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Suggested citation:

Wilm, Jensine, Volker Krömker, Carsten Kirkeby, and Maya Gussmann. 2023. "Lactational treatment of bovine mastitis—Development over time and factors influencing cytological cure." *Journal of Dairy Science* 106 (8): 5740–52. https://doi.org/10.25968/opus-3196.

Abstract

Lactational treatment of bovine mastitis is a major contributor to antibiotic consumption in dairy cattle and is, therefore, important to address in light of the increasing problem of antibiotic resistance. In this large-scale database-based retrospective observational study, we combined electronic health records and routinely measured somatic cell counts from individual cows to create an overview of lactational mastitis treatment in Danish dairy herds from 2010 to 2019. Furthermore, posttreatment somatic cell count was used to approximate treatment success in terms of cytological cure. A generalized logistic regression with mixed effects was performed to combine knowledge on cow-level factors (treatment-, pathogen-, and cow-related) with the new infection risk at the herd level, and to explore the relative effect on cytological cure. The investigation revealed that the total number of lactational treatments appears to have decreased steadily over the study period, whereas treatment duration increased slightly. The proportion of cases treated with penicillin-based protocols and the proportion of milk samples sent for pathogen analysis also decreased. Meanwhile, results from the statistical analysis confirm the importance of cow-related factors, such as parity and lactation stage, for the probability of cytological cure following lactational treatment of mastitis. However, they also disclose that factors that are easier to adjust, such as optimizing treatment duration, including knowledge on causative pathogens and improving the herd-level new infection risk that can be used to positively influence the outcome. Application of this knowledge could potentially assist in promoting a more prudent use of antibiotics for dairy cattle in the future.

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J. Dairy Sci. 106:5740–5752
https://doi.org/10.3168/jds.2022-22841
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Lactational treatment of bovine mastitis—Development over time and factors influencing cytological cure

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ABSTRACT

Lactational treatment of bovine mastitis is a major contributor to antibiotic consumption in dairy cattle and is, therefore, important to address in light of the increasing problem of antibiotic resistance. In this large-scale database-based retrospective observational study, we combined electronic health records and routinely measured somatic cell counts from individual cows to create an overview of lactational mastitis treatment in Danish dairy herds from 2010 to 2019. Furthermore, posttreatment somatic cell count was used to approximate treatment success in terms of cytological cure. A generalized logistic regression with mixed effects was performed to combine knowledge on cow-level factors (treatment-, pathogen-, and cow-related) with the new infection risk at the herd level, and to explore the relative effect on cytological cure. The investigation revealed that the total number of lactational treatments appears to have decreased steadily over the study period, whereas treatment duration increased slightly. The proportion of cases treated with penicillin-based protocols and the proportion of milk samples sent for pathogen analysis also decreased. Meanwhile, results from the statistical analysis confirm the importance of cow-related factors, such as parity and lactation stage, for the probability of cytological cure following lactational treatment of mastitis. However, they also disclose that factors that are easier to adjust, such as optimizing treatment duration, including knowledge on causative pathogens and improving the herd-level new infection risk that can be used to positively influence the outcome. Application of this knowledge could potentially assist in promoting a more prudent use of antibiotics for dairy cattle in the future.
Key words: dairy cattle, mastitis treatment, antibiotic consumption

Received September 29, 2022.
Accepted February 27, 2023.
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INTRODUCTION

The concern about antibiotic usage in livestock production being connected to growing antibiotic resistance in a one-health perspective (Tang et al., 2017) has inspired several initiatives investigating the potential for more judicious use. Mastitis treatments account for the largest share of antibiotics used in adult dairy cows' health (Korsgaard et al., 2020) and are thus important to address when promoting the prudent use of antibiotics. To facilitate this, it is necessary to gain a thorough understanding of the circumstances relating to treatment and their relative influence on the outcome. Treatment success can be measured in various ways, including clinical cure (where clinical symptoms are eliminated), bacteriological cure (where the causative pathogen is no longer detected), and cytological cure (determined by a reduction in SCC, preferably at quarter level; Ruegg, 2021). Furthermore, success can also be evaluated from an economic perspective, taking into account the cost of different treatment and management strategies in terms of increased expenses and production losses (Halasa et al., 2007). From the farmers' perspective, it is economically desirable to maintain a low bulk milk SCC. Therefore, long-lasting cytological cure at quarter, as well as cow level, is expected to be prioritized. The probability of cure following treatment (according to one or more of the given definitions) can vary greatly based on treatment regimen and causative pathogen- and cow-related factors, as studied in several field trials and reviewed thoroughly (Barkema et al., 2006). The pathogen-related factors are dependent on the susceptibility of the causative pathogen toward a given antibiotic treatment regimen, as well as the probability of spontaneous cure (Ruegg, 2018). The cow-related factors are manifold; among them, some of the most recognized are parity, lactation stage, clinical severity, previously elevated cell count, and history of mastitis (Pinzón-Sánchez and Ruegg, 2011; Ziesch and Krömker, 2016). In studies on the economic perspectives of mastitis management, often performed by simulation models, adjusting the treatment regimen according to cow-related factors alone does not improve the cost-effectiveness compared with blanket treatment practices (Steeneveld et al., 2011). However, several of these studies do indicate that the economic success of treatment strategies is dependent on the herd-level transmission dynamics (Barlow et al., 2009; van den Borne et al., 2010; Down et al., 2013), which describes the risk of cows experiencing new infections due to population-based infectious pressure. It is therefore recommended to include a herd-level approximation of the new infection risk in investigations on improving treatment strategies. In this retrospective data analysis, we have therefore aimed to use cow-level factors and the herd-level new infection risk to investigate the combined influence on cytological cure.

In Denmark, it has been required to document consumption of prescribed antibiotics for different indications at the herd level for many years. This allows for a good general overview of the development in antibiotic consumption for udder health (Korsgaard et al., 2020). According to this overview, changes at the herd level during the study period from 2010 to 2019 can be roughly described in 2 main trends. On one hand, there is a shift away from use of broad-spectrum antibiotics, reinforced by initiatives from industry and legislation such as phasing out third and fourth generation cephalosporines and fluoroquinolones in 2015. On the other hand, there has been a decrease in the amount of antibiotics used for udder health during lactation, whereas consumption for dry-cow treatments has increased. Meanwhile, the population of dairy cows has remained stable during the entire period with approximately 570,000 milking cows (Statistics Denmark, 2018), but the farm structure has changed in favor of fewer and larger herds. In the Nordic countries, the guidelines for antibiotic use are generally characterized as restrictive (Rajala-Schultz et al., 2021), whereas the presence of resistant bacteria is low (Chehabi et al., 2019). Lactational treatment of mastitis is also well documented; treatment records are mandatory, kept in an electronic database, and considered to be generally reliable (Wolff et al., 2012). Treatments can be initiated by either veterinarians or by farmers with permission to treat certain common diseases following instructions of the herd-affiliated veterinarian. The information within the treatment records on cow level is limited to the type of disease treated, and does not contain the exact treatment protocol. Instead, the database uses a coding system, where each type of disease has a label (a so-called diagnosis code). In case of lactational mastitis treatments, the records are generally labeled with either of the following 2 diagnosis codes: "common mastitis" or "acute mastitis." We have conducted a survey study of Danish cattle practitioners in 2020, showing that there appears to be a common understanding of treatment practice related to these 2 diagnosis codes (Wilm et al., 2021). According to this, a diagnosis code of "common mastitis" usually describes a case where penicillin-based protocols are applied (combined systemic and intramammary procaine penicillin or penethamate), due to suspected gram-positive etiology and often a mild to moderate clinical presentation. Meanwhile, a diagnosis code of "acute mastitis" usually describes a more severe case, with suspected gram-negative etiology, where broad-spectrum antibiotics are mainly used (typically systemic sulfonamide/trimethoprim, oxytetracycline or amoxycillinium, and either lincomycin/neomycin or first generation cephalosporine tubes). The same study also revealed that all lactational mastitis treatments are usually accompanied by nonsteroidal anti-inflammatory drugs and that milk sample analysis for causative pathogens is usually performed by herd-affiliated veterinarians in practice laboratories. During the study period, it was not mandatory to perform an analysis for all lactational treatments, although this changed afterward with new legislation in 2020. The vast majority of herds participate in the Danish milk recording service program (RYK), making it possible to obtain regular cow-level SCC measurements from milk control recordings. This available background information provides a unique opportunity to investigate Danish mastitis treatment procedures, and has been used previously to detect farm-level determinants relevant to the initiation of treatment (Gussmann et al., 2018). In the current study, we use information from the database to map how treatment parameters such as duration, use of pathogen analysis, as well as diagnosis codes have shifted over time. Furthermore, using the posttreatment cow-specific cell count level records to estimate cytological cure led to an extensive analysis investigating how different treatment parameters affect the subsequent likelihood of cytological cure.

This study had 2 objectives. The first objective was to investigate the changes in treatment parameters in light of changes in legislation and antibiotic usage over the 10-yr study period. The second objective was to analyze the relative influence of available cow-level factors (parity, DIM, mastitis history, diagnosis and duration, information on pathogen where available) and the herd-level new infection risk on the probability of achieving cytological cure in the subsequent period between 21 and 60 d posttreatment.

MATERIALS AND METHODS

Data Extraction

No human or animal subjects were used, so this analysis did not require approval by an Institutional Animal Care and Use Committee or Institutional Review Board. Anonymized data from the electronic treatment records of all Danish dairy farms and the Danish milk recording service were made available from the Danish cattle database administered by the Danish farmers' advisory company SEGES. A large data set was constructed for the purpose of describing treatment trends over time by combining records of lactational mastitis treatment and individual cow SCC from all farms in Denmark with 11 annual milk control recordings over a 10-yr period (2010–2019). A relatively large number of these observations could be further used to analyze the effect of given factors on the chance of cure. The data for this study were prepared and analyzed between October 2021 and April 2022. We extracted information on the individual cow's lifecycle (through calving records), routinely measured cow-level SCC, and health recordings with relevance to lactational mastitis treatment (treatment recordings and registered milk sample analysis). The DIM and parity for all included animals corresponding to each treatment record were calculated according to calving records. The diagnosis code was extracted to approximate the severity and likelihood of receiving broad-spectrum antibiotics.

Definitions

Case Definition. Treatments are registered electronically on a daily basis and categorized with either of the 2 diagnosis codes ("common" or "acute mastitis") by either veterinarians or farmers, depending on who administers the treatment. To calculate the duration of treatment, all consecutive dates with a registered treatment were added up to a total duration (in days). To determine whether 2 d of treatment should be counted as the same case, a maximum of 5 d were allowed between them; thus, a new case was defined as being at least 5 d apart from another record of lactational mastitis treatment (Jamali et al., 2018).

Diagnosis Codes. If 1 case event included both diagnosis codes, it was labeled as "mixed." For the final mixed effects logistic regression analysis, mixed cases were added to the "acute mastitis" diagnosis category to emphasize the topic of interest being a higher likelihood of a more severe case receiving broad-spectrum antibiotics compared with the "common mastitis" diagnosis.

Treatment Duration Categories. The treatment duration in days was categorized into 3 categories: short (1-d treatments), regular (2–5 d), or extended (6 or more days), relative to the known summary of product characteristics (**SPC**) for antibiotics used for lactational mastitis treatment in Denmark.

Pathogen Groups. The available milk sample analysis results were grouped in accordance with the expected difference in susceptibility toward antibiotic treatments and effect on cure rates (Ziesch and Krömker, 2016). The groups were *Staphylococcus aureus*, *Streptococcus* spp., gram-negative bacteria (*Escherichia coli*, *Klebsiella*, and other coliforms), no growth, nontreatable agents (*Mycoplasma*, yeast, and *Prototheca*), and others (minor pathogenic gram-positive bacteria). In order for a milk sample to be linked to a treatment, it had to be associated with the cow ID and dated within 7 d of initiation of the treatment event. Furthermore, sample results from the same cow in close proximity to one another (i.e., within 14 d) were excluded because 2 different sample results linked to the same case would not be able to provide a clear link between assumed etiology and the investigated outcome. It is not known how contaminated samples affected the recordings.

Available Results from Pathogen Analysis. Because a large number of treated cases had no available information on pathogen analysis, the statistical investigation was carried out on the full sample size of complete observations with a modified binary variable for pathogen analysis results, where the levels were set to "available" or "not available."

Parity and Lactation Stage. The parity and lactation stage of each cow were calculated for the day of treatment initiation. In accordance with other studies on risk factors for cure following mastitis treatment, they were each grouped into 3 categories according to the expected effect on the likelihood of cure. The final categories created were first, second, and ≥third parity, and early (0–100 DIM), mid (101–200 DIM), or late lactation (201–600 DIM; Samson et al., 2016).

Mastitis History. A cow was considered to have a history of mastitis (yes vs. no) if 2 or more preceding treatment events were recorded during the same lactation (Ziesch and Krömker, 2016).

Elevated Cell Count. A cow was considered to have a previously elevated cell count (yes vs. no) if it had 2 or more consecutive milk control recordings of >200,000 SCC in the same lactation leading up to the point of treatment (Samson et al., 2016). All SCC of zero were labeled NA.

Herd-Level New Infection Risk. The new infection risk was calculated at herd level as the percentage of cows at risk, changing from below 100,000 SCC to above this threshold in 2 consecutive herd testing dates, based on the 11 annual recordings.

Cytological Cure as a Dependent Variable. The outcome of interest was cytological cure approximated by cow-level composite SCC measurements from the Danish milk recording program. The threshold for cytological cure can be set at different levels and for composite samples, a threshold lower than 150,000 with long-term evaluation has been proposed (Ruegg, 2021).

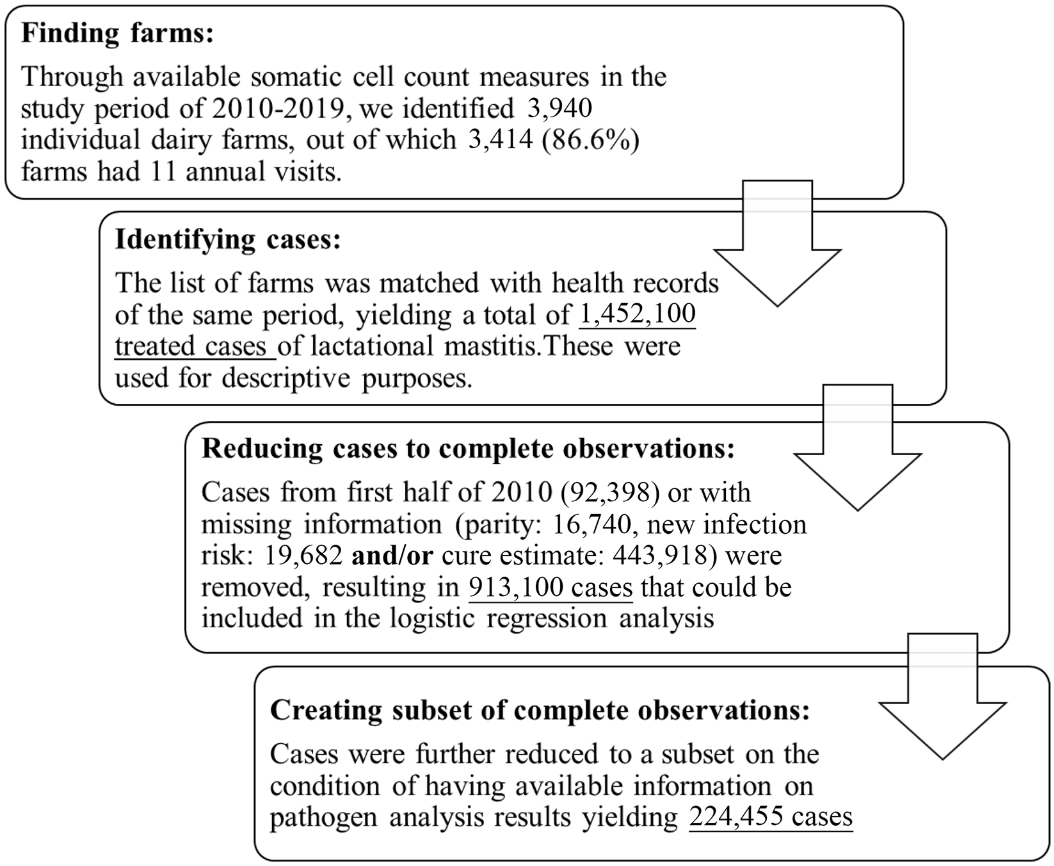

Figure 1. Diagram showing the steps of identifying sample sizes for descriptive purposes and regression analysis, respectively.

For this study, we chose to use a threshold of 100,000 SCC for the period 21 to 60 d posttreatment to distinguish between expected successful cytological cure (less than or equal to 100,000) and no cure (over 100,000). The definition was further determined by the absence of a second treated case within the same posttreatment period, as this could mask the effect of the investigated treatment. Such cases were not included in the analysis.

Statistical Analysis

All computation was carried out in R version 4.0.5 "Shake and Throw" (R Development Core Team, 2021). Data structuring was facilitated by the use of the "tidyverse" (Wickham et al., 2019) and "data.table" (Dowle M, 2021) packages. Statistical analysis was carried out with "lme4" (Bates et al., 2015), "performance" (Lüdecke et al., 2021), "pROC" (Robin et al., 2011), and "emmeans" (Lenth, 2022), whereas illustrations were supported by "ggplot2" (Wickham, 2016). To investigate the combined effect on the likelihood of cytological cure following a mastitis treatment during lactation, a generalized mixed effects logistic regression model was built using cow-level variables and herd-level new infection risk as independent variables and cytological cure as the dependent variable. This was done using all records of either the limited or the full sample of complete observations (with and without detailed information on pathogen group analysis, see Figure 1).

To assess the relevance of all independent variables identified in the literature on the chosen cure definition, they were tested for a univariable effect in our data set. The effect was evaluated using a significance level of $P < 0.05$ on the dependent variable of cytological cure. All mentioned variables fulfilled this criterion and were thus included as fixed effects in the multivariable regression model. Meaningful biological interactions between the fixed effects were also screened for effect only investigating the respective interaction and added to the model if significant ($P < 0.05$). If these fixed effects and interactions did not remain significant at a 0.05 level within the context of the full model, the contribution was evaluated based on effect on the Akaike information criterion (**AIC**). Nonsignificant fixed effects or interactions that increased the AIC were not included in the final model.

Treatment practices and thresholds for administering treatments vary among farms, and each cow's individual immune response as well as udder conformation are known to play a role in their ability to clear infections, but these cannot be quantified using the available data. The herd and cow ID were, therefore, directly included as random effects in the model to account for this. The effect of changes in treatment parameters and available antibiotics over time was less clear and, therefore, the effect of year on the chance of cure was investigated using a chi-squared test before we decided to include it as a third random effect in the model. The contribution of random effects to the full model was likewise evaluated by how they affected the AIC. The quality of the final model was assessed visually using the residual distribution and by calculating the area under the receiver operating characteristics curve for the outcome of cytological cure. Finally, we conducted a post hoc analysis of all fixed effects in the large model, where estimated means of the cure rate for the respective expressions of the fixed effects were calculated.

All fixed and random effects were included in the 2 final models (with and without detailed information on pathogen group). Two candidate interactions (mastitishistory × elevatedcellcount and pathogengroup × diagnosiscode) were initially found to be significant at the screening stage. Of these, only pathogengroup × diagnosiscode improved the full model and was therefore retained.

The general formulas for the final 2 models were

$$\begin{gathered}\text{Logit}[\text{P(cytological\_curei)}] = \beta\text{i0} + \beta\text{i1 paritygroupi} \\ + \beta\text{i2 lactationstagei} + \beta\text{i3 elevatedcellcowi} \\ + \beta\text{i4 mastitishistoryi} + \beta\text{i5 diagnosiscodei} \\ + \beta\text{i6 duration-categoryi} + \beta\text{i7 analysisresultavailablei} \\ + \beta\text{i8 new infection risk} + \text{cow\_IDi} \\ + \text{herd\_IDi} + \text{yearsi} + \varepsilon\text{i}\end{gathered}$$

for the full sample size including all complete cases but with limited information on pathogen analysis, and

$$\begin{gathered}\text{logit}[\text{P(cytological\_curei)}] = \beta\text{i0} + \beta\text{i1 paritygroupi} \\ + \beta\text{i2 lactationstagei} + \beta\text{i3 elevatedcellcowi} \\ + \beta\text{i4 mastitishistoryi} + \beta\text{i5 diagnosiscodei} \\ + \beta\text{i6 duration-categoryi} + \beta\text{i7 pathogengroupi} \\ + \beta\text{i5:}\beta\text{i7} + \beta\text{i8 new infection risk} + \text{cow\_IDi} \\ + \text{herd\_IDi} + \text{yearsi} + \varepsilon\text{i}\end{gathered}$$

for the limited sample size with more detailed information on pathogen analysis. In both models, P(cytological_cure) is the probability for cytological cure, while paritygroup, lactationstage, elevatedcellcow, mastitishistory, diagnosiscode, duration-category, analysisresultavailable, pathogengroup, and new infections risk are the variables described above. Cow_ID, herd_ID, and years are random effects for the respective cows, herds, and years. All variables are indexed by the observation they belong to i = 1, …, n. We assumed that random variables and random errors were normally distributed.

RESULTS

The data extraction process described in Figure 1 generated a large data set of 1,452,100 treated lactational mastitis cases originating from 3,391 farms and 819,436 cows in the period of 2010 to 2019. This crude data set was used for descriptive purposes. For the second objective (i.e., analyzing the influence of cow- and herd-level factors on the chances of cytological cure), only complete observations could be used. This meant that the descriptive data set was limited to observations where all investigated variables of interest were available, as well as the outcome "cured" or "not cured" within the defined timeframe. In addition, during the investigation, we identified unreliable information related to diagnosis codes in the first 6 mo of 2010; thus, this period was removed before the statistical analysis was conducted. In total, 913,100 cases qualified as complete observations and could be used for the logistic regression analysis. A smaller subset of the complete cases also had information on pathogen analysis available; thus, the analysis including the effect of pathogen group could be conducted on a limited sample size of 224,455 complete cases. Missing posttreatment SCC measurements could be due to dry-off, culling, or unregistered movements. Database recordings on movements and dry-off were so scarce that they could not be considered reliable in general; therefore, we did not attempt to differentiate between them (data not shown).

Observable Changes in Treatment Parameters

We had 3 observable changes in treatment parameters.

(1) Within our data set, there was a decline in the total number of treated lactational mastitis cases over the study period (Table 1). Meanwhile, the distribution of diagnosis codes shifted toward proportionally more treatments for a diagnosis of acute mastitis and mixed diagnosis treatments. There was a markedly lower level

Table 1. Total number (no.) of treated lactational mastitis cases in Danish dairy herds and the relative proportion of different diagnosis codes used in the electronic database during the study period (n = 1,452,100)

	Diagnosis code						
	Common mastitis		Acute mastitis		Mixed diagnosis		
Year	Total no.	%	Total no.	%	Total no.	%	Total cases
2010	179,553	97.7	2,973[1]	1.6[1]	1,151[1]	0.6[1]	183,677
2011	156,181	92.1	10,935	6.5	2,456	1.4	169,572
2012	143,019	89.9	13,446	8.5	2,559	1.6	159,024
2013	130,105	89.2	13,449	9.2	2,372	1.6	145,926
2014	130,816	87.8	15,302	10.3	2,808	1.9	148,926
2015	123,387	87.3	15,286	10.8	2,680	1.9	141,353
2016	116,990	87.2	14,801	11.0	2,388	1.8	134,179
2017	107,578	86.5	14,484	11.7	2,303	1.9	124,365
2018	108,011	86.7	14,284	11.5	2,318	1.9	124,613
2019	103,359	85.8	14,520	12.1	2,586	2.2	120,465

[1]Acute mastitis is considered to be underreported (registered as common mastitis due to technical problems) in January to June 2010.

of acute treatments registered in 2010, which can be attributed to technical problems related to recordings, which lasted until July 2010.

(2) Mean treatment duration has generally increased over the years, whereas the range has narrowed closer to an average of approximately 3 d (Figure 2). The y-axis has been truncated at a maximum of 10 d, thus removing outliers and providing a closer look at the overall picture (1,380 observations with a duration between 10 and 20 d, and 35 observations with >20 d), but few extreme values up to 40 d of treatment were present in our data. The increase in mean treatment duration corresponds with shifts in the categorized treatment durations. A yearly relative decrease in the proportion of 1-d treatments from 55% in 2010 to 15% in 2019 can be observed. Simultaneously, the proportion of treatments lasting between 2 and 5 d rose from 43% in 2010 to 81% in 2019. The relative number of extended treatments also rose during the study period from 1% of all cases in 2010 to 4% in 2019.

(3) Our data show a steady decrease in the absolute number and proportion of cases with registered samples over the years (Figure 3). Although 33% of all cases had a registered analysis result in 2010, this was only true for 20% of the lactational mastitis cases in our data set in 2019. The distribution of pathogen groups found in milk samples over the years appear to be relatively similar. However, the proportion of gram-

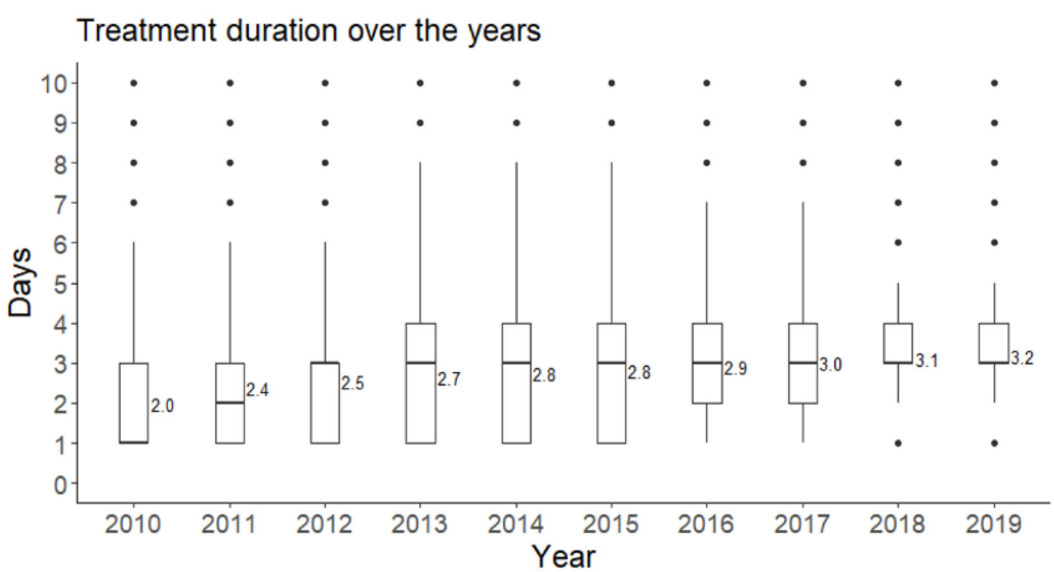

Figure 2. Boxplot overview of lactational mastitis treatment duration (in days) over the study period. The annotated numbers show the mean, whereas the median is shown by the line within the boxplot (n = 1,452,100). The edges of the box represent the 25th and 75th percentiles, and the dots show outliers.

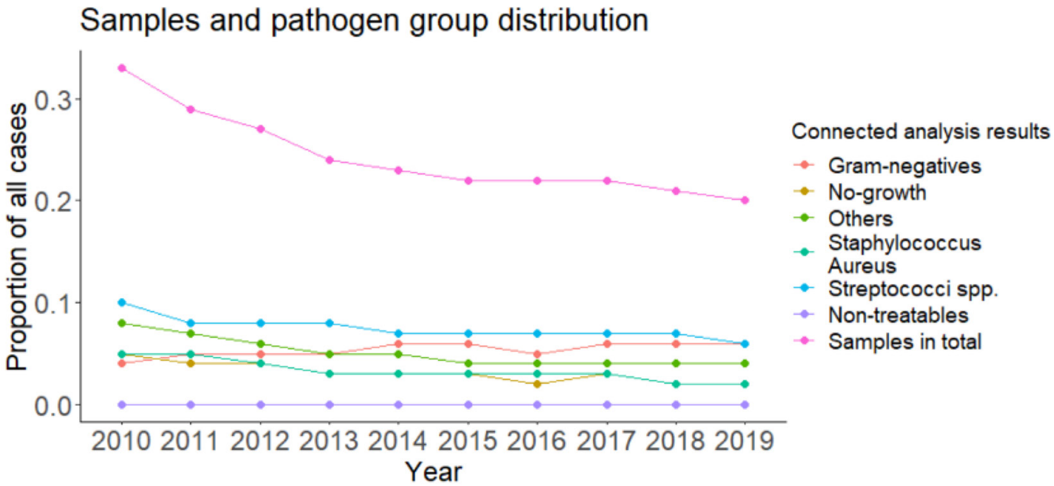

Figure 3. Proportion of all lactational mastitis cases where sample results on causative pathogens were available, as well as the absolute proportion of pathogen groups (n = 1,452,100).

negative bacteria has increased steadily over time, from 4% to 6% of all cases (including ones without available analysis results), whereas there has been a proportional decrease in "no growth," *S. aureus*, and other gram-positive bacteria. *Streptococcus* spp. and nontreatable microorganisms remained at approximately the same level over this period.

Generalized Logistic Regression of Risk Factors for Cytological Cure

The 3 random effects (cow ID, herd ID, and year) had considerably different degrees of variance. Cow ID showed the largest range whereas year was close to zero. However, all of the random effects were significant and improved the model evaluated by the AIC. As evident from the overview of the effect of year on the dependent variable (Figure 4), the proportion of cases followed by cytological cure appears to have remained within a rather similar range of 42% to 45% over the 10-yr period.

The results of the logistic regression analysis for all complete cases are listed in Tables 2 and 3 and for the subset of cases with available pathogen analysis results in Table 4. All of the investigated fixed effects were highly significant and are shown relative to the

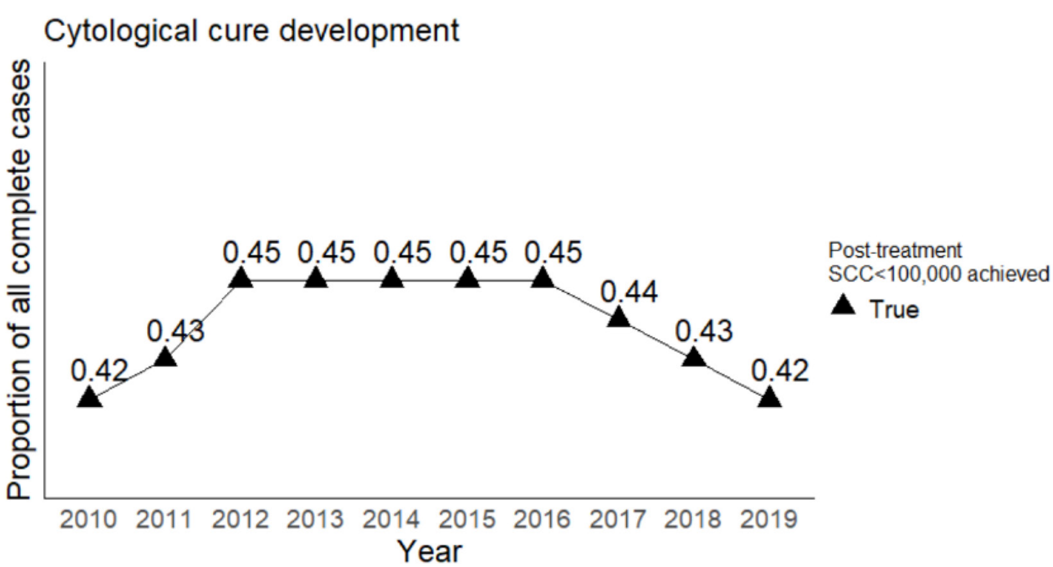

Figure 4. Development of cytological cure, defined by cow level <100,000 SCC 21 to 60 d after lactational mastitis treatment, during the study period based on all complete cases (n = 913,100).

Table 2. Logistic regression results of risk factors for cytological cure, defined by cow level <100,000 SCC 21 to 60 d after lactational mastitis treatment, based on all complete cases, with limited information on causative pathogens (n = 913,100)[1]

Type	Variable	Reference level	Comparator	Effect size	95% CI	*P*-value
Outcome	Cytological cure					
	Intercept			1.16	(1.13, 1.20)	<2e−16
Herd level	New infection risk	0	Increase of 1%	−0.013	(−0.014, −0.012)	<2e−16
Cow level	Parity	First parity	Second parity	−0.77	(−0.78, −0.75)	<2e−16
			≥Third parity	−1.26	(−1.27, −1.25)	<2e−16
Cow level	Lactation stage	0–100 DIM	101–200 DIM	−0.26	(−0.27, −0.25)	<2e−16
Cow level			201–600 DIM	−0.53	(−0.55, −0.52)	<2e−16
Cow level	Elevated cell count	No	Yes	−1.10	(−1.11, −1.08)	<2e−16
Cow level	Mastitis history	No	Yes	−0.42	(−0.44, −0.39)	<2e−16
Treatment-specific	Diagnosis code	Common mastitis	Acute mastitis	−0.24	(−0.25, −0.22)	<2e−16
Treatment-specific	Duration category	2–5 d	1 d	−0.04	(−0.06, −0.03)	4.59e−08
Treatment-specific			>5 d	−0.17	(−0.20, −0.13)	<2e−16
Pathogen-specific	Analysis result available	No	Yes	0.03	(0.02, 0.04)	7.72e−08

[1]Random effects include cow ID (variance: 0.37, SD: 0.61, 591,074 levels); herd ID (variance 0.10, SD: 0.32, 3,358 levels); and year (variance: 0.00, SD: 0.04, 10 levels).

reference level. The interaction between diagnosis and pathogen group showed a significant combined effect of the acute diagnosis code and gram-negative bacteria as well as *Streptococcus* spp. Table 3 furthermore shows the post hoc analysis of estimated marginal means of the first model including all complete cases. The mean probability of cytological cure per level for all variables varies between 18% and 43%, with the biggest range observed within the categorized variables of parity and elevated cell count.

DISCUSSION

Changes in Lactational Mastitis Treatments

Our investigation confirmed that the number of treatments during lactation decreased in accordance with decreased herd-level antibiotic use for udder health in lactation. We have aimed to include the majority of the entire Danish dairy population to justify comparing the absolute case numbers to the stable population size

Table 3. Post hoc analysis of the estimated marginal mean probability of cytological cure, defined by cow level <100,000 SCC 21 to 60 d after lactational mastitis treatment, based on the logistic regression results for all complete cases (n = 913,100)[1]

Type	Variable	Level	Estimated marginal mean probability of cure (%)	SE	Lower 95% (%)	Upper 95% (%)
Outcome	Cytological cure					
Herd level	New infection risk	18.2%	28.0	0.003	27.3	28.7
Cow level	Parity	First parity	43.4	0.004	42.5	44.2
		Second parity	26.2	0.003	25.5	26.9
		≥Third parity	17.8	0.003	17.3	18.3
Cow level	Lactation stage	0–100 DIM	33.6	0.004	32.9	34.4
		101–200 DIM	28.1	0.004	27.4	28.8
		201–600 DIM	22.9	0.003	22.3	23.6
Cow level	Elevated cell count	No	40.3	0.004	39.5	41.1
		Yes	18.4	0.003	17.8	18.9
Cow level	Mastitis history	No	32.4	0.004	31.7	33.1
		Yes	24.0	0.004	23.3	24.8
Treatment-specific	Diagnosis code	Common mastitis	30.5	0.004	29.8	31.2
		Acute mastitis	25.7	0.004	25.0	26.4
Treatment-specific	Duration category	2–5 d	29.4	0.004	28.8	30.1
		1 d	28.6	0.004	27.9	29.3
		>5 d	26.1	0.004	25.3	27.0
Pathogen-specific	Analysis result available	No	27.7	0.004	27.0	28.4
		Yes	28.3	0.004	27.6	29.1

[1]Results are calculated for each variable independently, using the averages of all other variables; *P*-values are adjusted using the Tukey method.

Table 4. Logistic regression results of risk factors for cytological cure, defined by cow level <100,000 SCC 21 to 60 d after lactational mastitis treatment, based on a limited sample size of complete cases with detailed information on the causative pathogen (n = 224,455)[1]

Type	Variable	Reference level	Comparator	Effect size	95% CI	*P*-value
Outcome	Cure100					
	Intercept			1.32	(1.27, 1.37)	<2e−16
Herd level	New infection risk	0	Increase of 1%	−0.014	(−0.015, −0.012)	<2e−16
Cow level	Parity	First parity	Second parity	−0.68	(−0.70, −0.65)	<2e−16
			≥Third parity	−1.12	(−1.15, −1.10)	<2e−16
Cow level	Lactation stage	0–100 DIM	101–200 DIM	−0.27	(−0.29, −0.25)	<2e−16
Cow level			201–600 DIM	−0.50	(−0.53, −0.47)	<2e−16
Cow level	Elevated cell cow	No	Yes	−1.10	(−1.13, −1.07)	<2e−16
Cow level	Mastitis history	No	Yes	−0.44	(−0.50, −0.38)	<2e−16
Treatment-specific	Diagnosis code	Common mastitis	Acute mastitis	−0.26	(−0.35, −0.17)	4.22e−08
Treatment-specific	Duration category	2–5 d	1 d	−0.05	(−0.08, −0.03)	2.18e−05
Treatment-specific			>5 d	−0.10	(−0.17, −0.04)	0.00132
Pathogen-specific	Pathogen group	No growth	Gram-negative	−0.45	(−0.49, −0.42)	<2e−16
			Others	−0.15	(−0.18, −0.11)	<2e−16
			Staphylococcus aureus	−0.36	(−0.40, −0.32)	<2e−16
			Streptococcus spp.	−0.12	(−0.15, −0.09)	8.33e−14
			Yeast, *Mycoplasma*, *Prototheca*	−0.68	(−0.81, −0.55)	<2e−16
Interaction	Acute mastitis: gram-negative			0.16	(0.06, 0.26)	0.00195
	Acute mastitis: others			0.07	(−0.04, 0.17)	0.28952
	Acute mastitis: *Staphylococcus aureus*			0.07	(−0.05, 0.19)	0.33559
	Acute mastitis: *Streptococcus* spp.			0.13	(0.02, 0.23)	0.02383
	Yeast, *Mycoplasma*, *Prototheca*			0.27	(−0.09, 0.62)	0.13203

[1]Random effects include cow ID (variance: 0.12, SD: 0.35, 197,129 levels); herd ID (variance: 0.09, SD: 0.30, 3,069 levels); and year (variance: 0.00, SD: 0.04, 10 levels).

within the study period. However, a smaller number of Danish farms without 11 annual SCC measurements are not represented in our sample.

The use of treatment codes has shifted to proportionally more treatments for "acute mastitis" over time. This indicates that, in general, more of the remaining cases have been treated with available broad-spectrum antibiotics. Likewise, there has been an increase in mixed cases, where both diagnosis codes were used in 1 case. The total number of cases labeled with the acute diagnosis code increased until 2015, after which it slightly decreased and then plateaued for the remaining period. Its peak thus occurred at the same time that some critical antibiotics were phased out, specifically third and fourth generation cephalosporines and fluoroquinolones. Thus, we assume that "acute mastitis" cases after this point were treated with remaining broad-spectrum options. Meanwhile, it appears that the numerical decrease in total treatments originates mainly from a reduction in the "common mastitis" treatments, typically representing penicillin-treated cases. Our data cannot provide the reasons behind this development, which should thus be interpreted with caution. However, because our previously conducted survey of Danish cattle practitioners revealed a link between case severity and initiation of antibiotic treatment (Wilm et al., 2021), it could be hypothesized that a growing awareness of prudent antibiotic use might have led to a more targeted approach for severe clinical cases.

Simultaneously with the decrease in total cases, the treatment duration steadily increased over the study period, from a mean of 2 in 2010 to a mean of 3.2 in 2019, accompanied by a proportional decrease in 1-d treatments over the entire study period. This could be interpreted as a positive development, considering that the SPC on the most commonly used antibiotics, mentioned in the introduction, require a treatment duration between 2 and 5 d. Following the SPC has since also become a regulated requirement with new EU legislation introduced in 2022. Within our study period, the number of extended treatments lasting more than 5 d also proportionally increased by a more subtle degree. This development could be viewed more critically from the perspective of using antibiotics prudently. Evidence of increasing cure rates through extended treatment beyond the recommended SPC can only be supported in some specific cases and is dependent on the causative pathogen (Swinkels et al., 2014; McDougall et al., 2019).

Lactational treatments, to which pathogen analysis results from milk samples could be linked, decreased numerically as well as proportionally over the study period. Knowledge of the causative pathogen can guide best treatment decisions and appropriate choice of antibiotics due to large differences in the chance of spontaneous cure and the effectiveness of antibiotics on different agents (Ruegg, 2018). Pathogen-based protocols have the advantage of being able to reduce antimicrobial use, especially when based on quick diagnostics such as on-farm culturing, which prevents unnecessary delays in treatment initiation (Vasquez et al., 2018). These quick diagnostic methods are nevertheless not applied in the Danish setting and, thus, a delay in availability of results is expected to play a role. A downside of treatment being dependent on culture results is that it can lead to reduced cost effectiveness (Down et al., 2017). However, the calculation by Down et al. (2017) is based on the assumption that gram-positive agents are responsible for a considerable proportion of cases. Whether that assumption applies at herd level can only be determined by monitoring pathogens. Given the relative rise of gram-negative agents observed within the available samples, obtaining more information on pathogens within the farm (either case-specific or as a monitoring tool) might prove useful in targeting treatment protocols even further in the future.

Effect of Cow- and Herd-Level Factors on the Chance of Cytological Cure: Random and Fixed Effects

All 3 random effects of cow ID, herd ID, and year were found to improve the model as expected. The cow ID had the largest effect on the outcome, indicating that between-cow variation is greater than between-herd variation and that the variation due to year of the study period is limited. Interestingly, given the context of limited available antibiotics and the shifting treatment parameters described previously, there was no linear trend of either increased or decreased cure observable over time. Although it is difficult to untangle the combined effect of these changes over time, it does support the argument that limiting the use of critically important antibiotics for human health does not necessarily decrease chances of effectively treating bovine mastitis (Nobrega et al., 2020) within the Danish context.

Fixed Effects

Herd-Level New Infection Risk. The new infection risk on herd level was consistently negatively associated with cytological cure. This emphasizes the epidemiological pattern of mastitis and the importance of preventing transmission for improved udder health, thereby reducing the need for antibiotic treatments (Breen et al., 2021). The effect size per percentage point might appear small compared with the other factors, but if we consider a more substantial change of, for example, 20%, the relative effect on chances of cure is similar to the difference between early- and mid-lactation cows. A key difference in its nature is that it is one of the few factors that can be influenced by management, and thus holds potential for easier change than the cow-level factors.

Cow-Related Factors. All cow-related factors, increasing parity and lactation stage, as well as elevated cell count and mastitis history, were negatively associated with cytological cure as expected based on previous studies (Pinzón-Sánchez and Ruegg, 2011; Ziesch and Krömker, 2016). Relative to the treatment- and pathogen-specific variables, the effect sizes within the model as well as the range of estimated marginal means were considerably larger. The level of SCC is known to be affected by several other factors than intramammary infection alone, including parity and lactation stage (Sumon et al., 2020), and the interpretation of cow-level SCC values should, therefore, always be considered in light of these. Nevertheless, it underlines the need to consider these factors when evaluating the expected added value of treating a case of lactational mastitis as opposed to culling or drying off the quarter or cow.

Treatment Parameters. The "acute mastitis" diagnosis code was negatively associated with the chance of cytological cure in both sample sizes. Because the categorization covers both the likelihood of a broad-spectrum antibiotic treatment protocol and the underlying suspicion of gram-negative etiology and higher case severity described previously, it is not as easily interpreted as others. Severe clinical cases have previously been associated with a lower chance of cure (Bradley and Green, 2009), but they do require immediate treatment and often allow the otherwise preferred restrictive approach of broad-spectrum antibiotic use to be disregarded (Ruegg, 2018). However, whether a certain treatment protocol is correctly targeting the infection can only be determined by an actual diagnosis of the causative agent (Barkema et al., 2006).

The effect of treatment duration was evaluated against the considered ideal of adhering to the SPC of available antibiotics for lactational mastitis treatments. Both extremes appear to have a negative effect on the chance of cytological cure. For the very short duration of 1-d treatments, the interpretation might be that the therapy is simply not complete, and that the full effect requires compliance with the SPC. For the extended duration, it is more likely that there could be unidentified cases of treatment failure.

Pathogen-Related Factors. Investigating the effect of pathogen results was only possible in the smaller subset of complete observations. In Denmark, pathogen analysis is outsourced to many private practice laboratories, and the quality of analysis within this setup has been criticized (Astrup et al., 2022). Additionally, the pathogen groups interact significantly with the treatment code and the effect should thus be evaluated with this relationship in mind. Compared with the reference level of "no growth," all pathogen groups decreased the probability of cure, but with considerably large variation in the effect size. The biggest effect was found in the nontreatable agents (yeast, *Mycoplasma*, and *Prototheca*), followed by gram-negative bacteria and *S. aureus*. In comparison, *Streptococcus* spp. and other gram-positive bacteria appeared to have a less negative effect on cure, which is in line with our expectations. We might expect the gram-negative group to have a relatively high level of bacteriological clearance, considering the high degree of spontaneous cure associated with *E. coli* infections (Ruegg, 2018). However, this is not necessarily translatable to cytological cure because many aspects of the immune response are pathogen-specific (Petzl et al., 2018). The significant interaction of pathogen group and treatment code suggests that, even though chances of cytological cure are generally lower for cases labeled with the "acute mastitis" diagnosis code, the difference in chances of cure between the 2 diagnosis codes decreases if the pathogen group is identified relative to "no growth" results. Within the larger model, even the simplified information on whether analysis results were available appears to be the only positive determinant of the outcome cure, suggesting that the obtained information should be used to make better treatment decisions and thus improving the chance of cure (Breen et al., 2021). The exact mechanism behind this positive effect cannot be determined from our data. Because results of pathogen analysis without on-farm diagnostics are often not available before initiation of treatment, we assume that the effect contributes to an adjustment in treatment after onset (change or cessation of antibiotic treatment or extended treatment duration), or that it plays a part as a monitoring tool within the herd. Regardless, it is another easily available tool and should be recommended to improve antibiotic treatment strategies for cases of clinical mastitis, despite the considerable room for improvement in the quality in general.

Strengths and Limitations

By using a data-based approach, we were able to investigate given factors on a much larger scale and explore the relative effect of given variables. The weakness of this approach is that interpretation of the data greatly relies on correct second-hand recordings, as well as the choices made for categorization. The relative similarity of effect sizes in the model on all complete observations and the subset with pathogen results, respectively, implies a certain degree of stability of the chosen categories. Likewise the threshold set for determining cytological cure can have affected the outcome. However, raising the threshold in initial test runs of the data to 150,000 and 200,000 cells/mL did not lead to any changes in tendencies of the result or the interpretation thereof. Furthermore, missing information on cow-level posttreatment SCC excluded some cases, where we do not know whether the treatment resulted in cure.

CONCLUSIONS

Our study shows that lactational mastitis treatments in Denmark have shifted over the 10-yr study period toward fewer treatments in total but relatively more use of available broad-spectrum options. Treatment duration has generally moved closer to the SPC standards, while the restrictions on broad-spectrum antibiotics increased. Despite these changes, the chance of cure did not decrease in the study population over the study period. Furthermore, our logistic regression analysis on the probability of cytological cure following lactational treatment confirmed the importance of known cow-level influencing factors such as parity and lactation stage on the chances of cure. However, it also highlights the effect of managing the herd-level new infection risk, as well as using pathogen analysis as a tool to improve cure rates and prevent any unnecessary use of antibiotics for bovine mastitis.

ACKNOWLEDGMENTS

This study has been part of the ACROBAT (Reduced use of AntimiCROBials in cAttle and poulTry) project funded by the Danish Veterinary and Food Administration. We thank Søren Saxmose Nielsen (University of Copenhagen, Denmark) and SEGES (Aarhus, Denmark) for providing the data. Handling of the data has been approved by the Danish Data Protection Agency (case no. 514-0696/22-3000). The authors have not stated any conflicts of interest.

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