A cohort study of the effect of *Streptococcus agalactiae* on milk yield and somatic cell count in Norwegian dairy cows

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ABSTRACT

The primary objective of the present study was to estimate the effect of *Streptococcus agalactiae* intramammary infection on milk production and somatic cell count (SCC) in Norwegian dairy cows. A secondary objective was to assess differences in the effect of common *Strep. agalactiae* sequence types (ST) found in Norwegian dairy herds. We performed a cohort study combining registry data with sequence-type data from *Strep. agalactiae* isolates. Herds in which *Strep. agalactiae* had been detected in individual animals (bacteriological culture or quantitative PCR) between 2012 and 2015 were included. We accessed monthly test-day milk yield records for the entire period to compare milk yield and SCC between cows that were *Strep. agalactiae* positive and all other cows, within each herd. The study sample consisted of 150 herds, 15,757 cows, 30,850 lactations, and 204,126 test days. We evaluated the effects of *Strep. agalactiae* on test-day milk yield and SCC using mixed linear regression models, controlling for clustering by herd, cow, and lactation. Multilocus sequence typing of *Strep. agalactiae* was available for isolates from 86 herds. Additional models were fit to a subset of herds ($n = 59$) in which ST1, ST23, ST103, and ST196 had been found, to compare the effects of ST on milk production and SCC. In the period 3 to 2 mo before diagnosis, *Strep. agalactiae*-positive cows produced an average of 1.3 kg more DIM-adjusted milk/d than their negative herd mates. At the time of diagnosis, production was on average 0.13 kg less DIM-adjusted milk/d in *Strep. agalactiae*-positive cows than in negative cows; 2 to 3 mo after diagnosis, they produced 1.24 kg less DIM-adjusted milk/d than negative cows. Losses persisted for the rest of the investigated period. Cows with

ST23, ST103, and ST196 followed a similar pattern as the overall analysis with respect to milk production, whereas ST1-affected cows produced similar amounts of milk before diagnosis as the negative cows. Cows with ST1 experienced the largest milk loss 1 to 2 mo after diagnosis but then recovered to some extent; for cows with ST103, the severe milk loss persisted for the rest of the investigation period. The cow-associated ST103 elicited a lower response in peak SCC compared with ST23, ST103, and ST196. The results indicate an effect of *Strep. agalactiae* on milk production and SCC. Production was lowest 2 to 3 mo after a positive sample. Peak SCC was reached the month before diagnosis, with notable differences between sequence types.

Key words: *Streptococcus agalactiae*, sequence type, milk production, SCC

INTRODUCTION

After being almost eliminated as a causative agent of IMI in Norwegian dairy herds, *Streptococcus agalactiae* has drawn attention again over the past decade (Sølverød et al., 2009; Østerås and Sølverød, 2011). The proportion of *Strep. agalactiae*-positive milk samples among those submitted to the national TINE Mastitis Laboratory increased from 0.1% in 2005 to 2.8% in 2013, indicating a re-emergence of the pathogen. At the herd level, the proportion of herds with at least one *Strep. agalactiae*-positive milk sample among herds that submitted more than 9 milk samples per year increased from 1.3% in 2005 to 6.4% in 2013 (Østerås, 2018). Although clinical cases do occur, *Strep. agalactiae* predominantly causes subclinical IMI. For many different intramammary pathogens, a subclinical infection causes elevated SCC (Schepers et al., 1997; Reksen et al., 2008). Elevated SCC ($>100,000$ cells/mL) is associated with milk production loss (Hortet et al., 1999; Halasa et al., 2009; Hand et al., 2012). Moreover, milk loss following clinical mastitis (independent of pathogen) is

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well documented in the literature (e.g., Rajala-Schultz et al., 1999; Wilson et al., 2004). In Finnish Ayrshire cows, the total loss during lactation due to clinical mastitis was estimated to be 110 to 348 kg in first-parity cows, 219 to 299 kg in second-parity cows, 352 to 510 kg in third-parity cows, and 328 to 550 kg in cows in their fourth parity or higher, depending on DIM when the case occurred (Rajala-Schultz et al., 1999). Estimates of the total milk loss following a clinical mastitis episode at 81 DIM in Holstein cows were 690 kg in first-parity cows and 1181 kg in cows in their second parity or higher (Wilson et al., 2004). Pathogen-specific estimates for milk loss have been provided for both the first case and repeated cases of clinical mastitis caused by select major and minor pathogens (*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* spp., *Trueperella pyogenes*, and *Streptococcus* spp.; Gröhn et al., 2004; Hertl et al., 2014; Heikkilä et al., 2018). Another study (de Haas et al., 2002) estimated lactation curves for SCC, showing the effect of a first case of clinical mastitis caused by *Staphylococcus aureus*, coagulase-negative staphylococci, *Escherichia coli*, *Streptococcus dysgalactiae*, *Streptococcus uberis* and other *Streptococcus* spp. Pathogen-specific estimates for milk loss and SCC for *Strep. agalactiae* IMI are currently lacking.

Streptococcus agalactiae has traditionally been regarded as an obligate intramammary pathogen (Keefe, 1997). However, a recent study showed that *Strep. agalactiae* could be isolated from several environmental sites in affected dairy herds, including floors, cow beds, and water troughs (Jørgensen et al., 2016). Multi-locus sequence typing (MLST) of the isolates was performed, and the results suggested that certain sequence types (ST) of *Strep. agalactiae* might be more persistent in the environment than others. Indeed, *Strep. agalactiae* ST1 was detected in 8% of environmental samples, whereas *Strep. agalactiae* ST103 was detected in 36% (Jørgensen et al., 2016). Strain-specific differences in infection route and duration of infection have also been described in *Strep. uberis* (Zadoks et al., 2001, 2003).

The primary objective of the present study was to estimate the effect of *Strep. agalactiae* IMI on milk production and SCC in Norwegian dairy cows. A secondary objective was to assess differences in these effects between common ST of *Strep. agalactiae* in Norwegian dairy herds.

MATERIALS AND METHODS

Study Population

We retrieved records from the Norwegian Dairy Herd Recording System (NDHRS) of bacteriological culture and quantitative PCR (qPCR) results from diagnostic

milk samples submitted to the TINE Mastitis Laboratory (Molde, Norway) and the Norwegian Veterinary Institute (Oslo, Norway) between January 2012 and December 2015. These 2 laboratories perform all diagnostics on milk samples in Norway. We extracted records for herds in which *Strep. agalactiae* had been detected either by bacteriological culture of quarter milk samples or by qPCR in cow composite samples (pooled from all 4 quarters in an udder). In larger herds, in particular, farmers tend to prefer submitting cow composite samples for qPCR analysis rather than quarter milk samples for bacteriological culture when they are sampling all lactating cows for control purposes. We did not have records of the sampling method (manual or automatic) for milk samples, but the general recommendation by both laboratories is manual aseptic sampling. We collected test-day records of milk yield and milk composition analyses from the NDHRS for the entire period to compare milk yield and SCC in *Strep. agalactiae*-positive cows with those of other cows in each herd. We also accessed records on calving date, parity, and disease history through the NDHRS.

Diagnostics

PCR Testing of Cow Composite Milk Samples.

Cow composite milk samples were tested using the PathoProof Mastitis PCR assay (Thermo Scientific Finnzymes Diagnostic Products and Services, Espoo, Finland) at the TINE Mastitis Laboratory. Analyses were performed according to the manufacturer's instructions (<http://tools.thermofisher.com/content/sfs/manuals/TS-Micro-MA-Pathoproof-Complete-Assay-IFU-v3.pdf>). The cycle threshold cutoff was set at 37 (i.e., values <37 were considered positive).

Bacteriological Culture of Quarter Milk Samples.

Bacteriological culture was performed by the TINE Mastitis Laboratory and the Norwegian Veterinary Institute as described by the National Mastitis Council (Hogan et al., 1999). When the microbiological diagnosis differed between quarters in the same cow, the cow was assigned more than one diagnosis.

Multilocus Sequence Typing. Multilocus sequence typing was performed previously as described in Jørgensen et al. (2016). Briefly, MLST was determined using a HiMLST protocol with PCR amplification and sequencing of the 7 housekeeping genes *adhP*, *atr*, *glcK*, *glnA*, *pheS*, *sdhA*, and *tkt* (Boers et al., 2012). At least one available isolate from each herd and each source (e.g., bulk tank milk, quarter milk, environmental sample) underwent MLST; MLST was available for 1 to 7 isolates from each herd, and in every case all isolates from the same farm belonged to the same ST. For farms followed over 4 mo, the isolates collected throughout

the period were of the same MLST type (Jørgensen, et al., 2016). Hence, for the purposes of the present study, each *Strep. agalactiae*-positive herd was designated to be positive with one ST.

Variables

Outcome Variables. The primary outcome of interest was monthly test-day milk yield, measured in kilograms of milk per day. The secondary outcome variable was SCC, measured in 1,000 cells/mL of milk. Analysis of milk composition, including SCC, was performed approximately every 2 mo.

Exposure Status. A lactation was defined as exposed if *Strep. agalactiae* was cultured from a quarter milk sample or detected in a cow composite sample by qPCR (as previously described) at 0 to 400 d after calving (DIM). All other lactations were considered unexposed, regardless of whether diagnostic milk samples were taken during the lactation.

Explanatory Variables. Parity was categorized as first, second, and third or greater. We retrieved the average annual herd size each year from 2012 to 2015 from the annual summary tables of the NDHRS, and used the most recent record of herd size. We extracted information on milking system (automatic milking system, pipeline milking, or milking parlor) and barn type (tiestalls or freestalls) from the same source. The main exposure of interest was detection of *Strep. agalactiae* in a lactation; however, we also included detection of any other pathogen to account for confounding effects. We recorded detection of each pathogen as a binary variable at the lactation level, and if detected, we classified the entire lactation as positive. We used only the first detection of each pathogen in the analysis; repeated findings of the same pathogen were not considered. All pathogens isolated by bacteriological culture or identified by qPCR were accounted for; however, bacteriological samples containing mixed flora (≥ 3 different colonies on the plate) were regarded as contaminated and recorded as negative. For *Strep. agalactiae*,

Staphylococcus aureus, *Strep. dysgalactiae*, and *Strep. uberis*, we classified lactations as positive based on bacteriological culture or qPCR. For all other pathogens, we based classification of lactations on bacteriological culture: CNS, *Streptococcus* spp., *Trueperella pyogenes*, *Corynebacterium bovis*, and environmental pathogens (including *Enterococcus* spp., *Escherichia coli*, *Corynebacterium* spp., coliform bacteria, *Enterobacteriaceae*, *Bacillus cereus*, *Bacillus* spp., fungi, *Pseudomonas aeruginosa*, *Pseudomonas* spp., *Proteus* spp., *Serratia* spp., *Klebsiella* spp., *Citrobacter* spp., *Enterobacter* spp., *Aeromonas* spp., *Clostridium perfringens*, and *Pasteurella* spp.).

Indices

Index for *Strep. agalactiae* and Other Pathogens. To allow for a time-dependent effect of diagnosis on test-day milk yield or SCC an index variable was created for each pathogen (or pathogen group). In lactations affected by any pathogen (or pathogen group), the number of days between a positive diagnostic milk sample and each test-day was calculated. Thereafter, test-days were classified according to the number of days before or after diagnosis, as initially described by Gröhn et al. (2004). We created a 16-level index variable: test days (milk yield/SCC) recorded ≥ 210 d, 209 to 180 d, 179 to 150 d, 149 to 120 d, 119 to 90 d, 89 to 60 d, 59 to 31 d, and 30 d to 1 d before diagnosis and 0 to 30 d, 31 to 60 d, 61 to 90 d, 91 to 120 d, 121 to 150 d, 151 to 180 d, 181 to 210 d, and >210 d after diagnosis; an example is shown in Table 1. The index was used to estimate milk production and SCC levels pre- and post-diagnosis. Lactations that did not have a diagnostic sample or did not have the pathogen in question served as the reference category (index 0). Lactations with *Strep. agalactiae* detected late in the lactation provided most of the information on yield and SCC before detection, and lactations with *Strep. agalactiae* detected early in the lactation provided most of the information on yield and SCC after detection.

Table 1. Example of *Streptococcus agalactiae* index for a cow diagnosed at 114 DIM

Test day	DIM	Diagnostic sample date	Days between test day and positive sample	<i>Strep. agalactiae</i> index
October 11, 2014	5	January 28, 2015	-109	5
November 4, 2014	29	January 28, 2015	-85	6
December 2, 2014	57	January 28, 2015	-57	7
January 24, 2015	110	January 28, 2015	-4	8
February 21, 2015	138	January 28, 2015	24	9
March 25, 2015	170	January 28, 2015	56	10
April 25, 2015	201	January 28, 2015	87	11
May 25, 2015	231	January 28, 2015	117	12
June 24, 2015	261	January 28, 2015	147	13

Index for Sequence Type. A corresponding 56 level time-dependent index was made for the ST assessed, the intervals ≥ 180 d, 179 to 150 d, 149 to 120 d, 119 to 90 d, 89 to 60 d, 59 to 31 d and 30 d to 1 d before diagnosis and 0 to 30 d, 31 to 60 d, 61 to 90 d, 91 to 120 d, 121 to 150 d, 151 to 180 d, and >180 d after diagnosis was used for each ST. Levels 1 to 14 included *Strep. agalactiae*-positive cows from herds with ST1; levels 15 to 28 included *Strep. agalactiae*-positive cows from herds with ST103; levels 29 to 42 included *Strep. agalactiae*-positive cows from herds with ST23; and levels 43 to 56 included *Strep. agalactiae*-positive cows from herds with ST196. Index 0 included test days from cows in corresponding herds that did not have a diagnostic sample or did not have *Strep. agalactiae*.

Indices for Veterinary Treatment of Mastitis and Veterinary Treatment of *Strep. agalactiae* Mastitis

Regulations regarding traceability of products meant for human consumption require all farmers in Norway to keep individual animal records of disease and treatment in their herd (Ministry of Agriculture and Food, 2010). Disease events are reported to the NDHRS either directly by the attending veterinarian (93%) or by farmers or advisors (7%; Østerås, 2018). Cases of veterinary-treated mastitis (VTM) were extracted from the NDHRS. Initial standard treatment for mastitis is parenteral administration of one dose of penicillin G combined with 5 d of penicillin G intramammary tubes applied to the affected gland (Norwegian Medicines Agency, 2012; Bruheim et al., 2018). Cases of VTM are recorded as subclinical, mild, or moderate to severe, but we classified a lactation as mastitic regardless of severity (i.e., we did not assess these diagnoses separately). We created an index variable for VTM as described above, but if a cow had several events of VTM in the same lactation, we reset the classification of test-day milk yields for test days succeeding each subsequent mastitis case. If detection and treatment of *Strep. agalactiae* occurred at approximately the same time (e.g., VTM between 5 d before and 15 d after detection of *Strep. agalactiae*), we assumed that treatment was related to the *Strep. agalactiae* diagnosis. In this interval, we disregarded the treatment in the VTM index (i.e., the VTM index was set to 0). These records were included in a separate index to account for treatment of the *Strep. agalactiae* mastitis. The *Strep. agalactiae* treatment index consisted of test days succeeding treatment, and the remaining test days of all cows acted as reference category. Test days after treatment were divided into time periods as described above. The *Strep. agalactiae* index remained unchanged. Whenever treatment occurred outside the defined time period

(i.e., VTM more than 5 d before or 15 d after detection of *Strep. agalactiae*), we assumed that it was related to a separate mastitis episode, and included it in the VTM index. Indices for detection of other pathogens were kept as is.

Statistical Analysis

Milk Yield. We calculated the incidence of first occurrence of *Strep. agalactiae*—the number of *Strep. agalactiae*-positive lactations divided by the total number of lactations—for the main exposures and selected cow and herd characteristics. We fitted a linear mixed model to study the effect of *Strep. agalactiae* on monthly test-day milk yields. Parity and year were included as fixed effects and herd, cow, and lactation were included as random effects. Maximum likelihood was used for estimation. The random effects were applied to account for dependency between test days within the same lactation, lactations within the same cow, and cows within the same herd. A first-order autoregressive covariance matrix at the test-day level was used to account for higher correlation in adjacent test days than in test days further apart. Only test-day milk yields up to 305 DIM were included in the statistical analysis. The lactation curve was modeled by including DIM and the natural logarithm of DIM in the model (Wilmink, 1987). A second model, incorporating the ST index instead of the *Strep. agalactiae* index as the exposure, was fit to a subset of herds in which the ST1, ST23, ST103, and ST196 of *Strep. agalactiae* had been found, to compare their effect on milk production (hereafter called the ST model). We explored the natural logarithm of SCC (lnSCC) as additional explanatory variables in both the general effect model and the ST model. Estimates are presented as kilograms of milk per day [95% confidence interval (CI)]. The full models were fit after assessment of causal diagrams, aiming to identify possible confounders for the effect of *Strep. agalactiae* on test-day milk yield. We assessed the overall significance of categorical variables by likelihood ratio tests. We evaluated any confounding effect of VTM, treatment of *Strep. agalactiae* mastitis, or indices of other pathogens (or pathogen group) by monitoring the estimates for the exposure of interest (*Strep. agalactiae*) as the potential confounders were included or removed from the multivariable model.

Somatic Cell Count. The effect of *Strep. agalactiae* on SCC was assessed by using a multilevel linear regression as described in the section above. A second model, incorporating the ST index instead of the *Strep. agalactiae* index as the exposure, was fit to a subset of herds in which the ST1, ST23, ST103, and ST196 of *Strep. agalactiae* had been found, to compare the effect

of sequence type on SCC. Estimates are presented as $\ln\text{SCC} \times 1,000$ cells/mL (95% CI) in tables and figures and as $\text{SCC} \times 1,000$ cells/mL in the text.

Model Assessment. Residuals at all levels were assessed by using normality plots, and plotted against predicted values to evaluate the assumption of homoscedasticity; no major shortcomings were detected. Data management and statistical analyses were performed using Stata (StataSE/14; Stata Corp., College Station, TX).

RESULTS

Descriptive Statistics

Eligible test days, exclusion criteria, and the number of test-day records used in the different analyses are depicted in Figure 1. The dairy company (TINE SA) does not accept milk from cows that yield less than 7 kg/d, and test-day milk yield records >55 kg/d are normally considered errors, so only records >7 and <55 kg/d were included. The study sample consisted of 150 herds, 15,757 cows, 30,850 lactations, and 204,126 test days. The number of test days used in the different analyses varied due to missing observations for milk yield (milk yield model); SCC (SCC model); ST other than 1, 23, 103, or 196 (ST yield model); and ST other than 1, 23, 103, or 196 plus missing SCC records (ST SCC model). The average herd size was 41.0 cows (SD: 23.8). The distribution of DIM at the time of first diagnosis of *Strep. agalactiae* and *Strep. agalactiae* ST1, ST23, ST103, and ST196 in each lactation are shown in Figures 2 and 3, respectively. The number of *Strep. agalactiae*-positive lactations and the incidence of first occurrence of *Strep. agalactiae* for the outcome and selected explanatory variables are shown in Table 2. The mean number of test-day yields per lactation was 6.6 (range 1–12). The mean number of test days containing information on SCC was 4.5 per lactation (range 1–12). Of the 30,850 lactations in the study sample, 11,415 had at least one diagnostic sample (bacteriological culture or qPCR) and 19,435 had no diagnostic sample. Overall, 70% of the sampled lactations had only one diagnostic sample. *Streptococcus agalactiae* was detected in 1,517 lactations, of which 17% had more than one positive sample. In total, 32% *Strep. agalactiae*-positive lactations were treated close to the time of (first) diagnosis (i.e., in the interval 5 d before to 15 d after diagnosis), and 21% were treated at dry-off. In comparison, 7% of *Strep. agalactiae*-negative lactations were treated at dry-off. At least one VTM was registered in 13% of *Strep. agalactiae*-negative lactations and 15% of *Strep. agalactiae*-positive lactations, excluding treatments close to the *Strep. agalactiae* diagnosis. In total, 17,769

diagnostic milk samples were submitted between 2012 and 2015. The distribution of pathogens in the milk samples is shown in Table 3.

Multivariable Models

Herd-level variables (milking system, barn type, and herd size) did not affect test-day milk yield or SCC. The indices for detection of *Staphylococcus aureus*, *Strep. dysgalactiae*, and *Strep. uberis*—as well as the VTM index and the treatment index for *Strep. agalactiae* mastitis—were included in the final models, because their removal resulted in a $>20\%$ change in the effect estimates for *Strep. agalactiae* IMI. To keep the most parsimonious model to answer our question, we removed the indices for environmental pathogens, *Streptococcus* spp., *Corynebacterium bovis*, and CNS despite their significant effect on model fit. Their removal resulted in only minor changes in the effect estimates for *Strep. agalactiae* IMI. The final models can be found in Supplemental Tables S1 to S6 (<https://doi.org/10.3168/jds.2018-16155>).

Milk Yield. The effects of *Strep. agalactiae* on milk yield presented in the current section are from the model that did not include $\ln\text{SCC}$ as an explanatory variable. Estimates were adjusted for the effect of treatment. Compared with *Strep. agalactiae*-negative cows, the *Strep. agalactiae*-positive cows produced an average of 1.31 kg more DIM-adjusted milk/d (95% CI: 0.95–1.68) in the 3 to 2 mo before diagnosis. At the time of diagnosis (0 to 1 mo after) *Strep. agalactiae*-positive cows produced an average of 0.13 kg less DIM-adjusted milk/day (95% CI: -0.49 to 0.23) than *Strep. agalactiae*-negative cows. In the period 2 to 3 mo after diagnosis, *Strep. agalactiae*-positive cows produced 1.24 kg less DIM-adjusted milk/d (95% CI: -1.74 to -0.74) than *Strep. agalactiae*-negative cows. Milk production remained lower for *Strep. agalactiae*-positive cows than for *Strep. agalactiae*-negative cows for the rest of the investigation period (Figure 4, Supplemental Table S1; <https://doi.org/10.3168/jds.2018-16155>). Cows with ST23, ST103, and ST196 followed the same pattern as the overall analysis with respect to milk production, but ST1-affected cows produced similar amounts of milk before diagnosis as negative cows. Cows with ST1 experienced the largest milk loss 1 to 2 mo after diagnosis and recovered thereafter to some extent, but for cows with ST103, the severe milk loss persisted for the rest of the investigation period (Figure 5, Supplemental Table S2; <https://doi.org/10.3168/jds.2018-16155>). Treatment of *Strep. agalactiae* mastitis influenced the recovery of milk production. In the general effects model, cows treated close to the time of diagnosis produced an average of 0.8 kg milk/d more in the

period 1 to 2 mo until 4 to 5 mo after treatment than cows left untreated (Figure 6, Supplemental Table S1). Corresponding estimates from the ST model were 1.4, 0.6, 1.1, and 3.4 kg milk/d for ST1, ST103, ST23, and ST196, respectively (Figure 7, Supplemental Table S2).

When explored as an additional explanatory variable, $\ln\text{SCC}$ was a significant factor affecting milk yield in both the general effect model and the ST model ($P < 0.001$, Supplemental Tables S3 and S4; <https://doi.org/10.3168/jds.2018-16155>). For each unit increase in

Figure 1. Flowchart of eligible test days, reasons for exclusions, and test days used in the different analyses.

lnSCC, milk yield decreased by approximately 0.5 kg ($\beta -0.52$, 95% CI: -0.54 to -0.50 ; and $\beta 0.50$, 95% CI: -0.54 to -0.47).

Somatic Cell Count. The estimated baseline SCC ($\times 1,000$ cells/mL) for *Strep. agalactiae*-negative cows was 183 (95% CI: 173–194). For *Strep. agalactiae*-positive cows, SCC increased before diagnosis. In the period 3 to 2 mo before diagnosis, the estimated SCC for *Strep. agalactiae*-positive cows was 260 (95% CI: 226–300). The peak of SCC occurred the month before diagnosis (364; 95% CI: 319–416). At peak SCC, the deviation from the *Strep. agalactiae*-negative cows was $181 \times 1,000$ cells/mL. For the rest of the investigation period, SCC tended to be higher for *Strep. agalactiae*-positive cows than for unexposed cows (Figure 8 and Supplemental Table S5; <https://doi.org/10.3168/jds.2018-16155>). All estimates presented are adjusted for the effect of treatment. The 4 ST of *Strep. agalactiae* generally followed the same pattern as the overall effect, with peak SCC the month before diagnosis. In contrast to cows infected with other ST, cows affected with ST103 did not have as pronounced a peak in SCC, and SCC levels persisted for an additional month before gradually decreasing (Figure 9, Supplemental Table S6; <https://doi.org/10.3168/jds.2018-16155>). The mean peak estimate for SCC ST103 ($\times 1,000$ cells/mL) was 330 (95% CI: 235–390), whereas the mean peak estimates for ST1, ST23, and ST196 were 371 (95% CI: 287–481), 510 (95% CI: 300–867) and 492 (95% CI:

350–691). The SCC in milk decreased after treatment. The average SCC from 0 to 1 mo until 2 to 3 mo after treatment was $152 \times 1,000$ cells/mL lower than in cows left untreated (Figure 10, Supplemental Table S5). Corresponding estimates from the ST model were 94, 193, 40, and $160 \times 1,000$ cells/mL for ST1, ST103, ST23, and ST196, respectively (Figure 11, Supplemental Table S6).

DISCUSSION

This study estimated the loss in milk production associated with *Strep. agalactiae* IMI. We also investigated milk production loss associated with common ST of *Strep. agalactiae* found in Norwegian dairy herds. In general, cows that were diagnosed later with *Strep. agalactiae* IMI produced more milk before diagnosis and less milk after diagnosis than their uninfected herd mates. A similar pattern has been observed for other agents causing IMI (Gröhn et al., 2004; Hertl et al., 2014). Also at the herd level, high milk production is associated with increased incidence of clinical mastitis (Schukken et al., 1990; Waage et al., 1998; O'Reilly et al., 2006). We found that cows infected with *Strep. agalactiae* in general produced 1.3 kg more DIM-adjusted milk/d more before diagnosis and 1.2 kg less DIM-adjusted milk/d after diagnosis, indicating that infected cows produced roughly 2.5 kg milk/d less after diagnosis than before. Milk production loss was

Figure 2. Distribution of DIM at first detection of *Streptococcus agalactiae* in 1,517 lactations.

Table 2. Total number of lactations (N), number of *Streptococcus agalactiae*-positive lactations (N pos.), and incidence of first occurrence of *Strep. agalactiae* for the main exposures and selected cow and herd characteristics

Item	N	N pos.	Lactational incidence
Overall	30,850	1,517	4.9
Sequence type			
ST1	3,182	329	10.3
ST23	1,746	66	3.8
ST103	4,593	338	7.4
ST196	2,615	123	4.7
Parity			
1	11,701	448	3.8
2	8,275	447	5.4
3 or greater	10,874	622	5.7
Season of calving			
December–February	6,966	404	5.8
March–May	5,449	298	5.5
June–August	8,094	352	4.4
October–November	10,341	463	4.5
Barn type			
Freestall (94 herds)	24,988	1,397	5.6
Tiestall (55 herds)	5,777	119	2.1
Missing (1 herd)	85	1	1.2
Milking system			
Automatic milking system (69 herds)	19,822	1,297	6.5
Milking parlor (24 herds)	4,836	99	2.1
Pipeline (55 herds)	5,777	119	2.1
Missing (2 herds)	415	2	0.48

detected as early as 2 to 1 mo before diagnosis. *Streptococcus agalactiae* primarily causes subclinical IMI, and the decrease in milk production before diagnosis may

reflect a delay in diagnosis in most cows. The milk loss associated with *Strep. agalactiae* IMI was considerably lower than reported for gram-negative pathogens *E. coli*

Figure 3. Distribution of DIM at first detection of *Streptococcus agalactiae* sequence type (ST)1, ST23, ST103, and ST196 in 329, 66, 338, and 123 lactations, respectively.

Table 3. Distribution of pathogens detected in quarter milk samples by bacteriology and cow composite samples and by PCR, 2012 to 2015

Pathogen	No.	%
Bacteriology (6,284 cows sampled)		
Negative	13,965	55.6
CNS	2,662	10.6
Mixed flora (contaminated)	2,560	10.2
<i>Staphylococcus aureus</i>	1,412	5.6
<i>Corynebacterium bovis</i>	926	3.7
<i>Streptococcus dysgalactiae</i>	731	2.9
<i>Streptococcus agalactiae</i>	589	2.3
<i>Streptococcus uberis</i>	491	2.0
<i>Trueperella pyogenes</i>	54	0.2
<i>Streptococcus</i> spp.	176	0.7
Environmental ¹	1,119	4.5
Growth inhibitor	13	0.1
Missing (empty or broken glass)	438	1.7
Total, quarter milk samples	25,136	100
PCR (11,485 cows sampled)		
Negative	7,563	61.0
<i>Streptococcus dysgalactiae</i>	1,693	13.7
<i>Streptococcus agalactiae</i>	1,410	11.4
<i>Staphylococcus aureus</i>	1,341	10.8
<i>Streptococcus uberis</i>	394	3.2
Total, cow composite samples	12,401	100

¹Includes *Enterococcus* spp., *Escherichia coli*, *Corynebacterium* spp., coliform bacteria, *Enterobacteriaceae*, *Bacillus cereus*, *Bacillus* spp., fungi, *Pseudomonas aeruginosa*, *Pseudomonas* spp., *Proteus* spp., *Serratia* spp., *Klebsiella* spp., *Citrobacter* spp., *Enterobacter* spp., *Aeromonas* spp., *Clostridium perfringens*, *Pasteurella* spp.

and *Klebsiella* spp., and for gram-positives *Staphylococcus aureus* and *Staphylococcus* spp. in Holstein cows with clinical mastitis, but comparable to losses caused by other *Streptococcus* spp. IMI (Gröhn et al., 2004).

The ST assessed in this study coincided with those described as the major ST in a Finnish–Swedish study (Lyhs et al., 2016). The authors of that study concluded that ST103 was the most common cow-associated ST, and ST1 (followed by ST23 and ST196) was the most common ST shared by humans and cows (Lyhs et al., 2016). We found little difference between ST in their effect on milk production assessed by overlapping CI. However, the study sample consisted of few lactations per ST and the estimates for ST23 and ST196 in particular were based on a small number of lactations, as reflected in the wide confidence intervals around the estimates of milk yield and SCC (i.e., the estimates were less precise). An increased number of *Strep. agalactiae*-positive lactations per ST would have increased the power of this study (i.e., the ability to detect differences if they exist).

The increase in SCC for *Strep. agalactiae*-positive cows (generic) starts 5 to 4 mo before diagnosis, suggesting that the pathogen is present for some time before diagnosis is made. The peak estimate of SCC we observed was comparable to estimates for other minor and major IMI pathogens reported from Nordic countries in cows with subclinical mastitis (Reksen et al.,

2008; Nyman et al., 2018). However, the SCC estimates in the present study were lower than those reported from the Netherlands in cows with clinical mastitis (de Haas et al., 2002). This is consistent with current understanding of the pathophysiology of mastitis. The peak SCC was lower in the cow-associated strain (ST103) than in the ST shared by humans and cows. This may be an example of a host–pathogen relationship favoring in situ strain survival by eliciting a more moderate immune response in the host-associated strain than in the shared strains. Strain survival in the host may also partly explain the more pronounced effect of ST103 on milk yield.

About 20% of first diagnoses of *Strep. agalactiae* occurred within the first 30 d of lactation, followed by an even distribution of DIM for first diagnosis throughout lactation for the rest. This pattern differed from the traditional epidemiology of *Strep. agalactiae*, in which increased risk is associated with milking (DIM) (Keefe, 2012). This could indicate that *Strep. agalactiae* shows opportunistic behavior (i.e., a combination of “contagious” and “environmental” modes) and coincides well with the contagious and environmental (oro–fecal) transmission cycles proposed by Jørgensen et al. (2016).

Methodological Considerations

Exposure status in this study was determined by detection of *Strep. agalactiae* using a qPCR test in a cow composite milk sample or by bacteriological culture of quarter milk samples. Although the specificity of bacteriological culture is close to 100, the somewhat lower specificity of qPCR may result in false-positive samples (Mahmmod et al., 2013; Holmøy et al., 2018). Moreover, qPCR detects *Strep. agalactiae* DNA, so it does not distinguish between live and dead bacteria. The general recommendation by the TINE Mastitis Laboratory and the Norwegian Veterinary Institute is a manual aseptic sampling. However, we did not have records indicating sampling methods and thus could not assess the risk of false-positive samples due to carryover effects or environmental contamination (Mahmmod et al., 2014). It is therefore uncertain whether a positive qPCR test always reflects an IMI. The median estimates of sensitivity for bacteriological culture (for *Strep. agalactiae*) using latent class analysis lie between 30 and 72 depending on the sample material and the cutoffs used for the comparison test (Mahmmod et al., 2013; Holmøy et al., 2018). The imperfect sensitivity of bacteriological culture leads to false-negative results (i.e., some misclassification among tested animals in the *Strep. agalactiae*-negative group). However, a more important source of misclassification error in this group is lack of diagnostic samples, because most of the lac-

Figure 4. Estimates and 95% CI for the effect of *Streptococcus agalactiae* diagnosis (all sequence types) on test-day milk yield records. Values show the months before (BD) and after (AD) diagnosis. Estimates were obtained from a mixed model with a first-order autoregressive covariance structure (Supplemental Table S1; <https://doi.org/10.3168/jds.2018-16155>). The number of test days at time point 0–1 after diagnosis was 1,238.

Figure 5. Estimates and 95% CI for the effect of *Streptococcus agalactiae* sequence type (ST)1, ST23, ST103, and ST196 on test-day milk yield records. Values show the months before diagnosis (BD) and after diagnosis (AD). Estimates were obtained from a mixed model with a first-order autoregressive covariance structure (Supplemental Table S2; <https://doi.org/10.3168/jds.2018-16155>). The numbers of test days at time point 0–1 after diagnosis were 289 for ST1, 67 for ST23, 283 for ST103, and 86 for ST196.

Figure 6. Estimates and 95% CI (test-day milk yield) for treated cows with *Streptococcus agalactiae* (all sequence types). The estimates are additive to what is shown in Figure 4. Values show the months after diagnosis (AD). Estimates were obtained from a mixed model with a first-order autoregressive covariance structure (Supplemental Table S1; <https://doi.org/10.3168/jds.2018-16155>). The number of test days at time point 0–1 after diagnosis was 492.

Figure 7. Estimates and 95% CI (test-day milk yield) for treated cows with *Streptococcus agalactiae* sequence type (ST)1, ST23, ST103, and ST196. The estimates are additive to what is shown in Figure 5. Values show the months after diagnosis (AD). Estimates were obtained from a mixed model with a first-order autoregressive covariance structure (Supplemental Table S2; <https://doi.org/10.3168/jds.2018-16155>). The numbers of test days at time point 1–2 after diagnosis were 94 for ST1, 51 for ST103, 11 for ST23, and 27 for ST196.

Figure 8. Estimates and 95% CI for the effect of *Streptococcus agalactiae* diagnosis (all sequence types) on test-day natural logarithm of SCC (LnSCC). Values show the months before diagnosis (BD) and after diagnosis (AD). Estimates were obtained from a mixed model with a first-order autoregressive covariance structure (Supplemental Table S5; <https://doi.org/10.3168/jds.2018-16155>). The number of test days at time point 0–1 after diagnosis was 966.

Figure 9. Estimates and 95% CI for the effect of *Streptococcus agalactiae* sequence type (ST)1, ST23, ST103, and ST196 on test-day natural logarithm of SCC (LnSCC). Values show the months before diagnosis (BD) and after diagnosis (AD). Estimates were obtained from a mixed model with a first-order autoregressive covariance structure (Supplemental Table S6; <https://doi.org/10.3168/jds.2018-16155>). The numbers of test days at time point 0–1 after diagnosis were 215 for ST1, 52 for ST23, 258 for ST103, and 68 for ST196.

Figure 10. Estimates and 95% CI (natural logarithm of SCC; lnSCC) for treated cows with *Streptococcus agalactiae* (all sequence types). The estimates are additive to what is shown in Figure 8. Values show the months after diagnosis (AD). Estimates were obtained from a mixed model with a first-order autoregressive covariance structure (Supplemental Table S5; <https://doi.org/10.3168/jds.2018-16155>). The number of test days at time point 0–1 after diagnosis was 388.

Figure 11. Estimates and 95% CI (natural logarithm of SCC; lnSCC) for treated cows with *Streptococcus agalactiae* sequence type (ST) 1, ST23, ST103, and ST196. The estimates are additive to what is shown in Figure 9. Values show the months after diagnosis (AD). Estimates were obtained from a mixed model with a first-order autoregressive covariance structure (Supplemental Table S6; <https://doi.org/10.3168/jds.2018-16155>). The numbers of test days at time point 1–2 after diagnosis were 75 for ST1, 43 for ST103, 8 for ST23, and 22 for ST196.

tations classified as negative had no diagnostic milk sample. Altogether, the sources of misclassification were nondifferential (not related to test-day milk yield or SCC), leading to an expected underestimation of effect (bias toward the null).

We assigned *Strep. agalactiae* ST to positive cows (lactations) in a herd based on a herd-level diagnosis of 1 to 7 *Strep. agalactiae* isolates per herd, which creates some uncertainty of the ST in each cow. However, the isolates from each herd were of different origins and included samples from floors, milking parlors, and water troughs in addition to milk samples. All isolates from the same herd belonged to the same ST (Jørgensen et al., 2016). A single predominating ST in each herd, indicative of a point source of infection, has also been reported from Denmark (Mahmmod et al., 2015). A more important issue rising from ST herd-level diagnosis is that it is difficult to untangle the effect of being an *Strep. agalactiae* affected cow in a specific herd from the “real” ST effect.

Despite the recommendation that all lactating cows should be sampled once *Strep. agalactiae* is detected in a milk sample, we found low compliance for some herds. Including only cows with a diagnostic sample could lead to selection bias if cows were sampled for a reason and hence were not representative of all cows in the herd. By including all lactating cows, regardless of whether a diagnostic milk sample was present, we sought to minimize selection bias at the cow level, but at the cost of larger chance of misclassification bias in the unexposed group.

Norwegian cattle health services recommend treatment of *Strep. agalactiae* cows as soon as possible after diagnosis as part of a control and eradication plan (TINE, 2016). However, this does not seem to be common practice. Treatment close to the time of diagnosis occurred in approximately one-third of *Strep. agalactiae*-positive lactations. The decision to treat (or not) is made by the veterinarian and the farmer, and most likely include considerations of prognostic factors. Recovery of milk production after treatment also depends on factors such as clinical status, how soon after infection diagnosis was made and treatment initialized, and previous cases of mastitis, among others. The general trend for cows that were treated for *Strep. agalactiae* mastitis was that they lost less milk and had lower SCC after treatment than cows that were left untreated. However, the effect of treatment was beyond the scope of this paper, and because treatment was carried out in a select group of cows, estimates were prone to bias and will not be discussed further.

Only a very small proportion (~2%) of Norwegian dairy herds was diagnosed with *Strep. agalactiae* from January 2012 to December 2015. The average herd

size in the study sample was larger than the overall average in Norway in 2015: 41 (range 9–120) and 25 cows, respectively (Østerås, 2018). Two-thirds of the holdings were free stalls, and 75% of the loose-housed systems used automatic milking systems. Overall, 30% of Norwegian herds are housed in free stalls. Thus, the herds in our sample were on average larger and more often housed in a free stall system compared with the average Norwegian herd.

In conclusion, our results indicate an evident effect of *Strep. agalactiae* on milk production and SCC. Production was lowest 2 to 3 mo after a positive sample. Peak SCC was reached the month before diagnosis, with notable differences between ST.

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REFERENCES

- Boers, S. A., W. A. van der Reijden, and R. Jansen. 2012. High-throughput multilocus sequence typing: Bringing molecular typing to the next level. *PLoS One* 7:e39630. <https://doi.org/10.1371/journal.pone.0039630>.
- Bruheim, J., H. M. N. Sleveland, G. Dalen, M. Bangen, and O. Reksten. 2018. Rapportering av utvalgte intramammariet til Veterinært legemiddelregister. Validering mot salgstall. *Nor. Vet. Tidsskr.* 130:78–84. [In Norwegian]
- de Haas, Y., H. W. Barkema, and R. F. Veerkamp. 2002. The effect of pathogen-specific clinical mastitis on the lactation curve for somatic cell count. *J. Dairy Sci.* 85:1314–1323. [https://doi.org/10.3168/jds.S0022-0302\(02\)74196-9](https://doi.org/10.3168/jds.S0022-0302(02)74196-9).
- Gröhn, Y. T., D. J. Wilson, R. N. González, J. A. Hertl, H. Schulte, G. Bennett, and Y. H. Schukken. 2004. Effect of pathogen-specific clinical mastitis on milk yield in dairy cows. *J. Dairy Sci.* 87:3358–3374. [https://doi.org/10.3168/jds.S0022-0302\(04\)73472-4](https://doi.org/10.3168/jds.S0022-0302(04)73472-4).
- Halasa, T., M. Nielsen, A. P. W. De Roos, R. Van Hoorne, G. de Jong, T. J. G. M. Lam, T. van Werven, and H. Hogeveen. 2009. Production loss due to new subclinical mastitis in Dutch dairy cows estimated with a test-day model. *J. Dairy Sci.* 92:599–606. <https://doi.org/10.3168/jds.2008-1564>.
- Hand, K. J., A. Godkin, and D. F. Kelton. 2012. Milk production and somatic cell counts: A cow-level analysis. *J. Dairy Sci.* 95:1358–1362. <https://doi.org/10.3168/jds.2011-4927>.
- Heikkilä, A. M., E. Liski, S. Pyörälä, and S. Taponen. 2018. Pathogen-specific production losses in bovine mastitis. *J. Dairy Sci.* 101:9493–9504. <https://doi.org/10.3168/jds.2018-14824>.
- Hertl, J. A., Y. H. Schukken, F. L. Welcome, L. W. Tauer, and Y. T. Gröhn. 2014. Pathogen-specific effects on milk yield in repeated

- clinical mastitis episodes in Holstein dairy cows. *J. Dairy Sci.* 97:1465–1480. <https://doi.org/10.3168/jds.2013-7266>.
- Hogan, J. S., R. N. González, R. J. Harmon, S. C. Nickerson, S. P. Oliver, J. W. Pankey, and K. L. Smith. 1999. Laboratory Handbook on Bovine Mastitis. Rev. ed. Natl. Mastitis Council, Inc., Verona, WI.
- Holmøy, I. H., N. Toft, H. J. Jørgensen, T. Mørk, L. Sølvørød, and A. Nødtvedt. 2018. Latent class analysis of real time qPCR and bacteriological culturing for the diagnosis of *Streptococcus agalactiae* in cow composite milk samples. *Prev. Vet. Med.* 154:119–123. <https://doi.org/10.1016/j.prevetmed.2018.03.019>.
- Hortet, P., F. Beaudeau, H. Seegers, and C. Fourichon. 1999. Reduction in milk yield associated with somatic cell counts up to 600 000 cells/ml in French Holstein cows without clinical mastitis. *Livest. Prod. Sci.* 61:33–42. [https://doi.org/10.1016/S0301-6226\(99\)00051-2](https://doi.org/10.1016/S0301-6226(99)00051-2).
- Jørgensen, H. J., A. B. Nordstoga, S. Sviland, R. N. Zadoks, L. Sølvørød, B. Kvitle, and T. Mørk. 2016. *Streptococcus agalactiae* in the environment of bovine dairy herds — Rewriting the textbooks? *Vet. Microbiol.* 184:64–72. <https://doi.org/10.1016/j.vetmic.2015.12.014>.
- Keefe, G. 2012. Update on control of *Staphylococcus aureus* and *Streptococcus agalactiae* for management of mastitis. *Vet. Clin. North Am. Food Anim. Pract.* 28:203–216.
- Keefe, G. P. 1997. *Streptococcus agalactiae* mastitis: A review. *Can. Vet. J.* 38:429–437.
- Lyhs, U., L. Kulkas, J. Katholm, K. P. Waller, K. Saha, R. J. Tomusk, and R. N. Zadoks. 2016. *Streptococcus agalactiae* serotype IV in humans and cattle, Northern Europe. *Emerg. Infect. Dis.* 22:2097–2103. <https://doi.org/10.3201/eid2212.151447>.
- Mahmmod, Y. S., I. C. Klaas, J. Katholm, M. Lutton, and R. N. Zadoks. 2015. Molecular epidemiology and strain-specific characteristics of *Streptococcus agalactiae* at the herd and cow level. *J. Dairy Sci.* 98:6913–6924. <https://doi.org/10.3168/jds.2015-9397>.
- Mahmmod, Y. S., M. M. Mweu, S. S. Nielsen, J. Katholm, and I. C. Klaas. 2014. Effect of carryover and presampling procedures on the results of real-time PCR used for diagnosis of bovine intramammary infections with *Streptococcus agalactiae* at routine milk recordings. *Prev. Vet. Med.* 113:512–521. <https://doi.org/10.1016/j.prevetmed.2014.01.002>.
- Mahmmod, Y. S., N. Toft, J. Katholm, C. Grønbaek, and I. C. Klaas. 2013. Estimation of test characteristics of real-time PCR and bacterial culture for diagnosis of subclinical intramammary infections with *Streptococcus agalactiae* in Danish dairy cattle in 2012 using latent class analysis. *Prev. Vet. Med.* 109:264–270. <https://doi.org/10.1016/j.prevetmed.2012.10.018>.
- Ministry of Agriculture and Food. 2010. FOR-2010-07-09-1131. Forskrift om sporbarhet og merking av storfe og storfekjøtt §21. Ministry of Agriculture and Food, Oslo, Norway. Accessed February 2018. <https://lovdata.no/SF/forskrift/2010-07-09-1131/>. [In Norwegian]
- Norwegian Medicines Agency. 2012. Bruk av antibakterielle midler til storfe. Pages 37–39 in Terapianbefaling: Bruk av antibakterielle midler til produksjonsdyr. Norwegian Medicines Agency, Oslo, Norway. Accessed February 2019. https://legemiddelverket.no/Documents/Veterin%C3%A6rmedisin/Terapianbefalinger/Terapianbefaling_bruk%20av%20antibakteriell%20midler%20til%20produks.pdf. [In Norwegian]
- Nyman, A. K., C. Fasth, and K. P. Waller. 2018. Intramammary infections with different non-aureus staphylococci in dairy cows. *J. Dairy Sci.* 101:1403–1418. <https://doi.org/10.3168/jds.2017-13467>.
- O'Reilly, K. M., M. J. Green, E. J. Peeler, J. L. Fitzpatrick, and L. E. Green. 2006. Investigation of risk factors for clinical mastitis in British dairy herds with bulk milk somatic cell counts less than 150,000 cells/ml. *Vet. Rec.* 158:649–653. <https://doi.org/10.1136/vr.158.19.649>.
- Østerås, O. 2018. Årsrapport helsekortordningen 2017, TINE SA. TINE SA, Oslo, Norway. Accessed January 2018. <https://storfehelse.tine.no/om-oss/%C3%A5rsmeldinger-og-rapporter/%C3%A5rsrapporter-for-helsekortordningen>. [In Norwegian]
- Østerås, O., and L. Sølvørød. 2011. *Streptococcus agalactiae* — et økende problem i store besetninger som må tas alvorlig. *Nor. Vet. Tidsskr.* 123:519–520. [In Norwegian]
- Rajala-Schultz, P. J., Y. T. Gröhn, C. E. McCulloch, and C. L. Guard. 1999. Effects of clinical mastitis on milk yield in dairy cows. *J. Dairy Sci.* 82:1213–1220. [https://doi.org/10.3168/jds.S0022-0302\(99\)75344-0](https://doi.org/10.3168/jds.S0022-0302(99)75344-0).
- Reksen, O., L. Sølvørød, and O. Østerås. 2008. Relationships between milk culture results and composite milk somatic cell counts in Norwegian dairy cattle. *J. Dairy Sci.* 91:3102–3113. <https://doi.org/10.3168/jds.2008-1006>.
- Schepers, A. J., T. J. Lam, Y. H. Schukken, J. B. Wilmink, and W. J. Hanekamp. 1997. Estimation of variance components for somatic cell counts to determine thresholds for uninfected quarters. *J. Dairy Sci.* 80:1833–1840. [https://doi.org/10.3168/jds.S0022-0302\(97\)76118-6](https://doi.org/10.3168/jds.S0022-0302(97)76118-6).
- Schukken, Y. H., F. J. Grommers, D. Van De Geer, H. N. Erb, and A. Brand. 1990. Risk factors for clinical mastitis in herds with a low bulk milk somatic cell count. 1. Data and risk factors for all cases. *J. Dairy Sci.* 73:3463–3471. [https://doi.org/10.3168/jds.S0022-0302\(90\)79045-5](https://doi.org/10.3168/jds.S0022-0302(90)79045-5).
- Sølvørød, L., K. P. Forshell, and A. C. Whist. 2009. Forekomst og risiko for spredning femten år etter at offentlig kontrollprogram for smittsom mastitt ble avsluttet. Pages 469–472 in Husdyrforsøksmøtet, Ås, Norway. <http://www.umb.no/statisk/husdyrforsoksmoter/2009/121.pdf>. [In Norwegian]
- TINE. 2016. Guidelines regarding *Streptococcus agalactiae*. TINE SA, Oslo, Norway. Accessed January 2019. <http://storfehelse.tine.no/jurhelse/mastittbakterier/streptococcus-agalactiae>.
- Waage, S., S. Sviland, and S. A. Ødegaard. 1998. Identification of risk factors for clinical mastitis in dairy heifers. *J. Dairy Sci.* 81:1275–1284. [https://doi.org/10.3168/jds.S0022-0302\(98\)75689-9](https://doi.org/10.3168/jds.S0022-0302(98)75689-9).
- Wilmink, J. B. M. 1987. Adjustment of test-day milk, fat and protein yield for age, season and stage of lactation. *Livest. Prod. Sci.* 16:335–348. [https://doi.org/10.1016/0301-6226\(87\)90003-0](https://doi.org/10.1016/0301-6226(87)90003-0).
- Wilson, D. J., R. N. González, J. Hertl, H. F. Schulte, G. J. Bennett, Y. H. Schukken, and Y. T. Gröhn. 2004. Effect of clinical mastitis on the lactation curve: A mixed model estimation using daily milk weights. *J. Dairy Sci.* 87:2073–2084. [https://doi.org/10.3168/jds.S0022-0302\(04\)70025-9](https://doi.org/10.3168/jds.S0022-0302(04)70025-9).
- Zadoks, R. N., H. G. Allore, H. W. Barkema, O. C. Sampimon, Y. T. Gröhn, and Y. H. Schukken. 2001. Analysis of an outbreak of *Streptococcus uberis* mastitis. *J. Dairy Sci.* 84:590–599. [https://doi.org/10.3168/jds.S0022-0302\(01\)74512-2](https://doi.org/10.3168/jds.S0022-0302(01)74512-2).
- Zadoks, R. N., B. E. Gillespie, H. W. Barkema, O. C. Sampimon, S. P. Oliver, and Y. H. Schukken. 2003. Clinical, epidemiological and molecular characteristics of *Streptococcus uberis* infections in dairy herds. *Epidemiol. Infect.* 130:335–349. <https://doi.org/10.1017/S0950268802008221>.