An assessment of risk factors impacting the prevalence of IMI in Danish dairy cattle

Author:	Nynke Schipper
Registration nr.:	1041595
Code:	QVE-70424
Supervisor (WUR):	Bart van den Borne
Host supervisor(s) (KU):	Michael Farre
	Carsten Kirkeby
Date:	January 10 to May 16, 2022

Introduction

Mastitis is a common disease that has a significant impact on the health, well-being, milk yield, and milk quality of dairy cattle (Le Roux et al., 2003; von Keyserlingk et al., 2009). It causes significant financial losses for dairy farmers worldwide (Nielsen, 2009; Gonçalves et al., 2018; Hogeveen et al., 2019).

When a quarter had clinical mastitis, it will most commonly be treated with antimicrobials (Afifi et al., 2018). However, the use of antimicrobials also correlates with antimicrobial resistance and can cause residues in the milk, which is undesirable. To combat upcoming antimicrobial-resistant pathogens, initiatives are currently being put forward to lower antimicrobial usage in animal husbandry (Bhutto et al., 2011). There is an increasing demand for non-antimicrobial, management-based options to lower infection risks.

Intramammary infections (IMI) can cause mastitis. To assess whether a quarter is infected, a microbiological culture can be used. Likewise, a MALDI-TOF test can be undertaken to identify the pathogen. For risk analysis, identifying pathogens is essential to distinguish infection patterns.

Classically, two different types of pathogens can be identified according to their route of transmission, namely contagious and environmental pathogens (Smith et al., 1985; Smith and Hogan, 1993; Klaas and Zadoks, 2018). Contagious pathogens transmit from cow to cow directly, primarily via milking procedures. Environmental pathogens infect cows through the environment, for example from the bedding or manure. In order to reduce the prevalence of both pathogen types, different measures need to be taken. This means influencing the transmission during milking procedures by using effective methods to disinfect and lower the transmission for contagious pathogens. With environmental pathogens, the infection rate can be reduced by having a clean environment.

For assessing the herd's health, it is imperative to use the correct methods. A commonly used method to monitor udder health is somatic cell counts (SCC). SCC are the number of somatic cells in milk and is positively associated with the prevalence of IMI (Wenz et al., 2007; Kelly et al., 2009; Ruegg and Pantoja, 2013). As a measure of herd health, the bulk tank SCC (BMTSCC) is used which is the pooled mean of the SCC of all cows in the herd. Farmers can earn more profit when their BMTSCC is lower because of fewer milk production losses and an increased price for the milk from the buyer. This means that when the herd's udder health is low, the revenue will also be lower (Kirkeby et al., 2016). Thus, dairy farmers want to have a healthier herd, supplying a lower BMTSCC.

Outside of management factors, the cow's cleanliness and its environment are said to influence the prevalence of IMI. To measure the cleanliness of the cow, the hygiene score can be used (Cook and Reinemann, 2007). It has been shown that a higher hygiene score is correlated with a high SCC and BMTSCC (Schreiner and Ruegg, 2003; DeVries et al., 2012).

Several management strategies exist during the milking procedure to reduce the infection risk. Both cleaning the teats and pre and post milking disinfection are effective in lowering the bulk tank SCC (Barkema et al., 1998; Godden et al., 2016; Fitzpatrick et al., 2021). These practices are recommended by the National Mastitis Council (NMC) and thus often implemented by farmers worldwide (National Mastitis Council, 2013). Where pre milking disinfection mainly targets the rate of infection of environmental pathogens, post milking disinfection is more effective for lowering contagious pathogens' infection risk.

Two main routes of post milking disinfection are most commonly used, namely spray and dip disinfection (Blowey and Edmondson, 1996). With spray disinfection, the teats and udder get sprayed with a germicidal solution in which the teats need to be entirely covered by the product for maximum efficacy. The dip method uses a cup containing a germicidal solution in which the teat get dipped in. This has a higher success rate, as it is harder not to cover the teat sufficiently. Both spray and dip disinfection are accepted by the NMC (National Mastitis Council, 2013). However, the product used must be used correctly and have a high efficacy.

No assessment of whether a difference can be found between different disinfection methods and what other factors are most influential on the prevalence of IMI and different pathogens has been made, as well as an assessment on whether currently used management options are effective. Additionally, the quality of indicators of udder health, for example the BMTSCC and hygiene score can be assessed. This study aims to assess the comparative efficacy of disinfecting milking procedures and the influence of herd characteristics on the prevalence of culture-negative quarters and environmental and contagious pathogens. Coming to the three research questions:

1. Which factors have an impact on the fraction of culture-negative quarters per herd?
2. Which factors have an impact on the average number of contagious pathogens per quarter?
3. Which factors have an impact on the average number of environmental pathogens per quarter?

Materials and methods

Data

From August 2019 to December 2020, a survey among Danish dairy farms (n=88) was conducted. One part of the on-farm data collection was an interview done by one investigator. Here questions were asked about management factors, including milking practices, the method of applying disinfection products, and the BMTSCC. All different outcomes for the categorical variables are summarized in Table 1.

Table 1: the categorical variables and the treatment used

Variable	**Category**
Pre milking sanitation	Foam cup
	Foam gun
	Soap spray
	Teat scrubber
	Nothing
Post milking disinfection	Dip cup
	Spray gun
	Other
	Nothing
Prewash	Dip
	Spray
	Other
	Nothing
Cow SCC status	Chronic inflammation
	New inflammation

Additionally, 25 cows of each herd were tested for IMI by MALDI-TOF, providing pathogen-specific prevalence data at quarter level. These cows were randomly selected from two groups, those that are chronically inflamed and those that are newly inflamed. Where the chronically inflamed cows had a SCC of 200,000 cells/ml or higher at the current and previous measurement, the newly inflamed cows had a SCC that was lower than 200,000 cells/ml at the previous measurement while the current one was higher than 200,000 cells/ml. IMI was defined by the presence of bacteria in the quarter milk sample, as diagnosed by microbiology. Quarter milk samples with three or more different pathogens were defined as contaminated and discarded. Non-inflamed quarters were classified as culture-negative. Consecutively, all pathogens were categorized into two groups, environmental and contagious. From a literature study, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae* and *Corynebacterium bovis* were defined contagious pathogens (Fox and Gay, 1993). Other pathogens found were classified as environmental, except for cases where its real classification was unclear from literature. Then these were excluded from the analysis.

The number of IMI caused by contagious/environmental pathogens or the number of culture-negative quarters per SCC group within each herd were summed and divided by the total number

of sampled quarters per herd, to normalize the data, leaving a fraction of pathogens per quarter present or fraction of culture-negative quarters per SCC group for each herd. These were separately analyzed for culture-negative, environmental and contagious pathogens.

Furthermore, the hygiene score was assessed by one person on all farms using the methods described by Cook and Reinemann (2007). The udder was scored on a scale from 1 until 4. Where 1 represents a clean or minimally dirty udder, 2 is a bit of dirt, 3 has a cover of mostly dirt and, 4 is completely covered in dirt. When possible, all lactating cows in the herd were assessed. The average hygiene score was calculated by averaging all hygiene scores of all cows within a herd.

Using the package "hydroTSM" (Zambrano-Bigiarini, 2020) the dates of the visits were collected and categorized into seasons. Where winter was defined as December, January and February, spring is March, April and May, summer is June, July and August, and autumn is September, October and November.

Statistics

The statistical analysis was carried out using R (R Core Team, 2021). At first, normality was assessed for all continuous variables. This was done by both making a qq-plot of the variables and running a Shapiro-Wilk test. Here a p-value of 0.05 or lower indicated an issue with assuming normality and warranted a non-parametric test. None of the response variables were normally distributed.

A linear mixed model was used to assess effects of the explanatory variables on the response variable using the lme4 (Bates et al., 2015) and lmerTest (Kuznetsova et al., 2017) packages in R. Where the model was built up like this:

$$Response\ variable = Intercept + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \cdots + \gamma + error$$

Three separate models were made, each with the same explanatory variables and random effect, but a different response variable. The three different response variables were: fraction of environmental pathogens per quarter present, fraction of contagious pathogens per quarter present or fraction of culture-negative quarters per SCC group for each herd. The explanatory variables were: prewash, pre milking sanitation, post milking sanitation, herd size, BMTSCC, mean hygiene score, season and the cow SCC status. Because two different fractions were present per herd, one for newly inflamed and one for chronically inflamed cows, a random effect for herd was added to all models. Using a backwards elimination procedure, the model was decreased until all variables were significantly contributing to the model. The different models were compared using the anova function from R. Giving whether significant differences could be found between the models.

Finally, boxplots were made in R using the package ggplot2 (Wickham, 2016).

Results

In Table 2, the characteristics of the variables used in the analyses are described. Missing data was present for the variables herd size, hygiene score, pre milking sanitation and BMTSCC (6, 10, 4 and 6 missing values, respectively). Overall, 65 herds were analyzed, with an average herd size of 344 cows (Table 2). The most commonly used treatments in prewashing, pre milking sanitation and post milking sanitation were dip (n=33), foam cup (n=35) and dip cup (n=40), respectively. Most pathogens were environmental compared to contagious pathogens (Table 2 and Figure 1). About the same numbers of cows were chronically inflamed as newly inflamed (n=944 and 865, respectively).

Table 2: The means or number of herds per factor per variable.

Variable		N herds	Mean
Herd size		65	344
Hygiene score			2.01
Prewashing	Dip	33	
	Spray	17	
	Other	1	
	Nothing	14	
Pre milking sanitation	Foam cup	35	
	Soap spray	13	
	Teat scrubber	2	
	Nothing	15	
Post milking disinfection	Dip cup	40	
	Spray gun	17	
	Other	1	
	Nothing	7	
Season	Winter	13	
	Spring	12	
	Summer	15	
	Autumn	25	
Bulk tank SCC (x1,000cells/mL)			125.9
Infection type[1]	Contagious		0.141
	Environmental		0.733
	Culture-negative		0.126
Status cow (%)	Chronically inflamed		52.2
	Newly inflamed		47.8

1: Total number of contagious/environmental IMIs or culture-negative quarters per herd divided by the sampled number of quarters per herd

The within-herd prevalence of contagious pathogens was higher in the group of chronically inflamed cows compared to the newly inflamed group (Figure 1). No real difference was present between chronically and newly inflamed cows and the herd level prevalence of environmental pathogens per quarter. The within-herd prevalence of culture-negative quarters was lower in the group of chronically inflamed cows compared to the newly inflamed group.

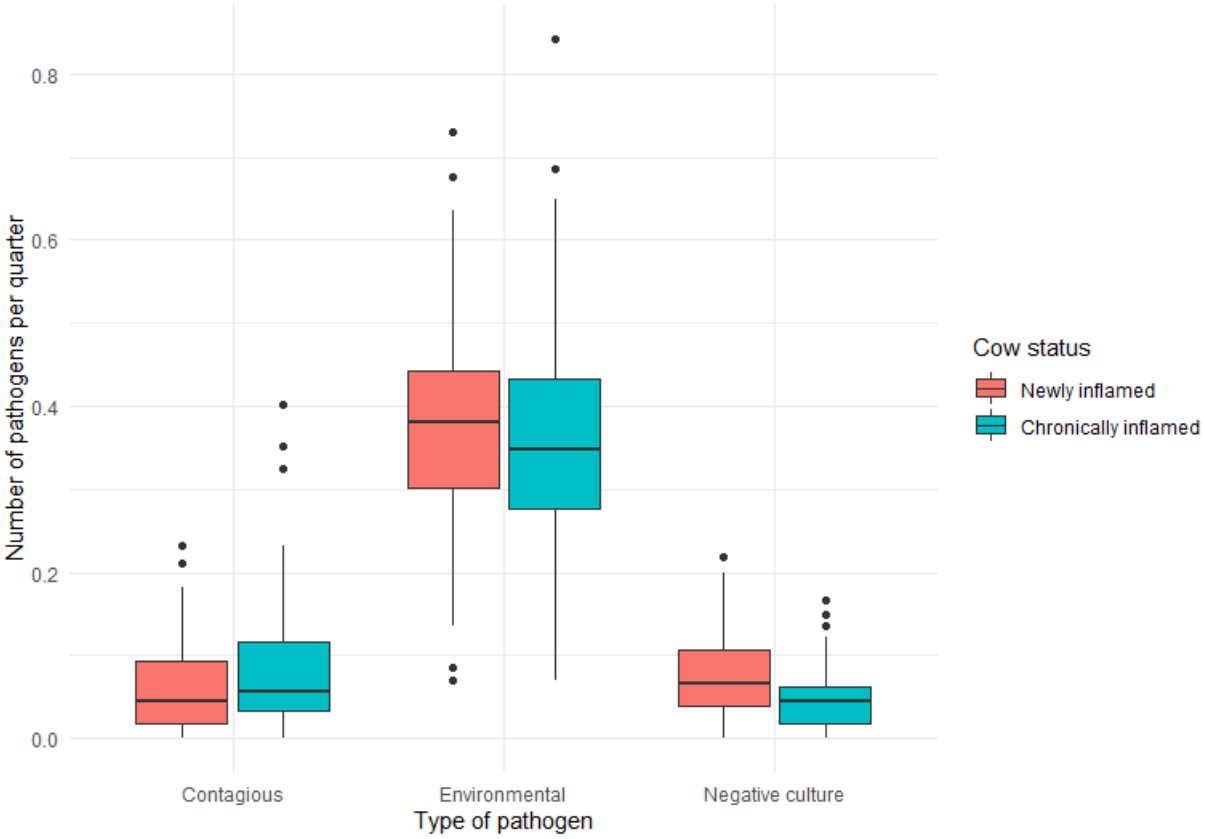

Figure 1: The average number of pathogens or culture-negative quarters, by the SCC status of the cow.

Culture-negative quarters

The fraction of culture-negative quarters within the herd was lower in chronically inflamed cows compared to newly inflamed cows (Table 3). Additionally, the fraction of culture-negative quarters within the herd was lower in autumn compared to spring.

Table 3:The results of the mixed linear model of risk factors for the within-herd prevalence of culture-negative quarters.

		β	Std. Error	t value	p-value
Intercept		0.06	0.01	8.43	4.85E-13*
Cow SCC status	Chronic	-0.03	0.01	-4.47	3.27E-05*
	Newly inflamed	Reference			
Season	Spring	0.04	0.01	3.43	1.04E-03*
	Summer	0.02	0.01	1.63	0.11
	Winter	0.01	0.01	0.49	0.62
	Autumn	Reference			

Contagious pathogens

The use of “other” post milking teat disinfection methods was associated with a higher fraction of contagious pathogens per quarter within the herd compared to doing nothing (Table 4). A higher within-herd fraction of contagious pathogens per quarter within the herd was seen among the chronically inflamed cows in a herd compared to the newly inflamed cows.

Table 4:The results of the mixed linear model of risk factors for the within-herd prevalence of contagious pathogens.

		β	Std. Error	t value	p-value
Intercept		0.04	0.02	1.96	0.05
Post milking treatment	Dip cup	0.01	0.02	0.31	0.76
	Other	0.11	0.06	2.02	0.05*
	Spray gun	0.04	0.02	1.72	0.09
	Nothing	Reference			
Status cow	Chronically inflamed	0.03	0.01	2.88	0.01*
	Newly inflamed	Reference			

Environmental pathogens

The use of a spray gun for post milking teat disinfection was associated with a lower fraction of environmental pathogens per quarter within the herd compared to doing nothing (Table 5).

Table 5: The results of the mixed linear model of risk factors for the within-herd prevalence with environmental pathogens.

		β	Std. Error	t value	p-value
Intercept		0.84	0.05	17.11	<2e-16*
Post milking treatment	Dip cup	-0.10	0.05	-1.84	0.07
	Other	-0.27	0.14	-1.92	0.06
	Spray gun	-0.15	0.06	-2.53	0.01*
	Nothing	Reference			

Other predictor variables

In the analysis, no effect of the BMTSCC on the fraction of quarters that were inflamed could be found (Figure 2). Most herds had a BMTSCC of 200,000 cells/ml or less (56/64, 87.5%).

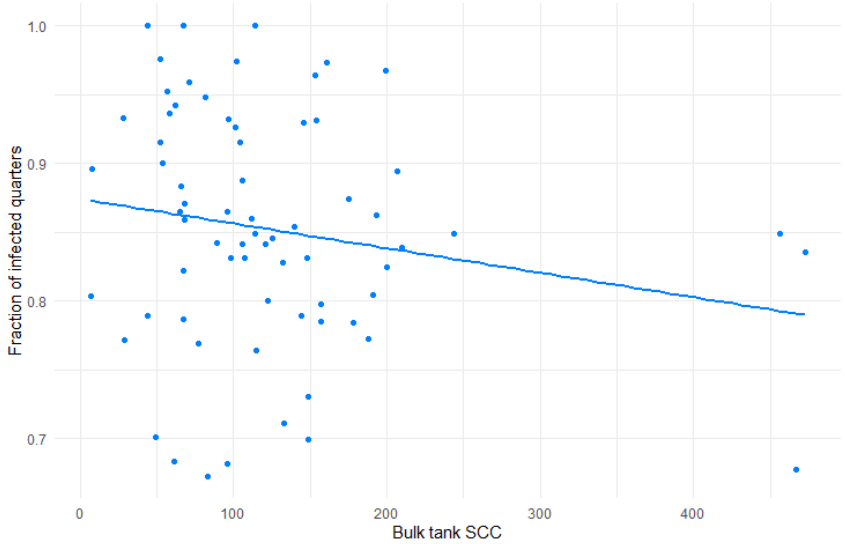

Figure 2: Bulk tank SCC per herd with fraction of infected quarters.

No correlation was seen between the mean hygiene score and the fraction of quarters infected either (Figure 3).

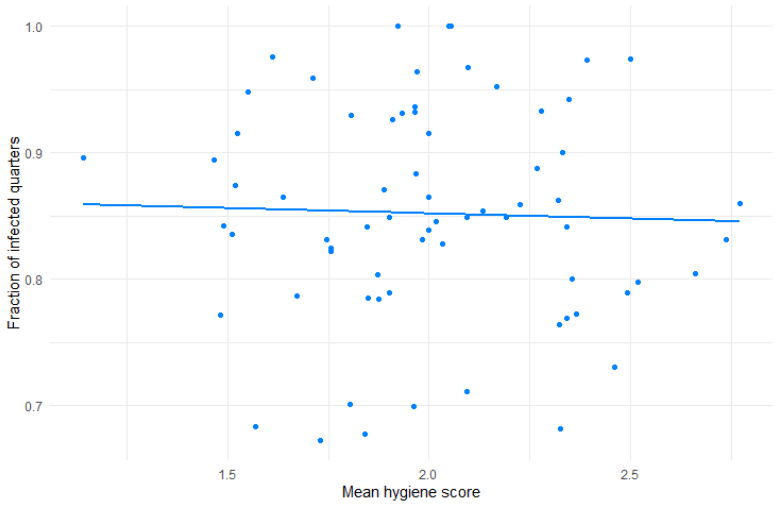

Figure 3: Mean hygiene score per herd with the fraction of quarters infected

Discussion

Management factors

The hygiene score of the udder has been shown before to be predictive for SCC and IMI (Schreiner and Ruegg, 2003; Cook and Reinemann, 2007; Zucali et al., 2011). In our analysis, at herd level, no correlation between hygiene score and the fraction of infected quarters, or the number of environmental pathogens could be found. The data used for the analysis was skewed towards a lower hygiene score (below 3). As none of the scores were above 3, it seems that, at herd level, cows sampled were too clean to identify significant differences.

Bulk milk tank SCC

No significant correlation was seen between the BMTSCC and the fraction of infected quarters. This is contrary to research previously conducted in this area, where bulk tank SCC was correlated with the fraction of infected quarters (Wilson et al., 1997). However, the study of Hutton et al. (1990) also showed no significant difference in infection rate between two groups with different BMTSCC, similar to the results in our study. It is possible that farmers are trying to keep the BMTSCC below the threshold, to assure they are not penalized. Thus, milk from cows with high SCC will be omitted, which can cause a discrepancy between BMTSCC and the infection rate.

Chronic and new infection

A distinction was made between chronic and newly inflamed cows. The analysis showed that among chronically inflamed cows the percentage of culture positive quarters was higher than with newly inflamed cows, indicating that newly inflamed cows had less IMI than chronically inflamed cows. Additionally, it has been shown that chronic infection causes significant changes in milk quality and production (Bobbo et al., 2020). Also, a positive correlation was present between chronically inflamed cows and the presence of contagious pathogens. Seemingly showing that contagious pathogens will cause longer term inflammation.

Additionally, the fraction of culture negative quarters in the current investigation is not representative of the fraction of culture negative quarters in the herd. The samples were only taken from cows that were inflamed, (eg cell count above 200.000 cells/ml for their last measurement), thus the non-inflamed cows are not included. This causes bias towards cows that are more likely to harbor an infection. As these cows, on average, have a higher somatic cell count, they are not representative of the herd. This could cause the discrepancies seen between BMTSCC, hygiene score and infection rate.

Season

The spring showed a significant increase in culture negative quarters compared to autumn (Table 3). Prior, similar effects were shown where season was correlated with the prevalence of contagious pathogens (Østerås et al., 2006). An increase in temperature and humidity has shown to increase the duration of infections in cows (Hamel et al., 2021). During summer and autumn the rainfall and thus humidity is higher which is positively correlated with higher SCC and infection rate (Reneau, 1986; Lopez-Benavides et al., 2005; Sant'Anna and Paranhos da Costa, 2011).

Post milking disinfection

To lower the fraction of infected quarters, post milking disinfection has been recommended by NMC (National Mastitis Council, 2013). Prior research showed that using post milking disinfection lowered the SCC and BMTSCC (Kelly et al., 2009). Post milking disinfection was associated with

a lower infection risk of environmental pathogens, however with contagious pathogens the risk was increased.

Additionally, farmers that are facing issues with high amounts of contagious pathogens will be more likely to implement methods to lower the risk of infection compared to farms where few contagious pathogens are present. Thus due to reverse causality, the results could be biased.

To expand on that, not all pathogens will cause inflammatory reactions. Some cultured pathogens will be cleaned up by the cow's immune system before it will cause inflammation. Thus other indicators of infection should have been added, such as milk yield and composition.

Most pathogens were environmental of nature (Table 2). The prevalence of these can be lowered by improving hygiene in the stable and pasture (Smith and Hogan, 1993). However, milking practices were mostly evaluated in this report, which should influence the infection rate of contagious pathogens. Here the effect of using post milking disinfection was shown to have a significant effect on lowering the fraction of infected quarters and prevalence of environmental pathogens. For contagious pathogens no such effect could be identified. Due to the prior focus on contagious pathogens, these levels may have been lowered already. The group of environmental pathogens is larger, thus giving more room to lower its prevalence.

Limitation of the analyses

All bacteria identified in the cows were put into two categories. This was done by using scientific literature, however not all could be categorized. For these species no clear proof could be found whether they belonged in either category. Overall the contagious group was mainly made up by major mastitis causing species. Whereas the environmental group was made up from major environmental species, including streptococci species other than *Streptococcus agalactiae* and *Streptococcus dysgalactiae,* coliform bacteria, and most minor pathogens (Smith, 1983). The species unable to be categorized were not a large portion of the data and are thus not considered very influential to the overall result.

Some species have antimicrobial properties and were thus not classified. An example is Lactobacillus, which is used in novel teat sealants to decrease new infection rate (Soleimani et al., 2010; Yu et al., 2017). Leaving those species in would have resulted in an overestimation of the mastitis causing pathogens, thus leading to an elevated infection rate.

Lastly, the classification of environmental and contagious pathogens is an old fashioned and disputed method (Klaas and Zadoks, 2018). It has been shown that many pathogens can be carried over both ways, as their primary ways do not affect them solely. Thus, combatting an infection with a certain method is not fully effective. It is therefore important to implement management measures against both routes of infection. However the added value of knowing where the majority of infections come from, can serve as an tool for prioritizing advice.

Conclusion

Given the low prevalence of contagious pathogens, the effect of pre and post milking practices on the overall infection rate was difficult to quantify in this study. The bulk of the pathogens defined were environmental pathogens, and some herds did not even have any contagious pathogens present. Therefore, risk factors potentially affecting the prevalence of environmental pathogens
The effect of milking disinfection, specifically post milking disinfection, lowers the overall fraction of infected quarters. Thus, it should be used as a common milking practice.

References

Afifi, M., F. Kabera, H. Stryhn, J.P. Roy, L.C. Heider, S. Godden, W. Montelpare, J. Sanchez, and S. Dufour. 2018. Antimicrobial-based dry cow therapy approaches for cure and prevention of intramammary infections: A protocol for a systematic review and meta-analysis. Anim. Heal. Res. Rev.. doi:10.1017/S1466252318000051.

Barkema, H.W., Y.H. Schukken, T.J.G.M. Lam, M.L. Beiboer, G. Benedictus, and A. Brand. 1998. Management Practices Associated with Low, Medium, and High Somatic Cell Counts in Bulk Milk. J. Dairy Sci. 81:1917–1927. doi:10.3168/JDS.S0022-0302(98)75764-9.

Bates, D., M. Mächler, B.M. Bolker, and S.C. Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. J. Stat. Softw. 67:1–48. doi:10.18637/JSS.V067.I01.

Bhutto, A.L., R.D. Murray, and Z. Woldehiwet. 2011. The effect of dry cow therapy and internal teat-sealant on intra-mammary infections during subsequent lactation. Res. Vet. Sci. 90:316–320. doi:10.1016/j.rvsc.2010.06.006.

Blowey, R., and P. Edmondson. 1996. Teat disinfection in dairy herds. In Pract. 18:254–260. doi:10.1136/INPRACT.18.6.254.

Bobbo, T., M. Penasa, and M. Cassandro. 2020. Combining total and differential somatic cell count to better assess the association of udder health status with milk yield, composition and coagulation properties in cattle. https://doi.org/10.1080/1828051X.2020.1784804 19:697–703. doi:10.1080/1828051X.2020.1784804.

Cook, N.B., and D.J. Reinemann. 2007. A Tool Box for Assessing Cow, Udder and Teat Hygiene Tools to Assess Udder Contamination. Page in NMC Annual Meeting Porceedings.

DeVries, T.J., M.G. Aarnoudse, H.W. Barkema, K.E. Leslie, and M.A.G. von Keyserlingk. 2012. Associations of dairy cow behavior, barn hygiene, cow hygiene, and risk of elevated somatic cell count. J. Dairy Sci. 95:5730–5739. doi:10.3168/JDS.2012-5375.

Fitzpatrick, S.R., M. Garvey, J. Flynn, B. O'brien, and D. Gleeson. 2021. Effect of Pre-Milking Teat Foam Disinfection on the Prevention of New Mastitis Rates in Early Lactation. Anim. 2021, Vol. 11, Page 2582 11:2582. doi:10.3390/ANI11092582.

Fox, L.K., and J.M. Gay. 1993. Contagious Mastitis. Vet. Clin. North Am. Food Anim. Pract. 9:475–487. doi:10.1016/S0749-0720(15)30615-0.

Godden, S.M., E. Royster, W. Knauer, J. Sorg, M. Lopez-Benavides, Y. Schukken, S. Leibowitz, and E.A. French. 2016. Randomized noninferiority study evaluating the efficacy of a postmilking teat disinfectant for the prevention of naturally occurring intramammary infections. J. Dairy Sci. 99:3675–3687. doi:10.3168/JDS.2015-10379.

Gonçalves, J.L., C. Kamphuis, C.M.M.R. Martins, J.R. Barreiro, T. Tomazi, A.H. Gameiro, H. Hogeveen, and M. V. dos Santos. 2018. Bovine subclinical mastitis reduces milk yield and economic return. Livest. Sci.. doi:10.1016/j.livsci.2018.01.016.

Hamel, J., Y. Zhang, N. Wente, and V. Krömker. 2021. Heat stress and cow factors affect bacteria shedding pattern from naturally infected mammary gland quarters in dairy cattle. J. Dairy Sci. 104:786–794. doi:10.3168/JDS.2020-19091.

Hogeveen, H., W. Steeneveld, and C.A. Wolf. 2019. Production Diseases Reduce the Efficiency of Dairy Production: A Review of the Results, Methods, and Approaches Regarding the Economics of Mastitis. https://doi.org/10.1146/annurev-resource-100518-093954 11:289–

312. doi:10.1146/ANNUREV-RESOURCE-100518-093954.

Hutton, C.T., L.K. Fox, and D.D. Hancock. 1990. Mastitis Control Practices: Differences Between Herds with High and Low Milk Somatic Cell Counts. J. Dairy Sci. 73:1135–1143. doi:10.3168/JDS.S0022-0302(90)78774-7.

Kelly, P.T., K. O'Sullivan, D.P. Berry, S.J. More, W.J. Meaney, E.J. O'Callaghan, and B. O'Brien. 2009. Farm management factors associated with bulk tank somatic cell count in Irish dairy herds. Ir. Vet. J. 62:45–51. doi:10.1186/2046-0481-62-S4-S45/TABLES/9.

von Keyserlingk, M.A.G., J. Rushen, A.M. de Passillé, and D.M. Weary. 2009. Invited review: The welfare of dairy cattle-key concepts and the role of science. J. Dairy Sci.. doi:10.3168/jds.2009-2326.

Kirkeby, C., K. Græsbøll, S.S. Nielsen, L.E. Christiansen, N. Toft, E. Rattenborg, and T. Halasa. 2016. Simulating the epidemiological and economic impact of paratuberculosis control actions in dairy cattle. Front. Vet. Sci. 3:90. doi:10.3389/FVETS.2016.00090/BIBTEX.

Klaas, I.C., and R.N. Zadoks. 2018. An update on environmental mastitis: Challenging perceptions. Transbound. Emerg. Dis. 65:166–185. doi:10.1111/TBED.12704.

Kuznetsova, A., P.B. Brockhoff, and R.H.B. Christensen. 2017. lmerTest Package: Tests in Linear Mixed Effects Models. J. Stat. Softw. 82:1–26. doi:10.18637/JSS.V082.I13.

Lopez-Benavides, M.G., J.H. Williamson, R.T. Cursons, S.J. Lacy-Hulbert, and M.W. Woolford. 2005. Streptococcus uberis population dynamics in the New Zealand pastoral dairy farm. 1st ed. H. Hogeveen, ed. Wageningen Academic Publishers, Wageningen.

National Mastitis Council. 2013. Recommended Milking Procedures. Accessed January 24, 2022. https://www.nmconline.org/wp-content/uploads/2016/09/Recommended-Milking-Procedures.pdf.

Nielsen, C. 2009. Economic Impact of Mastitis in Dairy Cows.

Østerås, O., L. Sølverød, and O. Reksen. 2006. Milk Culture Results in a Large Norwegian Survey—Effects of Season, Parity, Days in Milk, Resistance, and Clustering. J. Dairy Sci. 89:1010–1023. doi:10.3168/JDS.S0022-0302(06)72167-1.

R Core Team. 2021. R Core Team 2021. Accessed.

Reneau, J.K. 1986. Effective Use of Dairy Herd Improvement Somatic Cell Counts in Mastitis Control. J. Dairy Sci. 69:1708–1720. doi:10.3168/JDS.S0022-0302(86)80590-2.

Le Roux, Y., F. Laurent, and F. Moussaoui. 2003. Polymorphonuclear proteolytic activity and milk composition change. Vet. Res.. doi:10.1051/vetres:2003021.

Ruegg, P.L., and J.C.F. Pantoja. 2013. Understanding and using somatic cell counts to improve milk quality. Irish J. Agric. Food Res. 52:101–117.

Sant'Anna, A.C., and M.J.R. Paranhos da Costa. 2011. The relationship between dairy cow hygiene and somatic cell count in milk. J. Dairy Sci. 94:3835–3844. doi:10.3168/JDS.2010-3951.

Schreiner, D.A., and P.L. Ruegg. 2003. Relationship Between Udder and Leg Hygiene Scores and Subclinical Mastitis. J. Dairy Sci. 86:3460–3465. doi:10.3168/JDS.S0022-0302(03)73950-2.

Smith, K.L. 1983. Mastitis Control: A Discussion. J. Dairy Sci. 66:1790–1794. doi:10.3168/jds.S0022-0302(83)82007-4.

Smith, K.L., and J.S. Hogan. 1993. Environmental Mastitis. Vet. Clin. North Am. Food Anim. Pract. 9:489–498. doi:10.1016/S0749-0720(15)30616-2.

Smith, K.L., D.A. Todhunter, and P.S. Schoenberger. 1985. Environmental Mastitis: Cause, Prevalence, Prevention. J. Dairy Sci. 68:1531–1553. doi:10.3168/JDS.S0022-0302(85)80993-0.

Soleimani, N.A., R.K. Kermanshahi, B. Yakhchali, and T.N. Sattari. 2010. Antagonistic activity of probiotic lactobacilli against Staphylococcus aureus isolated from bovine mastitis. African J. Microbiol. Res. 4:2169–2173. doi:10.5897/AJMR.9000040.

Wenz, J.R., S.M. Jensen, J.E. Lombard, B.A. Wagner, and R.P. Oinsmore. 2007. Herd Management Practices and Their Association with Bulk Tank Somatic Cell Count on United States Dairy Operations. J. Dairy Sci. 90:3652–3659. doi:10.3168/JDS.2006-592.

Wickham, H. 2016. Ggplot2: Elegant Graphics for Data Analysis. Second Edition. Springer.

Wilson, D.J., H.H. Das, R.N. Gonzalez, and P.M. Sears. 1997. Association between management practices, dairy herd characteristics, and somatic cell count of bulk tank milk.. J. Am. Vet. Med. Assoc. 210:1499–1502.

Yu, J., Y. Ren, X.X. Xi, W. Huang, and H. Zhang. 2017. A novel lactobacilli-based teat disinfectant for improving bacterial communities in the milks of cow teats with subclinical mastitis. Front. Microbiol. 8:1782. doi:10.3389/FMICB.2017.01782/BIBTEX.

Zambrano-Bigiarini, M. 2020 hydroTSM: Time Series Management, Analysis and Interpolation for Hydrological ModellingR package version 0.6-0. URL https://github.com/hzambran/hydroTSM. DOI:10.5281/zenodo.839864.

Zucali, M., L. Bava, A. Tamburini, M. Brasca, L. Vanoni, and A. Sandrucci. 2011. Effects of season, milking routine and cow cleanliness on bacterial and somatic cell counts of bulk tank milk. J. Dairy Res. 78:436–441. doi:10.1017/S0022029911000598.