housing characteristics of these 21 farms. All farms offered half or full day access to pasture during the vegetation period from March/April to October/November (summer pasture) and milked in milking parlours. Calving took place throughout the year on all participating farms.

2.2. Data collection

2.2.1. Blood in milk

From July 2019 (around mid of summer pasture period) to January 2020 (around mid of winter barn period), the participating farmers continuously recorded all instances of BM (light pink to reddish colour in the foremilk of one or more udder quarters) via conventional visual method for which reliable detection of blood content ≥ 2.0% by experienced and inexperienced persons had been shown (Rasmussen & Bjerring, 2005). The documented instances were reported by the farmers monthly. However, one farm with alpine grazing during summer months did not document BM cases until September. Thus, only 5 months of data were available from this farm; all other farms provided data for 7 months. On this basis, a total of 145 monthly BM recordings at herd level

Fig. 1. Examples of visible damage to the udder potentially causing blood in milk (photos: University of Kassel).

Fig. 2. Examples of different horn-related integument damages (from left to right): hairless patches, older and fresh wounds, swellings (photos: University of Kassel).

were available. Along with BM cases the farmers also documented the parity and estimated social rank (categorisation in low, medium, high, based on daily observations during routine work with their animals) of the affected individuals and whether any wound or swelling of the udder (Fig. 1) was visible. A new instance of BM within an animal was counted when at least two milkings without BM had passed.

The farmers were not systematically trained to detect BM, but they were in general familiarized with the monitoring of horn-related problems by previous active involvement in the project and participation in project seminars over several years. Sixteen of the farmers involved in this study also participated in an inter-assessor agreement check regarding horn-related integument damage: each farmer independently, but at the same time with a trained and experienced person (silver standard), assessed ten individual cows of his/her own herd regarding a classification of individual animals ($\leq$ 5, 6–9, and $\geq$ 10 damages/cow). The median reflected very good agreement (prevalence and bias adjusted kappa (PABAK) = 0.85) following the interpretation of kappa values by Landis and Koch (1977).

2.2.2. Horn-related integument damage

Horn-related integument damage was systematically recorded once per herd during the winter period (November 2019 to January 2020) on 16 of the 21 farms that had improvable levels regarding horn-related integument damages (on average $>$ 5 damages/cows) in the previous year. Recording was done by a trained experimenter, whose inter-assessor reliability was tested prior to data collection. For this purpose, a total of 88 cows on four different farms were directly assessed regarding horn-related integument damage by the experimenter and three other trained individuals simultaneously, but independently of each other. Acceptable agreement was achieved with all individuals (Pearson correlations (r) = 0.83–0.96, with no indication of systematic bias (slopes of the trend line in the scatter plot: 0.75–1.20).

Depending on herd size, in a sample of 22 to 47 cows per herd (calculated according to Welfare Quality®, 2009), the integument of the individual animals (entire left and right side of the body including vulva and udder) was examined. Every horn-related damage was counted (modified after Schneider, 2010). This included hairless patches, old and fresh wounds, and swellings (Fig. 2). Technopathies, i.e., areas of hair loss, ulcers or swellings which are caused by inappropriate housing equipment, were differentiated from horn-related damage, and not considered in the analyses. Technopathies typically occur in several animals in similar body regions and in a similar form, particularly at the hocks, carpal joints, hip bones, shoulder, or neck. At herd level the mean number of horn-related damages/cow was calculated for later correlation analysis with BM incidences during winter season.

2.2.3. Housing and management factors

A total of 52 housing and management factors were recorded on the farms relating to recommendations from the guideline for the husbandry of horned dairy cows in loose housing (Johns et al., 2019). The various factors were grouped into five areas: 'milking', 'feeding', 'lying', 'walking and activity' and 'herd management' according to the structure of the guideline. The first four groups describe the design of the respective functional areas in the barn, but also contain related

Table 2
Guideline recommendations on factors in the areas of 'milking', 'feeding', 'lying', 'walking/activity' and 'herd management' and the respective numbers (proportions) of investigated farms complying with the recommendations.

Milking	**Recommendation**	**n (%) complying farms**
Waiting area	≥ 2.5 m^2/cow (at start of milking) or no waiting area	19 (91)
Design of waiting area	square (instead of tubular) or no waiting area	5 (24)
Free view of milker on waiting cows	yes	11 (52)
Type of milking parlour	individual milking stalls[a]	8 (38)
Concentrate feeding in milking parlour	no	15 (71)
Obstacles in the cows' head/horn area in the parlour	no	21 (100)
Feeding	Recommendation	n (%) complying farms
Number of feeding places per cow	≥ 1.1	9 (43)
Feeding gate type	palisade (instead of diagonal or neck rail)	11 (52)
Feeding place width	80–95 cm	8 (38)
Fixation of cows during main feeding	yes	18 (86)
Roughage availability	ad libitum (24 h)	16 (76)
Roughage provision frequency[b]	≥ 6 times a day	12 (57)
Fresh feed after milking	yes	15 (71)
Hay proportion in the feed ration[c]	high (≥ 95%)	10 (48)
Concentrate feeding at feeding table	no or only when restrained	20 (95)
Accessibility of concentrate stations	sufficient free space around[d] or no station	20 (95)
Rear protection at concentrate stations	yes or no station	19 (91)
Side protection at concentrate stations	yes or no station	20 (95)
Lying	Recommendation	n (%) complying farms
Number of lying places per cow	≥ 1.1	11 (52)
Cubicle type	deep bedded (instead of raised)	13 (62)
Head lunge space	≥ 100 cm[e]	0 (0)
Neck rail	flexible (instead of rigid)	7 (33)
Exit from cubicles	also possible to the front	4 (19)
Walking / activity	Recommendation	n (%) complying farms
Total barn area	≥ 13 m^2/cow	9 (43)
Feeding alley width	≥ 5 m	4 (19)
Walking alley width	≥ 4 m	2 (10)
Width of narrowest passage[f]	≥ 3 m	5 (24)
Dead end alleys[g]	none	5 (24)
Obstacle in alley (e.g., ramps, stairs)	none	10 (48)
Obstacle in passage[f] (e.g., brush)	none	10 (48)
Number of passages in the barn[f]	≥ 2	19 (91)
Distance between two passages	≤ 15 cubicles	20 (95)
Walking surface	slip-resistant	15 (71)
Accessibility of drinkers	sufficient free space around[d]	9 (43)
Accessibility of brush and lick stone	sufficient free space around[d]	12 (57)
Outdoor loafing area	≥ 4.5 m^2/cow	6 (29)
Enrichment of outdoor loafing area	e.g., trough, brush, hay rack	12 (57)
Accessibility of outdoor loafing area	always[h], via ≥ 2 passages	10 (48)
Number of cows per drinking place	< 10	14 (67)
Drinker height	60–90 cm	11 (52)
Herd management	Recommendation	n (%) complying farms
Separation of cows in heat	yes	6 (29)
Regroupings	never	8 (38)
Purchase of animals	no	19 (91)
Method of integration of heifers	single animals	3 (14)
Time of integration of heifers	before calving	14 (67)
Milking routine for heifers[i]	waiting outside of the waiting area	8 (38)
Additional monitoring after integration	yes	18 (86)
Breed	others than Holstein Frisian	17 (81)
Breeding for sociability	yes	11 (52)
Treatment of horns[j]	yes, in case of problems	12 (57)
Measures for aggressive animals	'education'[k] or exclusion from herd	16 (76)
Staff changes	< once a year	20 (95)

[a] tandem, drive-through or butterfly parlour
[b] daily frequency of fresh feed provision and feed remains pushed back to the feeding places
[c] except pasture
[d] accessible from ≥ 2 sides, no placement in confined areas
[e] distance between neck rail and wall or head rail
[f] passages between cubicle rows connecting feeding and walking alleys
[g] dead end alleys that are < 4.5 m wide
[h] apart from times at pasture
[i] during the first days after integration into the lactating herd
[j] e.g. rounding off the horn tips
[k] intervention by stockpersons in case of aggressive behaviour within the herd

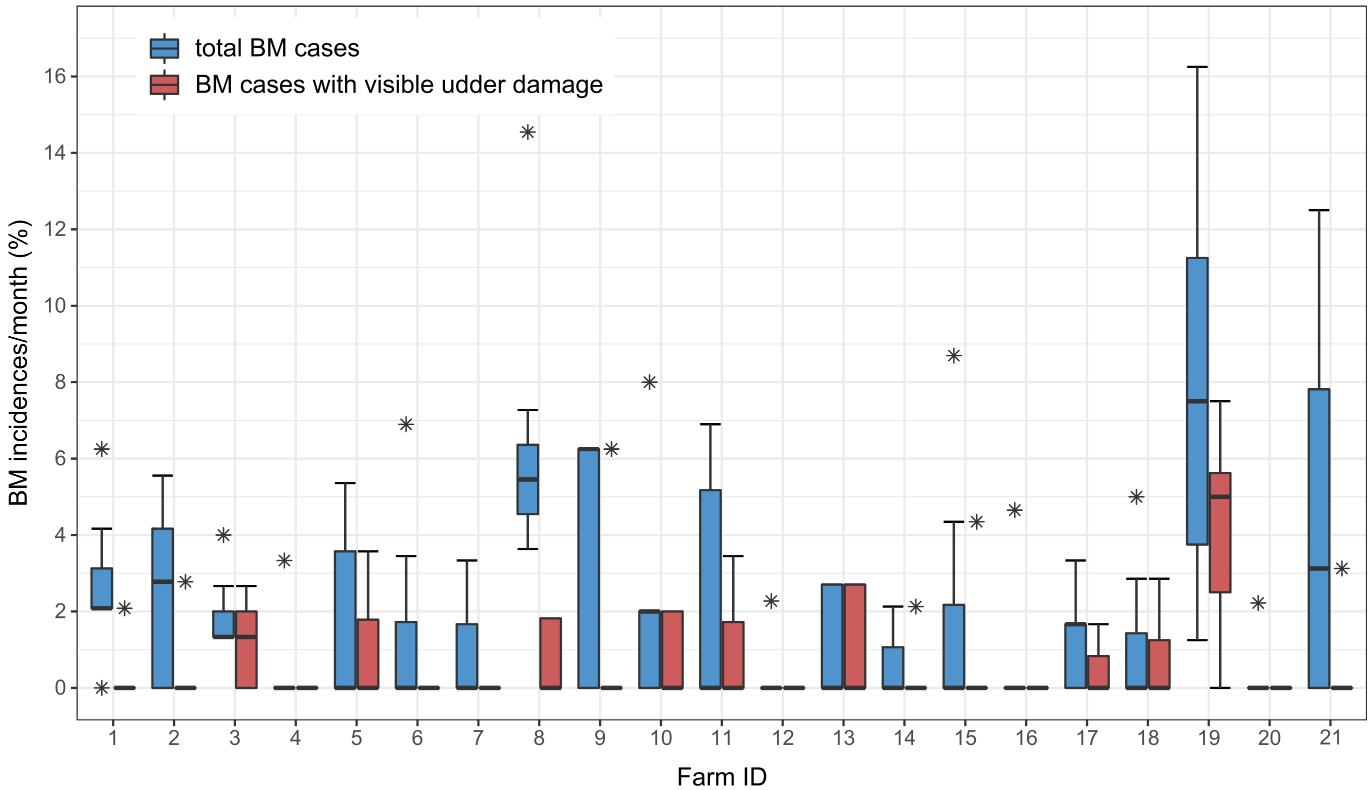

Fig. 3. Box plots (minimum, lower quartile, median, upper quartile, maximum, and potential outliers (interquartile range*1.5) of blood in milk (BM) incidences/month at farm level (n = 5 months in the case of farm 9, n = 7 months in the case of all other farms).

Table 3
Descriptive data on the percentages of fulfilled guideline recommendations at farm level (n = 21) in the areas 'milking', 'feeding', 'lying', 'walking/activity', and 'herd management'.

Variables	Median	Minimum	Maximum	Mean	SD
Milking (%)	66,67	33,33	83,33	62,70	12,81
Feeding (%)	75,00	41,67	91,67	70,63	12,53
Lying (%)	40,00	0,00	80,00	33,33	20,33
Walking/activity (%)	47,06	17,65	82,35	48,46	13,53
Herd management (%)	58,33	41,67	83,33	60,32	12,05

management factors. 'Herd management' exclusively includes factors that describe management decisions. Table 2 provides an overview of all factors covered, the respective recommendations and information on the number and proportion of investigated farms that complied with these recommendations.

Data on housing were collected during farm visits using standardised recording sheets; information on the management was gathered from the farmers via interviews using standardised recording sheets and via written questionnaires. However, some additional factors covered in the guideline were not considered because they were not reliably recorded. These were aspects of human-animal relationship (use of driving aid, quality of handling, contact times between stockpersons and animals), details on feeding gates (opening angle, functionality), drinkers (frost resistance, cleanliness, accessibility at the exit of the milking parlour) and heifer habituation before integration into the dairy herd.

2.3. *Statistical analyses*

Descriptive analyses at farm level were used to quantify monthly incidences of BM (% of cases in relation to the total number of lactating cows at farm) with visible damage to the udder (BMvD) compared to the total incidences of all BM (objective 1), and at animal level to characterize affected animals in terms of age and social rank (objective 2).

Graphical tests via normal quantile-quantile plots showed that monthly BMvD incidences (mean over the winter barn season 2019/20) and mean numbers of horn-induced integument damages/cow (recorded once during the winter season 2019/20) were non-normally distributed. Hence, to investigate associations between BMvD and integument damage (objective 1), the Spearman rank correlation coefficient (r_s) was calculated.

To test whether compliance with guideline recommendations can contribute to mitigate the occurrence of BMvD (objective 3), monthly

Table 4
Generalized mixed model regarding monthly BMvD occurrence (no/yes); fit by maximum likelihood method (n = 145 recordings from 21 farms): confidence intervals (CI) and p-values calculated via bootstrap procedure.

Fixed effects	Model estimate	95%CI	Odds ratios	p
Winter barn (ref. summer pasture)	0.867	0.021 – 1.905	2.39	0.0492
Milking (%)	-0.014	-0.064 – 0.024	0.99	0.5746
Feeding (%)	-0.066	-0.117 – -0.029	0.94	0.0141
Lying (%)	0.009	-0.043 – 0.065	1.01	0.7503
Walking/Activity (%)	-0.061	-0.142 – -0.002	0.95	0.1009
Herd management (%)	-0.003	-0.088 – 0.060	1.00	0.9312
VIF = 1.02–3.73, marginal R^2 = 0.26, conditional R^2 = 0.30				

Table A1
Spearman rank correlation coefficient (r_s) between independent variables (% of recommendations fulfilled in the areas milking, feeding, lying, walking/activity, herd management at farm level, n = 21) considered for multivariable analysis.

Variable		Milking	Feeding	Lying	Walking/Activity
Feeding	r_s	0.06			
	p	0.793			
Lying	r_s	0.20	0.19		
	p	0.396	0.421		
Walking/Activity	r_s	0.11	-0.18	0.52	
	p	0.624	0.432	0.015	
Herd management	r_s	-0.11	0.22	0.48	-0.11
	p	0.625	0.339	0.028	0.635

values at farm level were used. However, as data distribution of the proportion of affected cows was strongly right skewed, a binomial outcome variable (occurrence no/yes) was chosen, and the analysis carried out using a generalized mixed model fit via maximum likelihood method from the lme4 package (version 1.1–30, Bates et al., 2015) in RStudio (version 2022.12.0+353 for Mac OS X, Posit team, 2022). Each farm factor relating to the guideline recommendations (Table 2) was binomially categorised into 'recommendation fulfilled' or 'not fulfilled', and for each factor group (i.e., 'milking', 'feeding', 'lying', 'walking/activity', 'herd management'), the percentage of fulfilled recommendations was calculated at farm level and used as independent variables. Potential collinearity between these five factor groups was tested using Spearman rank correlations. All coefficients were in range of negligible to moderate correlation (r_s = 0.06–0.52, Appendix Table A1). Thus, a full model was set up with the fixed effects of the percentages of fulfilled recommendations regarding 'milking', 'feeding', 'lying', 'walking/activity', and 'herd management' at farm level, and 'season' (months with pasture access versus months with all-day housing in the barn) at the level of monthly recordings. The farm was included as random effect to account for repeated monthly recordings and possible within-farm variability.

Model diagnostics were done using a simulation-based approach (DHARMa package, version 0.4.5, Hartig, 2022). Model assumptions regarding the distribution of residuals were satisfied. No misspecification regarding over-/underdispersion, zero-inflation, or presence of outliers were identified. Confidence intervals of 95% were calculated via bootstrap procedure (number of simulations: 1,000), as were p-values for single fixed effects, the latter using 'PBmodcomp' from the 'pbkrtests' package (version 0.5.1).

3. Results

Cases of BM (with or without damage to the udder) occurred at all 21 participating farms during the seven-month survey period. The average monthly herd incidences ranged from 0.3 to 7.9%; the mean ± SD over all herds was 2.2 ± 1.9%. In addition, there was a high variance between monthly incidences within farms, ranging from 0.0 to 2.1% on farm 14 to 1.3 to 16.3% on farm 19 (Fig. 3). In absolute numbers, the incidences corresponded to 0 to 13 affected cows per herd and month in the present sample (mean ± SD = 1.1 ± 1.8). External damage to the udder indicating a horn thrust was visible in 38% of cases: the average monthly herd incidence varied between 0.0 and 4.1% (mean ± SD = 0.7 ± 0.9%). On six farms (farm 4, 6, 7, 12, 16, and 20), however, no cases of BM with visible damage to the udder (BMvD) were recorded during the period covered (Fig. 3).

Over the whole survey period, a total of 149 BM cases were recorded in 125 cows. With visible damage to the udder, 56 cases were recorded in 52 cows. Accordingly, four cows were affected twice. The majority of the 52 cows affected by BMvD were between the 2nd and 4th lactation (44%), followed by primiparous cows (33%). The proportion of older cows with more than four lactations was correspondingly 23%.

According to the farmers' subjective estimation of the cows' social position in the herd, most of the individuals affected by BMvD were medium ranking (58%), followed by low-ranking cows (31%). Two of the cows affected twice were primiparous and low-ranking, the other two were older (>4 lactations) and medium ranking. Within the group of primiparous cows, 9 out of 17 (53%) were classified as low-ranking, the others as medium ranking.

Between herd incidences of BMvD during winter in the barn and mean number of horn-related integument damages, a low positive but non-significant correlation existed (r_s = 0.39, p = 0.132, n = 16).

Regarding the percentages of fulfilled guideline recommendations, there were marked differences between farms (Table 3). However, farms that met a high percentage of recommendations in one area were not necessarily 'good' in the other areas. The correlation coefficients between areas were at maximum in the range of moderate strength (r_s = 0.06–0.52, Appendix Table A1).

The multivariable analysis (Table 4) revealed a higher BMvD risk during the barn season compared to the season with pasture access (odds ratio (OR) = 2.39). At farm level, each percentage point of higher fulfilment of recommendations concerning 'feeding' and 'walking/activity' was associated with a lower BMvD risk (OR = 0.94 and 0.95, respectively). However, the effect of compliance in the area of 'walking/activity', as well as in the areas 'milking', 'lying' and 'herd management' could not be statistically supported. The sum of fixed effects explained 26% of variance in the data set (marginal R^2). The random effect contributed an additional 4% (conditional R^2 = 0.30).

4. Discussion

The 21 herds studied were a convenience sample based on the voluntary participation of farms and thus probably reflect only part of the diversity within the German dairy farms with horned dairy cows in loose housing. However, official statistics and structural data on horned dairy herds at state or national level for an evaluation of the sample are not available.

4.1. Incidences of blood in milk

During the seven-month survey period, each farm had at least one case of blood in milk that could be due to various causes. A large proportion of BM was not associated with a visible injury or swelling at the udder (BMvD), and thus unlikely induced by horn thrusts, even though this cannot completely be excluded. Other causes of BM may for example be hind limbs bruising the udder during parturition, especially in primiparous or high-yielding cows (Blowey, 2016). Particularly after calving also physiological or pathological changes may be responsible. Particularly as consequence of prolonged or severe udder oedema, the increased pressure can promote tissue damage and ruptured blood vessels (Blowey, 2016; Moroni et al., 2018). Beyond calving, BM may also be caused by impaired rising or lying down due to insufficient cubicle dimensions, unusual gaits or pendulous udders being knocked by the legs during walking, but also by external trauma from kicking or head butting (without horns) by conspecifics (Blowey & Edmondson, 2010; Moroni et al., 2018).

BMvD occurred with distinctly lower incidences and only in 15 of the 21 participating herds. BMvD and horn-related integument damages were positively correlated, but statistically non-significant and of low strength. A possible explanation is that horn thrusts directed towards the udder and leading to BM occur in specific situations and are not necessarily influenced by all farm factors that affect horn thrusts directed at other higher body regions (e.g., hindquarter, flank, or shoulder). On the other hand, the recording method might have introduced uncertainties: while integument damages were recorded by a trained experimenter in a standardised way, BM cases and udder damage were based on recordings by the farmers. Even though the farmers were informed by several years of active participation in the project, limited reliability cannot be ruled out.

In the scientific literature, hardly any comparative data on BM incidences can be found. To our knowledge, the occurrence of BM specifically in association with horn-related damage to the udder has not yet been systematically recorded. In automatic milking systems discoloration of milk due to blood admixture is recorded as an indicator of milk quality and udder health (review in Hovinen & Pyörälä, 2011), but the method of data recording and data analysis in single studies preclude comparison with the incidence values used here. For example, in a study on breeding traits in relation to AMS, Dechow et al. (2020) recorded the occurrence of BM daily on three dairy farms in the US including altogether 1,714 hornless cows. They found a total average daily percentage of 6.01 with a high standard deviation of 23.76 (compared to monthly incidences of 2.2±1.9% found in the present investigation). However, if BM occurred over several milkings, one animal was included several times in the analysis. Moreover, the automated recording of discoloration that was used can lead to false positives, e.g., in case of yellow colour associated with higher milk fat contents (review in Hovinen & Pyörälä, 2011).

4.2. Characteristics of affected cows

We hypothesized that particularly primiparous cows would be lower ranking due to lower body size, and experience (review in Bouissou et al., 2001; Lindberg, 2001). At the same time, in the process of establishing their social status in the herd, they might be involved in more agonistic interactions (Hasegawa et al., 1997), altogether leading to a higher risk for horn-induced BM in primiparous cows. In the sample studied, only one third of the affected cows were primiparous (33%), of which, however, 53% were categorised as low-ranking and no cow as high-ranking. Overall, cows between the 2nd and 4th lactation (44%) and cows of medium social rank (58%) were most affected. There are three possible interpretations of these results: (1) The fact that the proportion of affected cows with different lactation numbers corresponded approximately to the typical distribution within German dairy herds (VIT, 2021) suggests that the risk of BM was rather evenly distributed over the different lactation/age categories. (2) The farmers' judgement of the social ranks of the affected cows might have been biased, although in a study in 12 Swiss dairy herds with horned and hornless cows, farmer's estimations of their cows' social rank was consistent with frequencies of initiated versus received agonistic interactions based on systematic behavioural observations (Lutz et al., 2019). However, the farmers could have partly been influenced by their knowledge of the cows' age. In addition, they might have tended to assign a medium rank to most cows, if they were not particularly conspicuous individuals from the lower and upper end of the social hierarchy. (3) Although medium-ranking cows are sometimes reported to be intermediate in terms of social interactions and behavioural restriction, for example regarding feeding time (e.g., Val-Laillet et al., 2008), findings in the literature are mixed. Medium-ranking cows may also be involved in social conflicts more often as a higher fluctuation in the dominance order of dairy herds was found in the medium range compared to the upper and lower third (Arave et al., 1973; Arave & Albright, 1976; Oberosler et al., 1982). In addition, contrary to our expectation that primiparous and low-ranking cows experience many displacements due to the high number of dominant counterparts, it is also possible that these animals more often avoid conflicts with physical contact, if they have the possibility of withdrawal (Lindberg, 2001).

4.3. Effects of pasture and compliance with recommendations

The recording period of the present study covered similarly long periods with pasture access and with all day housing on all investigated farms. We expected more BMvD cases to occur in the winter barn season when all animals are kept indoors all day and are less able to avoid each other compared to pasture (Irrgang, 2012; Miller & Wood-Gush, 1991). This was confirmed in the present study, although on some farms or on individual days only half-day grazing was provided and cows used resources in the barn during the second half of the day.

An earlier analysis of an extended sample of the herds studied here showed that the more guideline recommendations on barn resources and herd management were implemented on the farm, the less horn-related integument damage occurred (Knierim et al., 2020). Also, in relation to BMvD, the multivariable analysis revealed that a higher percentage of fulfilled recommendations in the areas of 'feeding' and in tendency of 'walking/activity' were associated with a lower risk of occurrence over the seven-month monitoring. No relationship to the sum of factors regarding 'milking', 'lying', and 'herd management' could be found. The factor 'feeding' summarises 12 recommendations, both on housing and on management aspects relating to an enhanced access to feed and better protection from other animals during feeding. The 'walking/activity' factor summarises 17 recommendations regarding housing and housing facilities that provide the animals with better opportunities for mutual avoidance in the walking area and in the use of resources (drinkers, cow brush, lickstone) located in the walking area (Table 2). The results suggest that horn thrusts against the udder in the sample studied occurred particularly in these barn areas and indicate that the risk for BMvD can be reduced accordingly by a combination of improvement measures in these areas. However, the lack of associations with recommendations in the areas of 'milking', 'lying' and 'herd management' should not be interpreted to mean that these areas are to be neglected. Although most of the recommendations developed for the prevention guide were considered in the present study (52 out of a total of 61), details on heifer integration as well as factors of the human-animal relationship, which were found to be associated to agonistic interactions and horn-related integument damage in previous studies (Menke et al., 1999; Schneider, 2010), could not be considered.

Specific indications whether the present guideline for the husbandry of horned dairy cows in loose housing should be adapted or extended regarding the prevention of horn-induced BM cannot be derived from the present study. For further examination and quantification of the effects of individual measures, broader epidemiological studies in larger samples or experimental studies under more controlled conditions would be necessary in the future.

4.4. Limitations of the study

In the present study, cases of BM were continuously recorded by the farmers and reported monthly. All farmers were familiar with BM and the evaluation whether milk is fit for human consumption due to legal requirements. However, they were not specifically trained in the assessment, and deviations in the evaluation of milk discoloration cannot be excluded. In future studies, it would be useful to provide farmers with a more precise definition of BM (i.e., using colour charts regarding the degree of coloration in milk, for example), and to train and test its use in advance to minimize potential differences in recording. Alternative detection methods, such as spectrophotometric measurements (Garro-Aguilar et al., 2023), could also be considered. Furthermore, despite the external inspection of the udder for visible damage, uncertainties remain regarding the causes of BM. For this, closer examination of affected udders, possibly including thermal imaging would be helpful.

5. Conclusion

We found large variation in incidences of blood in milk between different horned dairy herds. To a considerable degree they are likely due to agonistic interactions and resulting udder damage. The aim should be to reduce such cases. No age or social rank category of cows could be identified that are in general particularly vulnerable. However, in the investigated sample horn-induced blood in milk cases during summer with half- or full-day access to pasture were reduced compared to winter months with all-day indoor-housing. Apparently, pasture grazing may alleviate the social conflict potential between animals due to the extended space for movement and the different feeding situation compared to indoors. Moreover, higher compliance with existing practice recommendations for the prevention of horn-related integument damage on housing and management in the area of feeding and by tendency of walking/activity was associated with a lower blood in milk risk. The results suggest that the social conflict potential in these areas is also relevant for the occurrence of blood milk cases and can be mitigated by a combination of improvement measures.

Ethics approval

The participation of farms in the study was voluntary. Farmers were assured that all information would be kept confidential and that results would only be published anonymously. A written declaration of participation was obtained from each farm before the start of the survey.

The study was performed in accordance with the "Guidelines for ethical treatment of animals in applied animal behaviour and welfare research" (International Society for Applied Ethology, 2017). It involved no handling causing the animals pain, suffering, distress, or harm. The commercial farms kept the cows in line with national law and guidelines.

Data availability

As this study collected data from commercial farms, some of which are sensitive, none of the data are available in an official repository. On request, however, the data can be made available in anonymized form.

Author contributions

All authors have read and agreed to the published version of the manuscript.

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Ethical statement

Ethical review and approval were waived for this study, as approved by the Designated Veterinarian for Institutional Animal Care of the University of Kassel, because the cows did not undergo any experimental procedures and were only monitored by non-invasive clinical scoring. They were reared and kept for production for human consumption according to national law and guidelines, and not for experimental purposes. The study was conducted in accordance with the German Animal Protection Act (implementing the Directive 2010/63/EU on the protection of animals used for scientific purposes) and the Guidelines for the Ethical Treatment of Animals in Applied Animal Behaviour and Welfare Research of the International Society for Applied Ethology (2017). International Society for Applied Ethology. (2017). *Guidelines for Ethical Treatment of Animals in Applied Animal Behaviour and Welfare Research*. https://www.appliedethology. org/res/EthicalGuidelinesISAErevised2017 for council meeting. pdf

CRediT authorship contribution statement

Asja Ebinghaus: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft. **Julia Johns:** Conceptualization, Methodology, Investigation, Writing – review & editing, Project administration. **Ute Knierim:** Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Appendix

Table A1

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