Associations of husbandry management factors with the new infection risk of bovine intramammary infections in lactation of dairy herds in Northern Germany

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Abstract

The purpose of this investigation was to compare herd-level new infection risk of bovine intramammary infections regarding husbandry management factors. The new infection risk was derived from cow-level somatic cell counts that were provided by monthly dairy herd improvement tests and collected over a period of 3 years and 4 months from 60 commercial dairy farms located in Lower Saxony, Germany. Factors of the management of the farm, the livestock and milking were generated as potential predicting variables. Based on the results of linear mixed models, the herd-level new infection risk was significantly associated with the pre-milking routine and the housing of fresh-lactating cows. When forestripping of every cow was included in the pre-milking routine, this had a beneficial impact on the new infection risk, especially when a foremilking cup was used. Keeping fresh-lactating cows in pens separated from the herd had a negative impact on the udder health, especially when housed together with sick cows. The results of this study confirm that the management of the milking routine and the environment can contribute to the control of udder health and milk quality.

Key-words: bovine mastitis, new infection risk, husbandry management

Introduction

The health and productive performance of dairy cows is still significantly affected by the occurrence of inflammation of the udder because of intramammary infection with mastitis pathogens [1, 2]. Modern milk quality programs focus on the prevention of new infections of the mammary gland to provide and maintain satisfactory and sustainable udder health [3, 4]. In Germany and other European countries, the new infection risk of lactating cows is used as a tool to estimate the level of new infections of the udder occurring at herd-level. This parameter is available monthly from dairy herd improvement tests (DHIT). The average new infection risk in dairy herds in lactation located in Lower Saxony, Northern German, the focus of this study, was 20.8% using a cutoff of 100,000 somatic cell counts per mL milk [5].

Prevention can only be sufficient if risk factors that can cause mastitis are identified and research has focused on this factor over the last decades [4, 6]. A lack of information about farm-level risk factors affecting the udder health of dairy herds in Germany has been published. In a large field study recently conducted in Northern Germany, environmental pathogens were found to be the major cause of clinical mastitis with *Streptococcus uberis* being the most prevalent pathogen [7]. Full cure rates were shown to be low, emphasizing the great relevance of preventing new infection and therefore also clinical mastitis [7]. Bedding material and bovine feces are considered as the main reservoirs and vectors of environmental pathogens causing intramammary infection. Different bedding materials were shown to be a source of exposure to environmental pathogens like *Klebsiella* spp.[8, 9] or *Streptococcus uberis* [8], but they can also be prevalent in feces [10, 11] as well as the indoor and outdoor housing environment [11, 12, 13]. Another recent German scientific paper concluded that bacterial exposure could be reduced by daily replacement of bedding material and frequent cleaning of the lying area, as well as pre-cleaning teats before milking and

dipping teats after milking [14].
Possible risk factors and thus also preventive measures are diverse [6], so that it is necessary to focus on the most important factors under the given farm-level conditions. The aim of this study in the context of a joint research project was to determine factors of husbandry management, which are associated with the new infection risk measured by DHIT in Northern German dairy herds.

Material and Methods

Herd Selection:
As part of the joint research project "SAM, Analysis of Dairy Production", 60 dairy farms were selected. According to the selection criteria of the joint research project, all of these had to be commercial farms located in Lower Saxony, Germany. The herds had to have at least 60 dairy cows of mainly Holstein breed kept in free stalls with cubicles and to take part in dairy herd improvement testing monthly to be eligible to participate. At the outset of this study, the number of cows housed on the farms ranged from 62 to 620 cows (arithmetic mean: 143.4; median: 116.5) and from 7,500 to 11,750 liters of milk yield per cow per year (arithmetic mean: 9,437.4; median: 9,500.0). The original topic of the joint research project was to compare indoor husbandry with pasture-based husbandry to identify differences in animal health and welfare, and specifically in udder health, while determining the factors of pasture management and husbandry that are associated with udder health parameters. Factors regarding pasture management as part of this research were analyzed separately and published in a previous paper [15]. Further details on material and methods are presented in that publication.

Udder Health Data:
The new infection risk of lactating cows is defined for dairy herd improvement tests in Germany [16] as the percentage of lactating animals with > 100,000 somatic cells per mL milk of all lactating animals at monthly dairy herd improvement test having had ≤ 100,000 somatic cells per mL milk at the previous dairy herd improvement test. This parameter was calculated at farm-level using the individual somatic cell counts from monthly DHIT from January 2012 to April 2015. Arithmetic means of the new infection risk were determined at herd-level for the years 2012, 2013, 2014, the summer seasons (May to October) in 2012, 2013, 2014 and the winter seasons (November to April) in 2012/2013, 2013/14 and 2014/2015 based on the data provided monthly.

Farm Data:
Each farm was visited by trained scientists at the beginning of the joint research project. On this occasion farm-specific data was documented following a standardized, structured questionnaire. The data regarding management of the dairy farm and its cattle was collected via interview of the farmer and investigating the livestock facilities (Table 1).

Statistical Analysis:
For analyzing the dataset, the program SPSS 26.0, SPSS Inc. (Chicago, IL, USA) was used with the herd as the statistical unit. The subject was the herd (random) with repeated measurements. We found the unstructured covariance matrix structure to provide the best fit to these data. Associations between new infection risk (NIR) at herd-level level for the years 2012, 2013, 2014, the summer seasons (May to October) and the winter seasons (November to April) in 2012, 2013, 2014 and 2012/2013, 2013/14 and 2014/2015 (target) and risk factors (independent variables) were examined with generalized linear mixed models after pre-screening for variable selection in univariable analysis. The normal distribution of the outcome variable NIR was tested and confirmed using the Kolmogorov-Smirnow test. The relationship between

Table 1: Farm-level variables considered in the univariable analysis of their associations with the new infection risk of lactating cows*. Farm-level variables from the univariable analysis exhibiting a significant association ($p \leq 0.1$) were considered in the multivariable analysis of their associations with the new infection risk of lactating cows*

Category	Variable	p ≤ 0.1**
Farm		
performance	number of dairy cows	
	number of workers	**
	number of workers per dairy cow	
	average milk yield	
	length of productive life per cow	**
	average lifetime milk production per cow	**
	milk yield per length of productive life per cow	
	milk yield per life days per cow	
	replacement rate	
	age at first calving	**
	calving interval	
management	regular pregnancy check	
	purchase or rearing of heifers	
	age of the farmer	
	years of job experience of the farmer	
	member of an advisory council	**
	use of dairy herd management software	
	udder health of cows is a priority	
	fertility of cows is a priority	
	hoof health of cows is a priority	
	longevity of cows is a priority	**
	good overall health of cows is a priority	
	milk yield per cow is a priority	**
feed	forages are tested	
	grass silage is tested	
	corn silage is tested	
hoof health	prevalence of lameness diseases	
	prevalence of dermatitis digitalis	**
	prevalence of sole ulcers	**
	prevalence of white line disease	**
	prevalence of dermatitis interdigitalis and laminitis	**
	prevalence of dermatitis digitalis and white line disease	**
	number of hoof trimmings per cow per year	**
	hoof trimming is done by	
	method of hoof trimming	
	use of footbath	

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udder health	most prevalent mastitis pathogen	
	number of milk samples for microbiological testing	
	species of mastitis pathogens prevalent in milk samples	
Milking		
technology	design of the milking parlor	**
	age of the milking parlor	
	maintenance interval of the milking machine	**
	number of milking clusters	**
	use of automatic cluster removers	
	switch level of automatic cluster removers	**
	assisted positioning of milking clusters	
	use of automatic post-milking stripping	
	milk yield recording	
	operating vacuum of milking machine	**
	type of pulsator	
	pulse ratio	
	design of the milk line	**
	diameter of the milk line	
	diameter of the short milk hose	
	material of the short milk hose	**
	diameter of the long milk hose	
	length of the long milk hose	
	material of the long milk hose	
	material of the liners	
	design of the liners	**
	replacement interval of the liners followed according to specifications	**
	sight glass in the milking parlor	**
	air ingress during milking	**
	driver in the waiting area	
work	who is milking	
	number of milkers per milking	**
	parlor work routine	**
	use of forestripping during pre-milking routine	**
	examination of the milk during forestripping	**
	preparation lag time	**
	use of automatic stimulation	
	work-quality of attaching the milk clusters	**
	use of oxytocin	**
hygiene	number of cows milked per milking cluster	
	milking-order of cow groups	
	milking-order of cows with mastitis	
	milking-order of fresh cows	
	use of gloves in the milking parlor	**
	cleaning of hands in the milking parlor	
	sanitation of hands in the milking parlor	**
	cleaning of teats before milking	**
	method of cleaning of teats before milking	**
	number of cows per pre-cleaning-towel	**
	cleaning of pre-cleaning towels	**
	pre-milking teat sanitation	
	post-milking teat sanitation	**
	method of post-milking teat sanitation	**
	intermediate rinsing of clusters	
	intermediate disinfection of clusters	**
	removal of udder-hair	**
	water quality in the milking parlor	**
	cleaning of the milking stall floor	**
	tools for cleaning the milking stall floor	**
	cleansing of the milking machine	
	temperature of rinsing water	**
	alkaline cleansing of the milking machine per day	**
	acid cleansing of the milking machine per day	**

the NIR and the independent variables was first determined using appropriate univariable parametric test procedures. Independent variables associated with the dependent variable at $p \leq 0.1$ except for predictors in the same model, which indicated a correlation of $r > 0.70$ with one another (to avoid multicollinearity; for this reason, no variables were excluded) were submitted to generalized linear mixed models with an identity link.
The multivariable analysis was performed using a backward stepwise selection and elimination procedure until each independent variable had a p-value of ≤ 0.05. Confounding effects were monitored by observing regression coefficient changes. Variables that modified regression coefficients by > 20% were considered confounding factors. No confounding was observed. The models were evaluated using the Akaike information criterion (AIC), where an AIC closest to zero was used as final model. In the final model, all biologically credible two-way interactions were tested but eliminated again due to lack of significance. Model fit was evaluated by checking normality of the residuals. The random farm effect was not significant in the models but was kept as a design variable. Least square means from the model were calculated. The significance level for the linear mixed model was 0.05.

Results

New Infection Risk:

Between January 2012 and April 2015, the monthly herd-level new infection risk of lactating cows varied between 7.4% and 43.0%. The arithmetic mean was 20.6% and the median 18.9%.
The monthly herd-level new infection risk of lactating cows calculated from individual somatic cell counts from monthly DHIT are shown in Table 2 for the respective years, summer (May to October) and winter seasons (November to April) in the period from January 2012 to April 2015.

Univariable analysis:

Numerous factors of the management of the farm, milking, the lactating cows, the dry cows as well as the young stock were found to be associated with the new infection risk in the univariable analysis. In Table 1, the herd-specific factors that were statistically associated with the risk of new infection ($p \leq 0.1$) in the univariable analysis are listed.

Lactating cows		
housing	number of groups of lactating cows	**
	design of the barn	**
	number of cows per stall	
	number of cows per feed bunk space	**
	housing area per cow	
	design of the stalls	**
	type of mattress in the stalls	
	primary bedding material	
	secondary bedding material	**
	type of limestone added to the bedding material	
	proportion of limestone of the bedding material	
	frequency of adding fresh bedding material	
	frequency of cleaning the stalls	
	complete replacement of the bedding	**
	lactating cows on deep straw	
	material of the housing floors	**
	design of the housing floors	**
	frequency of manure removal from the housing floor	
	method of manure removal from the housing floor	**
	additional housing yards	
	access to pasture	
	days per year access to pasture	
	hours per day access to pasture in March	**
	hours per day access to pasture in April	
	hours per day access to pasture in May	
	hours per day access to pasture in June	
	hours per day access to pasture in July	
	hours per day access to pasture in August	**
	hours per day access to pasture in September	
	hours per day access to pasture in October	
	hours per day access to pasture in November	
	access to pasture from November to April	
	selection gates	
	consolidation of livestock trails	
	consolidation material of livestock trails	
	separate pen for fresh cows	**
	time in separate pen for fresh cows	**
feed	source of drinking water	
	access to additional feed of lactating cows if kept on pasture	**
	type of additional feed of lactating cows if kept on pasture	
	type of watering place on pasture	
	number of different rations for lactating cows	**

Dry cows		
drying off	drying-off procedure	
	average length of the dry period	
	dry cow treatment	**
	method of dry cow treatment	
	blanket dry cow treatment	
housing	number of groups of dry cows	**
	design of the dry cow pen	**
	design of the dry cow stalls	**
	type of mattress in the dry cow stalls	
	bedding material in the dry cow stalls	**
	type of limestone added to the bedding material of dry cows	
	design of the housing floor in the dry cow pen	**
	frequency of manure removal on housing floor of the dry cows	
	method of manure removal on housing floor of dry cows	
	access to pasture of dry cows	
	days per year access to pasture of dry cows	
	start of the pasture-season of the dry cows	**
	end of the pasture-season of the dry cows	**
	requirements (weather, grass growth) for access to pasture of dry cows	
	design of the calving pens	
	number of calving pens	
	average size of calving pens	
	overall size of the calving area	
	average square meters of calving area per cow	
	bedding material in the calving pens	**
	calving pens separated from sick cow pens	
feed	number of dry cow rations	
	separate ration fed to dry cows	**
Young stock		
calves	design of the calf pen in the first two weeks after birth	
	design of the calf pen after the first two weeks after birth	
	bedding material of the calves	
	access to pasture of calves	
	age at first access to pasture of calves	
young heifers	design of the young heifer pen	
	design of the young heifer stalls	
	type of mattress in the young heifer stalls	
	bedding material of the young heifers	
	access to pasture of young heifers	
	days per year access to pasture of young heifers	
	age at first access to pasture of young heifers	

heifers	design of the heifer pen	
	design of the heifer stalls	
	type of mattress in the heifer stalls	
	bedding material of the heifers	**
	access to pasture of heifers	
	days per year access to pasture of heifers	
	age at first access to pasture of heifers	**
	age group at first access to pasture	**

* percentage of lactating animals with > 100,000 somatic cells per mL milk of all lactating animals at monthly dairy herd improvement test having had ≤ 100,000 somatic cells per mL milk at the previous dairy herd improvement test
** the respective variable was statistically associated ($p \leq 0.1$) in the univariable analysis

Multivariable analysis:
Four different risk factors remained in the final generalized mixed models. These factors were related to milking and the environment of the lactating cows. The new infection risk was lower in herds with a pre-milking procedure including forestripping of every cow as a standard, and even lower if foremilking cups were used for this purpose compared to herds with no forestripping included in the pre-milking routine. If only animals which were conspicuous regarding udder diseases were forestripped, the new infection risk was higher than in those herds with no forestripping procedure. Very good quality work in attaching the milk-clusters to the udder was significantly associated with a lower new infection risk. The existence of fresh cow pens resulted in a higher new infection risk, especially if the fresh cows were housed together with the sick cows. The method of manure removal from housing floors provided significant results. Robots and vehicles with an attached scraper and manual scraping were associated with a lower new infection risk and manually controlled manure scrapers with a higher new infection risk compared to an automatic scraper.
The results of the final multivariable analysis of the new infection risk of lactating cows including these risk factors are displayed in Table 3, and least square means describing differences between these risk factors in Table 4.

Discussion

The aim of this study was to investigate the influence of manifold management factors on the new infection risk of dairy cattle in Lower Saxony, Germany. This was one of the first investigations focusing on such udder health risk factors in this geographic region. The regional impact on the results must be considered in the interpretation, even though no significant factors were region-specific in the narrower sense. It must also be recognized that the number of evaluated variables was high compared to the number of herds and respective data regarding udder health due to the given study design of the joint research project. Changes in the examined variables of the participating farms over the period of the investigation were not considered.
Despite this, the study provides evidence that management factors regarding milking and the environment of the dairy cows have an impact on udder health. Due to the limited power of the study, only the most important risk factors could probably be identified.
One of these factors is the pre-milking routine. Forestripping every cow before attaching the milking clusters had a beneficial effect on the new infection risk of the lactating cows, especially if the milk was collected with a foremilking cup. Forestripping only cows conspicuous of mastitis resulted in a higher risk of new infection.
Previous studies have shown a positive impact of using a foremilking cup on somatic cell counts at cow level [17] in the forestripping practice, but a negative impact on the number of cases of clinical mastitis [18] and clinical mastitis caused by *Staphylococcus aureus* [19, 20, 21] and *Escherichia coli* [20] when cows were forestripped manually. More recent investigations either found no association between forestripping during milking preparation and the somatic cell count of the bulk tank milk [22] or showed a tendency for forestripping to reduce the occurrence of clinical mastitis and a significant reduction thereof if forestripping was part of a complete milking routine (forestripping, predipping and drying before milking unit attachment) [23].
The procedure of forestripping could also be related to the preparation lag time, which was only a significant factor in the univariable analysis. Preparation lag time was associated with clinical and subclinical mastitis [24, 25].
Forestripping is a recommended practice for a complete milking routine as well as mastitis control programs [3, 23, 26] and is a mandatory practice by legislation of the European Union [27]. Nonetheless, the

Table 2: Farm-level new infection risk of lactating cows* [%] calculated from individual somatic cell counts from monthly DHIT in the period from January 2012 to April 2015

Period	Minimum	Lower Quartile	Median	Upper Quartile	Maximum	Mean
All	7.4	15.1	18.9	25.2	43.0	20.6
2012	10.0	17.0	22.0	28.0	39.0	22.6
2013	8.0	15.0	18.0	24.0	35.0	19.7
2014	10.0	15.0	18.0	24.3	37.0	19.7
2012S	8.1	18.7	23.5	30.8	8.1	25.0
2013S	7.4	16.6	18.6	23.8	7.4	20.7
2014S	10.4	15.2	18.6	25.2	10.4	20.8
2012/2013W	8.8	13.1	17.1	21.5	8.8	18.2
2013/2014W	8.7	14.1	17.8	25.3	8.7	19.8
2014/2015W	8.0	14.0	17.3	23.7	8.0	18.9

* percentage of lactating animals with > 100,000 somatic cells per mL milk of all lactating animals at monthly dairy herd improvement test having had ≤ 100,000 somatic cells per mL milk at the previous dairy herd improvement test
S = summer-season (May to October)
W = winter-season (November to April)

Table 3: Final multivariable analysis of the new infection risk of lactating cows*

Variable	Coefficient	Standard Error	Confidence interval 2,5%	Confidence interval 97,5%	p
Intercept	28.328	2.052	24.306	32.349	0.000
Use of forestripping during pre-milking routine					
Yes	-5.094	1.252	-7.548	-2.640	0.000
Yes, cup is used	-6.151	1.238	-8.576	-3.725	0.000
Yes, only conspicuous cows	13.179	2.373	8.527	17.830	0.000
No	0				
Work-quality of attaching milk clusters					
Very good	-5.412	1.270	-7.901	-2.922	0.000
Good	-1.046	1.300	-3.594	1.503	0.421
Satisfactory	2.931	2.289	-1.554	7.417	0.200
Just sufficient	0				
Method of manure removal from housing floors					
Manual scraping	-5.103	1.796	-8.622	-1.584	0.004
Robot	-6.650	2.221	-11.003	-2.297	0.003
Vehicle	-4.257	1.547	-7.289	-1.225	0.006
Manually controlled scraper	5.653	1.416	2.878	8.428	0.000
None	-2.307	1.908	-6.047	1.433	0.227
Automatic scraper	0				
Separate pen for fresh cows					
Yes	3.426	1.243	0.990	5.863	0.006
Yes, kept together with sick cows	5.350	1.558	2.297	8.404	0.001
No	0				

*percentage of lactating animals with > 100,000 somatic cells per mL milk of all lactating animals at monthly dairy herd improvement test having had ≤ 100,000 somatic cells per mL milk at the previous dairy herd improvement test

quality of how it is performed in terms of pre-milking hygiene, especially when contagious mastitis pathogens are prevalent, could be crucial for the outcome.

Another finding associated with the milking practice was a lower new infection risk related to high-quality work in attaching the milk-clusters to the udder. Quality indicators included the extent of air admission, hygiene and the speed of attaching the milk-clusters. A French study [28] showed a negative impact of air admission at teat cup attachment, this being an indication of low-quality work. Air admission could lead to a varying vacuum in the milking system and even fall-offs of the milking clusters with poorer udder health resulting [29].

The method of manure removal from the housing floors of the lactating cows was statistically significant in the final model of our study. The methods were recorded as manual scraping, robot, vehicle, manually controlled scraper, none and automatic scraper, with the manually controlled scraper having the highest risk of new infection in lactation and the robot having the lowest risk. Possibly the method of manure removal refers to the new infection risk if it has a sufficient impact on

Table 4: Least square means describing differences between farm-level variables associated with the new infection risk of lactating cows*

Variable	Mean	Standard Error	Confidence interval 2,5%	Confidence interval 97,5%
Use of forestripping during pre-milking routine				
Yes	18.527	0.737	17.081	19.970
Yes, cup is used	17.470	0.990	15.529	19.410
Yes, only conspicuous cows	36.799	1.802	33.266	40.332
No	23.620	1.193	21.281	25.960
Work-quality of attaching milk clusters				
Very good	19.573	0.932	17.747	21.400
Good	23.939	0.641	22.683	25.196
Satisfactory	27.916	1.815	24.359	31.474
Just sufficient	24.985	1.287	22.463	27.507
Method of manure removal from housing floors				
Manual scraping	21.111	1.554	18.065	24.157
Robot	19.564	1.443	16.737	22.393
Vehicle	21.957	1.564	19.690	24.223
Manually controlled scraper	31.867	1.288	29.342	34.393
Automatic scraper	26.214	1.199	23.864	28.565
None	23.907	1.651	20.670	27.144
Separate pen for fresh cows				
Yes	29.245	1.180	26.933	31.558
Yes, kept together with sick cows	31.169	1.407	28.412	33.927
No	25.819	0.629	24.587	27.051

* percentage of lactating animals with > 100,000 somatic cells per mL milk of all lactating animals at monthly dairy herd improvement test having had ≤ 100,000 somatic cells per mL milk at the previous dairy herd improvement test

the hygiene of the environment. Numerous other studies could show a relation between the hygiene of floors and passages as well as udder health indicators [30, 31, 32, 33].

The collected data provided evidence that the existence of a pen for fresh cows was statistically associated with a higher new infection risk. No references with the same or with differing findings are available. The study dataset did not provide clear information about the conditions and the management of the fresh cow pens. However, the dataset provided information that it was a common practice to house fresh cows in group pens on deep straw during the first days after calving in at least 29 of the 60 farms. This type of housing could be associated with poorer udder health because of worse environmental hygiene compared to free stall housing [18, 34, 35].

Several investigations stated the importance of optimal environmental hygiene for the transition area [31] and the calving area [18, 19, 20, 21, 30, 31, 36, 37]. The environmental hygiene fresh cows are exposed to after calving could be of similar importance.

When the fresh cows were housed together with the sick cows, the described effect was intensified. This practice possibly increases the exposure to mastitis pathogens. Comparable results were stated concerning the incidence rate of clinical mastitis caused by Escherichia coli when calving pens and sick cow pens were not separated [19].

Conclusions

Farm-level risk factors for new infections of the udder of dairy cows are multitudinous and depend on the given conditions of the respective farm. Minimizing the new infection risks can be effectively achieved by optimizing the management of the milking routine and the environment that cows are housed in. The results of this research suggest keeping fresh cows under hygienic conditions and separated from sick cows and to include forestripping to the pre-milking routine. The use of a foremilking cup can be a purposeful tool to maximize hygiene in this practice.

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