J. Dairy Sci. 101:9493–9504
https://doi.org/10.3168/jds.2018-14824

Pathogen-specific production losses in bovine mastitis

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ABSTRACT

Reduction in long-term milk yields represents a notable share of the economic losses caused by bovine mastitis. Efficient, economic, and safe measures to prevent these losses require knowledge of the causal agent of the disease. The aim of this study was to investigate pathogen-specific impacts of mastitis on milk production of dairy cows. The materials consisted of milk and health recording data and microbiological diagnoses of mastitic quarter milk samples of 20,234 Finnish dairy cows during 2010, 2011, and 2012. The 6 most common udder pathogens were included in the study: *Staphylococcus aureus*, non-*aureus* staphylococci (NAS), *Escherichia coli*, *Corynebacterium bovis*, *Streptococcus uberis*, and *Streptococcus dysgalactiae*. We used a 2-level multilevel model to estimate curves for lactations with and without mastitis. The data on lactation periods to be compared were collected from the same cow. To enable comparison among lactations representing diverse parities, the estimated lactation curves were adjusted to describe the cow's third lactation. Mastitis caused by each pathogen resulted in milk production loss. The extent of the reduction depended on the pathogen, the timing of mastitis during lactation, and the type of mastitis (clinical vs. subclinical). The 2 most commonly detected pathogens were NAS and *Staph. aureus*. *Escherichia coli* clinical mastitis diagnosed before peak lactation caused the largest loss, 10.6% of the 305-d milk yield (3.5 kg/d). The corresponding loss for *Staph. aureus* mastitis was 7.1% (2.3 kg/d). In *Staph. aureus* mastitis diagnosed between 54 and 120 d in milk, the loss was 4.3% (1.4 kg/d). The loss was almost equal in both clinical and subclinical mastitis caused by *Staph. aureus*. Mastitis caused by *Strep. uberis* and *Strep. dysgalactiae* resulted in losses ranging from 3.7% (1.2 kg/d) to 6.6% (2.1 kg/d) depending on type and timing of mastitis. Clinical mastitis caused by the minor pathogens *C. bovis* and NAS also had a negative effect on milk production: 7.4% (2.4 kg/d) in *C. bovis* and 5.7% (1.8 kg/d) in NAS when both were diagnosed before peak lactation. In conclusion, minor pathogens should not be underestimated as a cause of milk yield reduction. On single dairy farms, control of *E. coli* mastitis would bring about a significant increase in milk production. Reducing *Staph. aureus* mastitis is the greatest challenge for the Finnish dairy sector.
Key words: bovine mastitis, pathogen, milk yield, multilevel modeling

INTRODUCTION

Bovine mastitis mainly results from IMI, and is mostly derived from common udder pathogens such as staphylococci, streptococci, and coliform species (Ruegg, 2017). Mastitis results in substantial problems in terms of animal welfare, food safety, and profitability of milk production. Prevention is always the best measure to avoid the negative effects of mastitis. To develop efficient incentives for prevention, information on the true costs of all types of mastitis is needed. To be efficient, economic, and safe, prevention measures should be adjusted according to the causal agent because different approaches are needed to address different pathogens (Lago et al., 2011a,b; Down et al., 2013; Griffioen et al., 2016). Moreover, public health issues have become increasingly important in the milk industry because of the fear concerning antimicrobial resistance, which increases the pressure to reduce antimicrobial drug usage. Prevention and treatment of mastitis are the main reasons for antimicrobial drug use in the dairy industry (EMA-EFSA, 2017).

Economic losses due to mastitis include direct costs due to diagnostic testing, veterinary service, medication, discarded milk, and labor, as well as indirect costs associated with future milk production loss, reduced reproduction, and premature culling and replacement of mastitic cows (e.g., Santos et al., 2004; Hagnestam-Nielsen and Østergaard, 2009; Hogeveen et al., 2011; Rollin et al., 2015). The costs of preventive measures should also be considered in the total costs of mastitis (van Soest et al., 2016). The extent of economic loss var-

Received March 26, 2018.
Accepted June 16, 2018.
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ies significantly among countries, depending on factors such as milk price, treatment costs, and replacement costs (Halasa et al., 2007). Despite country-specific variation, long-term milk yield losses constitute a notable share of the economic losses attributable to mastitis (Seegers et al., 2003; Heikkilä et al., 2012; Liang et al., 2017). Because of evaluation difficulties, particularly for indirect costs, dairy farmers typically underestimate the costs of mastitis (Huiips et al., 2008). Moreover, costs such as the loss of future milk returns are difficult to gauge.

The estimated milk yield reduction caused by mastitis varies across studies due to differences in follow-up periods, estimation methods, cattle breeds, management, and so on. One reason for the variation in results is the use of different diagnostic methods or definitions for bovine mastitis and IMI (Andersen et al., 2010; Dohoo et al., 2011; Reyher and Dohoo, 2011). The lactation phase when the cow becomes infected is critical because milk losses are significantly greater in early than in late lactation (Hagnestam et al., 2007). High milk yield predisposes cows to mastitis (Oltenacu and Broom, 2010; Taponen et al., 2017) but the decrease in milk production may be greater than that in less-productive cows (Koivula et al., 2005). Clinical and subclinical mastitis have different effects on milk production as do different mastitis-causing pathogens. Milk yield losses resulting from clinical mastitis or high SCC have been studied widely (e.g., Rajala-Schultz et al., 1999; Hagnestam-Nielsen et al., 2009; Detilleux, 2018) but pathogen-specific research has been limited in scope. In some studies carried out in New York State dairy herds, data were categorized according to the causal agent into gram-positive and gram-negative groups and sometimes to the species level (Gröhn et al., 2004; Schukken et al., 2009a; Hertl et al., 2014). Pathogen groups causing the greatest losses were gram-negative species, coliforms, and streptococci. The pathogens causing the greatest losses differed between primiparous and multiparous cows (Hertl et al., 2014). Potential effects of minor pathogens on milk production are largely unknown. In the study of Hertl et al. (2014), clinical mastitis caused by CNS did not result in milk yield losses.

Pathogen-specific information is a prerequisite for detailed estimation of economic losses and tailored control of mastitis. Hence, information on the occurrence of different mastitis-causing agents in herds is needed. Milk sampling for bacteriological diagnostics from all or most clinical and subclinical mastitis cases would create routinely available data on different causal agents. Unfortunately, this practice remains limited in many countries where mastitis is treated empirically, and mostly without sampling (Griffioen et al., 2016). On dairy farms, pathogen information could be used to improve mastitis management (Samson et al., 2016). For scientific research, it would open new possibilities to identify pathogen-specific risk factors and effects of mastitis and facilitate development of responsible treatments for introduction on dairy farms.

In Finland, milk sampling in mastitis cases is routine. Most mastitic milk samples are analyzed in the laboratories of Valio Ltd. (Helsinki, Finland), where the results are recorded in a bacteriological database (Vakkamäki et al., 2017). Moreover, 70% of dairy herds and 80% of dairy cows participate in the Finnish dairy herd recording system, where abundant cow- and herd-specific information is stored (ProAgria, 2017). The aim of this study was to investigate pathogen-specific impacts of mastitis on milk production of dairy cows. We aimed to explain these effects under farm conditions where current mastitis control practices are followed. As such, the results can be utilized in our upcoming study on the pathogen-specific costs of mastitis and the profitability of preventive measures on dairy farms. The field data, where the microbiological database of Valio Ltd. was merged with the database of the Finnish dairy herd recording systems, comprised the materials of the study. Six common udder pathogens were included in the evaluation of reduction in milk yields.

MATERIALS AND METHODS

Data

The initial materials consisted of data from milk and health recordings and microbiological diagnoses of mastitic quarter milk samples from 93,529 cows during the years 2010, 2011, and 2012. The data are part of the broader data set described in Vakkamäki et al. (2017).

Data for cows fulfilling the following criteria were included in this study: (1) the cow was of Nordic Red or Holstein breed; (2) the data from 2010 to 2012 included information from at least 2 lactations of a single cow; (3) at least one of the lactations was free from IMI; (4) from each cow, 1 to 4 quarter milk samples were sent for microbiological analysis to the laboratory of Valio Ltd. only once, on the same day; (5) only one pathogen was detected in the milk samples from a cow; and (6) the pathogen detected was *Staphylococcus aureus*, non-*aureus* staphylococci (**NAS**, formerly described as CNS), *Escherichia coli*, *Corynebacterium bovis*, *Streptococcus uberis*, or *Streptococcus dysgalactiae*. The number of cows fulfilling these criteria was 20,580. After excluding the cows with missing values, the numbers of cows and herds providing data for the study were 20,234 and 3,953, respectively. The pathogen frequencies among

those cows were as follows: NAS, 9,304; *Staph. aureus*, 5,160; *Strep. uberis*, 1,691; *Strep. dysgalactiae*, 1,604; *C. bovis*, 1,352; and *E. coli*, 1,123.

The reason for milk sampling was detected or suspected clinical or subclinical mastitis (elevated milk SCC) in the quarter. Milk samples were taken by herd staff or a supervising veterinarian and submitted to the laboratory of Valio Ltd. The bacteriological diagnosis was mainly based on a single quarter milk sample. The average number of samples per cow was 1.2 for cows having NAS or *C. bovis*, 1.1 for cows with *Staph. aureus* or *Strep. dysgalactiae*, and 1.0 for cows with *Strep. uberis* or *E. coli*.

Microbiological diagnoses of quarter milk samples from mastitic cows analyzed at the laboratory of Valio Ltd. were retrieved from the database of Valio Ltd. For microbiological analyses, the PathoProof Mastitis PCR Complete-12 assay (Thermo Fisher Scientific, Waltham, MA) was used. This test contains oligonucleotides for the staphylococcal β-lactamase gene (*blaZ*) and for the following microbial species or groups of species: *C. bovis*, *Enterococcus* spp. (including *Enterococcus faecalis* and *Enterococcus faecium*), *E. coli*, *Klebsiella oxytoca* and *Klebsiella pneumoniae*, *Serratia marcescens*, *Staphylococcus* spp., including all major NAS, *Staph. aureus*, *Streptococcus agalactiae*, *Strep. dysgalactiae*, *Strep. uberis*, and *Trueperella pyogenes*/*Peptoniphilus indolicus*. In March 2012, the test kit was replaced by PathoProof Mastitis PCR Complete-16 assay, which also includes *Mycoplasma* spp., *Mycoplasma bovis*, *Prototheca* spp., and yeasts.

Milk and health recording data for the cows were received from the databases of the Finnish dairy herd recording system and the Finnish cattle health monitoring system. These data were merged with the data of Valio Ltd., resulting in a data set in which microbiological results for mastitic milk samples could be analyzed with cow-specific information, including breed, date of birth, dates of calvings, dates of milk recordings, milk yield and SCC for each milk recording, possible mastitis diagnosis made by the supervising veterinarian, and recorded mastitis treatments.

Statistical Analyses

Our objective was to examine whether mastitis caused by a specific pathogen affects milk production during lactation with respect to DIM. Information on lactation periods with and without mastitis was collected from the same cow. The estimated lactation curves were adjusted to describe the third lactation of a cow. In the adjustment, we used weightings derived from the results of Lidauer et al. (2000). This procedure enabled the comparison of predicted milk yields of the same cow for lactation periods with and without mastitis.

The collected variables from each cow were daily milk production (response), DIM, type of mastitis (categorical, 2 levels), SCC (categorical, 2 levels), stage (DIM at which the pathogen was discovered in the milk sample, 3 levels), and pathogen (categorical, 6 levels). A dummy status predictor defined lactation periods as being with or without mastitis. A lactation was defined as being with mastitis if a milk sample was sent to the laboratory and a pathogen was detected from the sample. A lactation was defined as being free of mastitis if no milk sample was sent for analysis and no mastitis diagnosis and treatment was recorded for the cow.

All milk samples were taken from cows in which herd staff had suspected mastitis or observed clinical signs of mastitis. In this study, we described the type of disease by the diagnosis made by a veterinarian and recorded in the Finnish cattle health monitoring system. We considered the record of "acute clinical mastitis" ±14 d from the bacteriological diagnosis of the pathogen causing IMI as an indicator of clinical mastitis (**CM**). If such a diagnosis was not made, we recorded that IMI had caused subclinical mastitis (**SCM**). Cows in that category had either the diagnosis "subclinical mastitis" or no veterinary diagnosis. Milk SCC ±14 d from the bacteriological diagnosis was divided into 2 categories (≤500,000 cells/mL and >500,000 cells/mL) and was also used to describe the type of mastitis. Two variables measuring the same feature cannot be included in the same model and hence we tested the superiority of these 2 variables before selecting the final model.

Because the mean of peak lactation of nonmastitic lactations was at 53.3 DIM, the first lactation stage with increasing milk production was from 1 to 53 DIM ("pre" period). The second stage was from 54 to 120 DIM ("post 1" period) and the third stage from 121 DIM to next calving, at a maximum 400 DIM ("post 2" period). These periods formed the 3 categories indicating the time of diagnosis with respect to peak production.

For each cow, we had several daily milk production measurements, up to 14 per lactation (12 per year). For an individual cow, the daily milk production values are correlated. Furthermore, each cow belongs to a specific herd and it is realistic to assume that data for cows within the same herd are correlated. Thus, we have a multilevel structure of 2 nested levels: cows within herds and measurements for each cow within a herd. The model response is a vector of milk yield measurements for an individual cow. We applied a 2-level multilevel model, which can be written as a modification of Laird and Ware (1982):

$$\mathbf{y}_{ij} = \mathbf{X}_{ij}\boldsymbol{\beta} + \mathbf{Z}_{i,j}\mathbf{b}_i + \mathbf{Z}_{ij}\mathbf{b}_{ij} + \varepsilon_{ij},\ i = 1,\ \ldots,\ M,\ j = 1,\ \ldots,\ M_i \quad [1]$$

$$\mathbf{b}_i \sim N(0,\boldsymbol{\Psi}_1),\ \mathbf{b}_{ij} \sim N(0,\boldsymbol{\Psi}_2),\ \varepsilon_{ij} \sim N(0,\sigma^2\mathbf{I}),$$

where $\mathbf{y}_{ij}$ denotes the response vector at the innermost level of grouping (individual daily milk production measurement for a cow), M is the number of first-level groups (number of herds), and M_i is the number of second-level groups within the first-level group i (number of cows within herds i). The length of $\mathbf{y}_{ij}$ is n_{ij}, which is the number of milk yield measurements for an individual cow j in herd i; $\boldsymbol{\beta}$ is the p-dimensional vector of fixed effects, and the fixed-effects model matrices are $\mathbf{X}_{ij}$, $i = 1,\ \ldots,\ M$, $j = 1,\ \ldots,\ M_i$ of size $n_{ij} \times p$. The first-level random effects are $\mathbf{b}_i$ and the second-level random effects are $\mathbf{b}_{ij}$, with lengths q_1 and q_2, respectively; $\mathbf{b}_i$ represents the deviation from the population milk yield mean for herd i and $\mathbf{b}_{ij}$ represents the deviation from herd i milk yield mean for cow j, within herd i, for specific predictor values. The corresponding model matrices are $\mathbf{Z}_{i,j}$ and $\mathbf{Z}_{ij}$ of sizes $n_{ij} \times q_1$ and $n_{ij} \times q_2$, respectively. Random effects $\mathbf{b}_i$ are assumed to be normal and independent for different i, and random effects $\mathbf{b}_{ij}$ are assumed to be normal and independent for different i or j and to be independent of random effects $\mathbf{b}_i$. Matrices $\boldsymbol{\Psi}_1$ and $\boldsymbol{\Psi}_2$ denote the covariance matrices of b_i and b_ij of sizes q_1 × q_1 and q_2 × q_2, respectively. The within-group errors ε_{ij} are assumed to be normal and independent for different i or j and to be independent of the random effects. Matrix $\mathbf{I}$ denotes an identity matrix of size n_ij × n_ij. For our model, M = 3,953, M_i ranged from 5 to 70, and n_{ij} ranged from 1 to 14 with a median of 10, $q_1 = 3$, $q_2 = 3$, and $p = 216$.

We considered 2 lactation curves that define the exact formulation of the 2-level multilevel model: a model proposed by Wilmink (1987; equation [2]) and a model proposed by Guo and Swalve (1995; equation [3]). These models can be written as follows:

$$y = \beta_0 + \beta_1 \exp\left(-k \times DIM\right) + \beta_2 DIM; \quad [2]$$

$$y = \beta_0 + \beta_1 \sqrt{DIM} + \beta_2 \ln\left(DIM\right). \quad [3]$$

These model formulations are written at a scalar level without subscripts. The models were incorporated into the 2-level multilevel model [1] matrices $\mathbf{X}_{ij}$, $\mathbf{Z}_{i,j}$, and $\mathbf{Z}_{ij}$. We considered every possible interaction of the predictors in the model matrix $\mathbf{X}_{ij}$. Because of the random structure, we considered random intercept and random coefficients with respect to $\exp(-k \times DIM)$ and DIM for Wilmink [2], and random intercept and random coefficients with respect to $\sqrt{DIM}$ and $\ln(DIM)$ for Guo and Swalve [3]. The constant k in the Wilmink model [2] was −0.05, based on Wilmink (1987).

Different random and correlation structures were used to model the dependence among observations. The random structure specifies the mixed-effects parameters. We considered several cases: random intercept (β_0) and "slopes" (β_1 and β_2); random intercept and one slope (β_1); and only random intercept and no random structure using the notation of the Wilmink [2] and Guo and Swalve [3] models. In the multilevel model structure, these terms are then incorporated into the fixed- and random-effects vectors. The correlation structures are used to model the dependence among the within-groups errors. For correlation structures, we used the first-order autoregressive [AR(1)] and compound symmetry structures; AR(1) assumes that the correlation of the observations is larger for observations closer to each other in time and diminishes as the time lag increases. For the time information, we used both DIM and the discrete ordered time points. Compound symmetry assumes that the correlation between observations is constant. We also considered a nonrandom model structure, which does not assume correlation among any observations. Details of these correlation structures can be found, for example, in Pinheiro and Bates (2000).

Models were fit first using the method of maximum likelihood to allow comparison between models using the Akaike information criterion (**AIC**). When the most adequate model was found, the method of REML was used to obtain the model parameters.

As model selection and validation tools we used AIC, conditional type II and III F-tests, and likelihood ratio tests. Conditional F-tests were used to test the significance of fixed-effects terms. Likelihood ratio tests were used to test significance of terms in the random-effects structure. As a goodness-of-fit measure, we used the R^2 (coefficient of determination) described by Nakagawa and Schielzeth (2013). They presented marginal and conditional R^2 coefficients representing the variance explained by fixed factors and both fixed and random factors, respectively. Finally, model assumptions were examined using residual diagnostics.

Missing values were assumed missing completely at random or missing at random. Observations for which any of the model predictors consisted of missing values were omitted from the analysis. Hence, the number of valid observations was smaller than the number of cows eligible to be modeled.

Because AIC is a meaningful comparison tool only for models fit to identical data sets, modeling was first conducted on valid observations determined by predic-

tor SCC, which was the most restrictive predictor with respect to missing values. Two Wilmink models, with random terms corresponding to β_0, β_1, and β_2 [2], AR(1) correlation structure with all predictors except for SCC, and with all predictors except for treatment, had essentially the same value for the AIC. The number of cows in these analyses providing data was 8,096, indicating that for over 50% of the cows in the full data set, there were missing values with respect to SCC. This is highly problematic and suggests, for example, the possibility of selection bias. Because the other predictors did not have such a large number of missing values, we considered the model with all predictors except SCC to be more robust. Subsequently, we considered only models not including the predictor SCC and fit accordingly using valid observations, not restricted by the missing values corresponding to SCC.

The model minimizing the AIC was for the Wilmink formulation, including predictors DIM, status, treatment, stage, and pathogen, with random terms corresponding to β_0, β_1, and β_2 [2] for both herd and cow levels and AR(1) correlation structure. Although the above model had the minimum AIC, we further compared it with similar models with different random structures using likelihood ratio testing. The likelihood ratio tests suggested that each random term was statistically significant compared with models with 2 random slopes, 1 random slope, and no random terms. The statistical significance of the fixed effects was examined via conditional *F*-tests. Most importantly, the type II and III tests corresponding to the 5-way interactions were statistically significant for terms β_1 and β_2 [2], providing yet more evidence that each predictor should be included in the model.

All computations were performed with R software (R Core Team, 2017) using packages car (Fox and Weisberg, 2011), MuMIn (Barton, 2017), and nlme (Pinheiro et al., 2017).

RESULTS

The results are based on model [1], whose Nakagawa marginal and conditional R^2 values were 0.39 and 0.67, respectively. Thus, almost 40% of the variance in milk production was explained by fixed factors only. A significant portion of data variability also resided in the random factors, which can be seen from the difference between the R^2 values. Almost 70% of milk production variability was explained using both fixed and random factors.

Residual diagnostics based on the within-group residuals and fitted values were examined graphically. No deficiencies were established regarding homoscedasticity or structural violations. The residuals showed deviation from normality in the sense of having longer tails. Robust or resampling methods could be adopted to investigate the effects of the long tails. Such approaches were not conducted in this study. The random terms corresponding to terms β_0 and β_1 [2] seemed to follow the normal distribution at both levels; β_2 [2] had heavier tails for the cow level and some left-skewness for the herd level. There was some correlation between the random terms for both levels but it was not statistically significant.

The 305-d milk yields, estimated by using predictions and corresponding confidence intervals, are presented in Table 1. The difference between lactations with and without mastitis is interpreted to be significant only in the categories where the confidence intervals of the estimated 305-d yields do not overlap. Supplemental Figures S1 to S6 (https://doi.org/10.3168/jds.2018-14824) present the predicted lactation curves along with pointwise 95% CI for *Staph. aureus*, NAS, *E. coli*, *C. bovis*, *Strep. uberis*, and *Strep. dysgalactiae*, respectively. The curves are presented separately in relation to the type of mastitis (CM, SCM) and the time of diagnosis with respect to peak production (pre, post 1, and post 2 periods). The figures also indicate the number of cows in each category.

Staphylococcus aureus

Staphylococcus aureus accounted for 25.5% of the pathogens detected in the cows included in this study. The type of mastitis caused by *Staph. aureus* was mainly SCM, which accounted for 77% of all cases (Supplemental Figure S1a–f; https://doi.org/10.3168/jds.2018-14824). *Staphylococcus aureus* mastitis occurred typically either in the beginning of lactation or in the end of lactation, 40% before peak lactation and 41% during the last stage of lactation (post 2 period). When *Staph. aureus* mastitis was diagnosed before peak lactation, milk production remained lower than in lactations free of mastitis for the entire lactation. The difference slightly decreased toward the end of lactation in the case of CM (Supplemental Figure S1a) but remained unchanged with SCM (Supplemental Figure S1b). The average daily milk losses calculated until 305 DIM were 2.3 and 2.2 kg for cows with CM and SCM, respectively. Daily milk production for lactations with and without mastitis was equal during early lactation when the diagnosis was made after peak production (post 1 period) for both CM and SCM. After the peak, the yield for lactation with mastitis decreased and remained lower than that for lactation free of mastitis, regardless of type (Supplemental Figure S1c, d). The

average daily milk loss calculated until 305 DIM was 1.4 kg in both cases. If the diagnosis was made in the last stage of lactation (post 2 period), the early lactation yield was slightly higher for lactation with mastitis than for lactation free of mastitis, but at the end of lactation the ratio was reversed (Supplemental Figure S1e, f).

Mastitis caused by *Staph. aureus* and diagnosed before 120 DIM (pre and post 1 periods) resulted in a decrease in 305-d milk yield compared with lactation without mastitis. The decrease, calculated from the point estimates of the yields, was 7.1% of the total 305-d yield when the diagnosis was made before peak lactation. If the diagnosis was made after peak production (post 1 period), the proportional reduction in the milk yield was 4.3% in CM and 4.4% in SCM (Table 1).

Non-aureus Staphylococci

Non-*aureus* staphylococci was the most common finding (46.0%) among the pathogens included in the

Table 1. The 305-d milk yield and 95% confidence interval on lactations with mastitis and free of mastitis presented by causative agent, timing in lactation, and mastitis type, as well as significant differences in the yield between nonmastitic and mastitic lactations

	305-d milk yield (kg) with 95% CI						Significant difference in 305-d yield between lactations		
	Lactation with mastitis			Lactation free of mastitis					
Pathogen, timing,[1] and type of mastitis[2]	Lower bound	Point estimate	Upper bound	Lower bound	Point estimate	Upper bound	Milk yield loss, kg	Milk yield loss, %	Milk yield loss, kg/d
Staphylococcus aureus									
Pre peak CM	8,994	9,093	9,193	9,682	9,784	9,886	691	7.1	2.3
Pre peak SCM	8,686	8,868	9,050	9,357	9,542	9,728	674	7.1	2.2
Post 1 CM	9,221	9,329	9,437	9,653	9,752	9,852	423	4.3	1.4
Post 1 SCM	9,044	9,222	9,400	9,477	9,648	9,819	426	4.4	1.4
Post 2 CM	9,572	9,722	9,873	9,608	9,752	9,896			
Post 2 SCM	9,378	9,628	9,878	9,419	9,661	9,903			
Non-*aureus* staphylococci									
Pre peak CM	9,114	9,188	9,262	9,668	9,744	9,820	556	5.7	1.8
Pre peak SCM	9,102	9,300	9,498	9,428	9,630	9,832			
Post 1 CM	9,202	9,277	9,353	9,511	9,583	9,656	306	3.2	1.0
Post 1 SCM	9,076	9,255	9,434	9,360	9,533	9,705			
Post 2 CM	9,484	9,602	9,720	9,436	9,551	9,667			
Post 2 SCM	9,268	9,541	9,814	9,357	9,622	9,886			
Escherichia coli									
Pre peak CM	8,654	8,874	9,094	9,703	9,927	10,151	1,053	10.6	3.5
Pre peak SCM	8,691	9,049	9,407	9,242	9,602	9,963			
Post 1 CM	8,917	9,136	9,355	9,242	9,448	9,654			
Post 1 SCM	8,837	9,193	9,550	9,199	9,542	9,884			
Post 2 CM	9,497	9,795	10,092	9,390	9,673	9,956			
Post 2 SCM	9,033	9,454	9,874	9,095	9,497	9,899			
Corynebacterium bovis									
Pre peak CM	9,042	9,197	9,352	9,770	9,928	10,085	731	7.4	2.4
Pre peak SCM	8,304	8,842	9,380	9,046	9,592	10,139			
Post 1 CM	9,036	9,256	9,476	9,137	9,332	9,526			
Post 1 SCM	8,461	9,017	9,574	9,191	9,674	10,158			
Post 2 CM	9,540	9,818	10,096	9,357	9,618	9,879			
Post 2 SCM	9,019	9,623	10,227	9,079	9,649	10,218			
Streptococcus uberis									
Pre peak CM	9,327	9,501	9,674	9,605	9,781	9,956			
Pre peak SCM	8,798	9,064	9,330	9,441	9,709	9,976	645	6.6	2.1
Post 1 CM	8,998	9,176	9,353	9,415	9,583	9,751	407	4.2	1.3
Post 1 SCM	9,136	9,453	9,769	9,511	9,817	10,122			
Post 2 CM	9,657	9,907	10,157	9,842	10,083	10,325			
Post 2 SCM	9,091	9,480	9,869	9,168	9,550	9,932			
Streptococcus dysgalactiae									
Pre peak CM	8,989	9,164	9,340	9,605	9,787	9,968	623	6.4	2.0
Pre peak SCM	8,704	9,023	9,343	9,197	9,522	9,847			
Post 1 CM	9,082	9,251	9,420	9,449	9,606	9,763	355	3.7	1.2
Post 1 SCM	9,023	9,333	9,642	9,325	9,631	9,936			
Post 2 CM	9,596	9,880	10,164	9,512	9,783	10,055			
Post 2 SCM	9,065	9,546	10,028	9,016	9,492	9,969			

[1]Pre peak = 1–53 DIM; post 1 = 54–120 DIM; post 2 = >120 DIM.

[2]CM = clinical mastitis; SCM = subclinical mastitis.

study. Mastitis caused by NAS was mainly subclinical (89%; Supplemental Figure S2a–f; https://doi.org/10.3168/jds.2018-14824). It occurred typically either in the first or the last stage of lactation, the proportions being 44% and 41%, respectively (Supplemental Figure S2a–f). The lactation curves for cows with NAS mastitis (Supplemental Figure S2a–f) followed the same pattern as those for cows with *Staph. aureus* mastitis (Supplemental Figure S1a–f), but the differences between lactations with mastitis and lactations free of mastitis were smaller. When the pathogen was diagnosed before peak lactation, the average daily milk loss calculated until 305 DIM was 1.8 kg for cows with CM (Supplemental Figure S2a), but with SCM, we detected no significant difference between the lactation curves for lactations with and without mastitis (Supplemental Figure S2b). For cows diagnosed after peak lactation (post 1 period) and having CM, the average daily milk yield loss until 305 DIM was 1.0 kg (Supplemental Figure S2c).

Mastitis caused by NAS affected 305-d yield when the diagnosis was made before or soon after (post 1 period) peak lactation and the cow was diagnosed with CM. The decrease was 5.7% for the earlier-diagnosed cow and 3.2% for the later-diagnosed cow (Table 1).

Escherichia coli

Escherichia coli was the causal agent in 5.6% of mastitis cases. Mastitis caused by *E. coli* was clinical in 28% of cases and diagnosed in 41% of the cases before peak lactation (Supplemental Figure S3a–f; https://doi.org/10.3168/jds.2018-14824). The effect of *E. coli* mastitis on daily milk yield was evident for cows diagnosed before peak lactation and having CM (Supplemental Figure S3a). The average loss until 305 DIM was 3.5 kg/d and the maximum loss 3.9 kg/d at 61 DIM. When the diagnosis of CM was made after the peak (post 1 period), the pre-peak milk yield was higher than in lactation without mastitis, but mastitis caused a significant reduction in the yield (Supplemental Figure S3c). After the intersection of the lactation curves in Supplemental Figure S3c, the total loss until 305 DIM was 486 kg (2.3 kg/d). At 305 DIM, the reduction was 4.6 kg/d, which was the highest daily loss caused by the 6 pathogens investigated in the period from 1 to 305 DIM.

Mastitis caused by *E. coli* affected 305-d milk yield only when the diagnosis was made before peak lactation and the cow had CM. The decrease was 10.6% of the total 305-d yield (Table 1). When the diagnosis was made after peak lactation, losses in 305-d yields were compensated by the high yields in early lactation.

Corynebacterium bovis

Corynebacterium bovis was detected in 6.7% of the cows with mastitis. Half of the mastitis cases caused by *C. bovis* were diagnosed at the end of lactation (post 2 period). As with NAS mastitis, *C. bovis* mastitis was mainly (89%) subclinical (Supplemental Figure S4a–f; https://doi.org/10.3168/jds.2018-14824). *Corynebacterium bovis* mastitis caused a significant yield loss for the whole lactation when the diagnosis was made before peak lactation and the cow was diagnosed with CM (Supplemental Figure S4a). If the diagnosis was made after the peak (post 1 period), the yield in early lactation was higher in lactation with mastitis than in lactation free of mastitis, but decreased clearly after the diagnosis (Supplemental Figure S4c). The average daily yield loss for a cow with CM and diagnosed before peak was 2.4 kg (Supplemental Figure S4a). The total loss for lactation with CM after the peak (post 1 period) was 304 kg (1.9 kg/d) for the period from the intersection of the lactation curves until 305 DIM (Supplemental Figure S4c).

The effects of *C. bovis* mastitis on the 305-d yield was observed only if the pathogen was diagnosed before peak lactation and the cow was diagnosed with CM. The loss was 7.4% compared with the lactation free of mastitis (Table 1).

Streptococcus uberis

Streptococcus uberis was detected in milk of 8.4% of the cows. Clinical cases accounted for 27% of *Strep. uberis* mastitis, which was diagnosed equally (40%) before peak production and in the last stage of lactation (post 2 period; Supplemental Figure S5a–f; https://doi.org/10.3168/jds.2018-14824). When mastitis was caused by *Strep. uberis*, the curves for lactations with and without mastitis were mostly quite close to each other (Supplemental Figure S5a–f). However, for cows that had SCM and were diagnosed before peak lactation, average daily loss until 305 DIM was 2.1 kg (Supplemental Figure S5b). The corresponding loss was 1.3 kg for cows having CM and diagnosed after peak lactation (Supplemental Figure S5c).

When mastitis was caused by *Strep. uberis*, the total loss in 305-d yield was 6.6% in the category of cows having SCM and diagnosed before peak lactation. The corresponding loss for cows diagnosed after peak

production (post 1 period) and having CM was 4.2% (Table 1).

Streptococcus dysgalactiae

Streptococcus dysgalactiae was detected in milk samples of 7.9% of the cows. Mastitis caused by *Strep. dysgalactiae* typically occurred at the beginning of lactation, with 47% of the cases occurring before peak lactation (Supplemental Figure S6a–f; https://doi.org/10.3168/jds.2018-14824). Clinical cases accounted for 22% of all *Strep. dysgalactiae* mastitis cases (Supplemental Figure S6a–f). The lactation curves for cows with mastitis caused by *Strep. dysgalactiae* were similar to those for cows with mastitis caused by *Strep. uberis*. However, yield losses were slightly smaller when mastitis was caused by *Strep. dysgalactiae*. The average loss in daily milk yield was 2.0 kg if the diagnosis of CM was made before peak lactation (Supplemental Figure S6a) and 1.2 kg if the diagnosis was made after the peak (Supplemental Figure S6c).

At the 305-d yield level, the losses were 6.4 and 3.7% for cows with CM that were diagnosed before and after peak lactation (post 1 period), respectively. When the diagnosis was made in the last stage of lactation (post 2 period), we observed no significant differences in the lactation curves or, consequently, in 305-d yields (Table 1).

DISCUSSION

Our extensive field data from Finnish dairy farms provided excellent possibilities to investigate the pathogen-specific effects of mastitis on milk production. Although we had to adapt the original data set to generate appropriate material for the purposes of this study, numerous observations were available for each pathogen. The data enabled comparison of milk yields for lactations with mastitis and those free of mastitis from the same cow. Typically, researchers have been forced to make comparisons between a cow with mastitis and her healthy herdmates (Gröhn et al., 2004; Hertl et al., 2014, El-Tarabany and Ali, 2015), which may distort the results because cows with mastitis are often higher producers before diagnosis than their nonmastitic herdmates (Gröhn et al., 2004; Hertl et al., 2014).

Mastitis is an inflammation of the mammary gland and always results in decreased production of milk, which may be of short duration or can last to the end of that lactation period. Histological and other analyses have been used to assess the damage to the secretory tissue caused by mastitis (Zhao and Lacasse, 2008). The results clearly indicate that the presence of microorganisms (i.e., IMI) is associated with tissue damage. Mammary tissue damage reduces the number and activity of epithelial cells and, consequently, milk production of the quarter is disturbed (Zhao and Lacasse, 2008). The mechanisms causing the damage to the mammary tissue may differ (Zhao and Lacasse, 2008) but the resulting irreversible damage is the main reason for the milk loss (Oliver and Calvinho, 1995). Moreover, clear differences exist in pathogenesis between bacterial species that cause mastitis. The host immune response depends on the invading bacterial species and cow-specific factors, and contributes to the severity of udder inflammation as well as to the outcomes of mastitis, including milk loss (Burvenich et al., 2003; Schukken et al., 2011).

Because IMI was detected in milk of every cow included in our study, losses in milk yields were expected. Pathogen-specific differences in the losses reflect the pathogenicity of the detected bacterial species. When comparing results of different studies from different eras, our results are mainly in line with those of previous studies. However, some deviations were apparent, particularly regarding the effects of minor pathogens.

Staphylococcus aureus is perhaps the most important udder pathogen that causes subclinical and clinical mastitis with mild to moderate clinical signs (Schukken et al., 2011). In our data, the proportions were 72% for SCM and 28% for CM. *Staphylococcus aureus* mastitis responds poorly to treatment and often remains persistent in the quarter (Barkema et al., 2006). The bacterium is able to adhere to epithelium and invade the interstitial tissues of the mammary gland, causing a deep infection (Schukken et al., 2011). Pathological changes in the affected quarter caused by *Staph. aureus* are substantial, in particular if the disease becomes chronic (Zhao and Lacasse, 2008).

In our study, *Staph. aureus* mastitis caused significant and long-term loss in milk production. The decrease was larger when the diagnosis was made in early lactation but almost equal in CM and SCM. Our results on milk losses and their persistence in *Staph. aureus* mastitis agree with those of earlier studies (Wilson et al., 1997; Gröhn et al., 2004; Reksen et al., 2007). Gröhn et al. (2004) reported that milk loss in *Staph. aureus* CM persisted until at least 70 d after diagnosis. Cows with *Staph. aureus* mastitis already showed a significant decrease in milk production up to 30 d before the actual case, indicating that CM was a flare-up from SCM. More recently, Bobbo et al. (2017) reported a small but statistically significant production loss in the case of SCM caused by contagious pathogens. In the category of contagious pathogens, *Staph. aureus* alone accounted for 88% and *Staph. aureus* with another pathogen for 6% of the pathogens detected (Bobbo et al., 2017).

The most commonly detected pathogens in this study were NAS. Our results showed that cows with NAS mastitis with a diagnosis of CM (11% of all NAS mastitis) produced less milk in lactations with mastitis than in those free of mastitis. If the disease occurred at the end of lactation, the 305-d yield losses were compensated by the higher yields of early lactation. Subclinical mastitis caused by NAS decreased milk production if the diagnosis was made between 54 and 120 DIM. If NAS SCM was diagnosed earlier or later, an indication of production loss, although not statistically significant, was seen. Our results are in contrast to those of most previous studies. Hertl et al. (2014) investigated repeated clinical mastitis episodes in Holstein dairy cows and found no association between milk yield loss and occurrence of CNS. Similarly, no milk losses were attributed to mastitis caused by CNS in 3 other studies (Paradis et al., 2010; Tomazi et al., 2015; Bobbo et al., 2017). Pearson et al. (2013) used data from 19 twin pairs to estimate the effects of pericalving CNS mastitis in primiparous cows. Again, CNS mastitis was not associated with decreased milk yield.

Non-*aureus* staphylococci are traditionally regarded as minor pathogens that seem to lack the ability to cause severe mastitis (Taponen and Pyörälä, 2009). Some studies have even suggested a positive association between NAS IMI and milk production (Schukken et al., 2009b; Piepers et al., 2013). Cows included in those studies, however, differed from those in our study because all cows in the herds were sampled, in contrast to our study, where only cows with signs of mastitis or suspected mastitis were enrolled. Consequently, the proportion of NAS originating from the teat canal or causing only short-duration IMI was probably high in the cited studies, explaining the absence of a negative effect on milk yield. Some NAS species affect udder health more than others, and the prevalence of NAS species may differ between countries (Nyman et al., 2018). In our study, the mean number of samples for cows with NAS or *C. bovis* mastitis indicated that many cows had more than one inflamed quarter. Insignificant milk loss in one quarter might thus become significant loss at the cow level. Our study does not support the existence of a positive milk yield effect related to NAS mastitis.

Escherichia coli typically causes acute clinical mastitis with moderate to severe clinical signs (Hogan and Smith, 2003). In our data, the mean proportion of CM was the largest for *E. coli* (28%). During the middle stage of lactation, the proportion was even higher (34%). Endotoxin produced by *E. coli* triggers a rapid and strong inflammatory reaction, which results in substantial damage to the secretory tissue (Schukken et al., 2011). Consequently, a significant decrease in milk production ensues, which may continue until the end of lactation (Burvenich et al., 2003; Zhao and Lacasse, 2008). In our study, the most severe and sudden decrease in milk production was recorded for cows with *E. coli* mastitis. The significant milk loss due to *E. coli* mastitis has been verified by Gröhn et al. (2004) and Hertl et al. (2014). If the diagnosis of CM caused by *E. coli* was made after 53 DIM in our study, the daily milk yield of lactation with upcoming mastitis was initially higher than that of a lactation free of mastitis. In general, dairy cows with high milk yields are more prone to mastitis than those with low yields, but this phenomenon was common to all pathogens considered in this study except *C. bovis* (Taponen et al., 2017). Hence, a special susceptibility to mastitis due to *E. coli* because of high production does not explain this result.

Corynebacterium bovis has been regarded as a minor pathogen that causes only a slight increase in milk SCC and rarely clinical mastitis (Djabri et al., 2002). It has been considered a colonizer of the teat canal (Bexiga et al., 2011). However, in a study in which quarter milk samples were taken both via the teat canal and directly from the udder cistern, *C. bovis* was detected in both sites in almost equal numbers, indicating that it does cause mastitis (Hiitiö et al., 2016). Our study showed that *C. bovis* is able to cause CM, although the proportion of CM was, along with the CM proportion of NAS, the lowest (11%) among the pathogens studied.

Very little is known about the effect of mastitis due to *C. bovis* on milk production. This bacterium has not been a major focus and relevant data may have been pooled with other "less important" pathogens. However, Wilson et al. (1997) reported that 305-d milk production of cows with *C. bovis* in composite milk was lower (9,002 kg) than that of cows without any isolated pathogens (9,578 kg). In contrast, Gonçalves et al. (2016) reported that subclinical *C. bovis* infection had no effect on milk yield. In the present study, we showed that mastitis caused by *C. bovis* is associated with milk losses. Our results indicated a larger proportional decrease (7.4 vs. 6.0%) in 305-d milk yield than did Wilson et al. (1997), providing that *C. bovis* was detected before peak lactation and the cow was diagnosed with CM. Based on our results, *C. bovis* deserves more attention, although milk losses caused by *C. bovis* mastitis were significant only in CM.

Streptococcus uberis and *Strep. dysgalactiae* are common udder pathogens causing subclinical mastitis and clinical mastitis with moderate clinical signs (Leigh, 1999; Rato et al., 2011). *Streptococcus uberis* has received more attention because of its pathogenic characteristics and ability to cause persistent or recurrent infections (Pedersen et al., 2003; Milne et al., 2005). Milk losses from IMI caused by *Strep. uberis* and

Strep. dysgalactiae have seldom been studied separately but the species have been included in the group of *Streptococcus* spp. We found that both species caused moderate production losses, but *Strep. uberis* more than *Strep. dysgalactiae*. In the study of Gröhn et al. (2004), milk yield losses caused by *Streptococcus* spp. were among the most significant, together with those caused by *E. coli* and *Staph. aureus*. Hertl et al. (2014) also indicated that production losses resulted from CM caused by *Streptococcus* spp. Mastitis caused by *Strep. uberis* was associated with a lower milk yield in the twin pairs comparison of Pearson et al. (2013).

Milk losses were greater if a cow had CM compared with SCM. The only exception was for *Staph. aureus*, which caused almost equal losses regardless of the type of mastitis. Differences between CM and SCM were expected because CM affects the general health of the cow and causes pathological changes in the udder, which can be severe. Cows suffering from CM probably received antimicrobial treatment and, in moderate or severe cases, also supportive therapy (Barlow, 2011). In SCM, treatment may often be postponed until drying-off (Barlow, 2011). In our study, treatment of CM probably affected the outcome of mastitis and decreased production losses by relieving clinical signs and shortening the duration of the disease. Nonetheless, the difference between CM and SCM remains. Poor prognosis to eliminate IMI is generally associated with *Staph. aureus* mastitis (Barkema et al., 2006). In the current study, milk loss due to *Staph. aureus* CM slightly decreased to the end of lactation (Supplemental Figure S1a), in contrast to SCM (Supplemental Figure S1b). This could result from a treatment effect but might also indicate that the stronger host response in *Staph. aureus* CM was able to support elimination of IMI more efficiently than in SCM, which often may be of chronic nature. Clinical mastitis caused by penicillin-susceptible *Staph. aureus* responds well to penicillin, which is the treatment of choice in Finland (Taponen et al., 2003).

In this study, our aim was to investigate production losses at the cow level to produce appropriate data for economic analysis rather than to indicate a treatment response. Differences in numbers of quarters infected with the 6 pathogens studied and antimicrobial treatments given to cows might have affected the results. However, it is in line with our purpose to show the effect of pathogen-specific mastitis on milk production as it appears in Finnish dairy herds.

CONCLUSIONS

In terms of milk yield losses, the most harmful pathogen causing mastitis is *E. coli*. The milk yield reduction resulting from the presence of *E. coli* was both large and long lasting. However, this result is valid only at the cow level because *E. coli* is the least common pathogen among the 6 most common pathogens detected in Finnish dairy herds and investigated in this study. *Corynebacterium bovis* proved to be a more harmful pathogen than expected when causing CM because it can cause milk losses comparable to those of *Staph. aureus*. When considering the entire dairy sector, the largest milk yield losses result from mastitis due to the widespread pathogen *Staph. aureus*, which causes moderate yield losses with both CM and SCM. The NAS have a substantial negative impact because they are very common and can reduce milk production, especially when associated with CM. *Streptococcus uberis* and *Strep. dysgalactiae* belong to the middle category, in terms of both frequency and loss of milk production. Milk yield losses are invariably larger the earlier in lactation the diagnosis is made. With late onset of mastitis, daily milk yields for early lactations may be even higher for lactations with mastitis than for those without. We conclude that mastitis caused by the pathogens investigated in this study results in reduced milk yield on Finnish dairy farms. The extent of the reduction depends on the causal agent, the timing of mastitis within a lactation, and the type of disease (clinical or subclinical). The minor pathogens should not be underestimated as causes of milk production losses. On single dairy farms, control of *E. coli* mastitis would bring about a significant increase in milk production. Reducing *Staph. aureus* mastitis is the greatest challenge for the dairy sector.

ACKNOWLEDGMENTS

Financial support from Walter Ehrström Foundation (Helsinki, Finland) and the Ministry of Agriculture and Forestry of Finland (Helsinki) is gratefully acknowledged. We also wish to thank Valio Ltd. (Helsinki, Finland) for financing this study and for permitting access to their bacteriological database. ProAgria (Helsinki, Finland) provided us data from the Finnish dairy herd recording systems, which we greatly appreciate.

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