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Large-scale cross-sectional study of relationships between somatic cell count and milking-time test results in different milking systems

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ABSTRACT

Milking-time testing (MTT) is a method for evaluating the vacuum conditions in the teatcup during milking. The purpose is to evaluate the possible impact of the milking and milking equipment on udder health and milk quality. The method is commonly implemented by herd health advisory services, but results are interpreted empirically due to lack of scientific documentation on relationships between MTT result variables and objective measures of udder health.

The current study was conducted to increase our understanding of associations between cow-level differences in composite milk somatic cell count (CMSCC) and MTT results in dairy cows milked in 3 different milking systems; automatic milking systems (AMS), milking parlors, and pipeline milking systems. Data from 7069 cows (predominantly Norwegian Red breed) in 1009 herds were used in a cross-sectional study. Multilevel linear regression models with a random intercept at herd level were used to describe relationships between CMSCC (on logarithmic scale) and the following MTT explanatory variables: average vacuum level in the short milk tube and mouthpiece chamber in the main milking and overmilking periods, the duration of these two periods, and vacuum stability, measured by sudden vacuum drops in the short milk tube. The models were corrected for the herd effect, mastitis history and differences in milk yield, lactation stage and parity between cows. Separate models were run for AMS, milking parlors, and pipeline milking systems, because this approach allowed for comparison between systems and for evaluation of the herd effect independently of milking system.

The models described 8–10 % of the variation in CMSCC, indicating that MTT could only explain a relatively small proportion of a large total variation in CMSCC. In most observations, vacuum levels in the short milk tube during main milking were within the range recommended by the International Organization for Standardization. The results from our multivariable models showed decreasing CMSCC with increasing vacuum level in the short milk tube during the main milking period in AMS and milking parlors. Similarly, decreasing CMSCC was also associated with increasing duration of the main milking period in all 3 systems. These relationships are important for the interpretation of MTT results under practical conditions; finding high vacuum levels and long milking durations in a MTT is not associated with elevated CMSCC. In AMS herds, we also found indications that the relationships were different for cows where a case of mastitis had been treated before the MTT.

1. Introduction

Somatic cell count (SCC) is a widely used indicator of milk quality and udder health (Schukken et al., 2003), and composite milk SCC (CMSCC) is used as a cow-level indicator of subclinical mastitis in herd-health improvement programs. Mastitis causes significant losses in milk production (Heikkilä et al., 2018) and prevention is therefore essential for successful dairy farming. Bacterial infection is the most important cause of mastitis and elevated CMSCC (Schepers et al., 1997), with the dominant route of infection through the teat canal (Jain, 1979).

During machine milking, the teat is exposed to external factors that have the potential to alter the integrity of the teat orifice and teat canal, thereby affecting their ability to act as a barrier against mastitis-causing pathogens (Mein, 2012). Milking-time testing (MTT) is a method for evaluating the vacuum conditions in the teatcup in a milking system during milking (International Organization for Standardization (ISO),

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2007b; National Mastitis Council (NMC), 2012). A range of result variables can be calculated in a MTT. The teat end vacuum is an important parameter because it drives the milk flow across the teat canal, and causes the liner to collapse during the pulsation cycle. Increasing teat end vacuum levels will therefore increase the physical forces acting on the teat end (Mein et al., 1987; Leonardi et al., 2015). High vacuum levels at the teat end are associated with increasing occurrence of teat-end hyperkeratosis (Nørstebø et al., 2018), which in turn is a risk factor for clinical mastitis (Neijenhuis et al., 2001). However, it is not known whether a high teat end vacuum observed in a MTT is a risk factor for increased SCC, although this is commonly assumed by advisory personnel in the sector. Vacuum level in the mouthpiece chamber (MPC) is a proposed measure of how well the teat fits the liner (Borkhus and Rønningen, 2003), and relationships between MPC vacuum and udder health indicators have been reported (Rasmussen, 1997; Rønningen and Postma, 2012). High MPC vacuum causes edema at the teat end (Penry et al., 2017), possibly negatively influencing the risk of mastitis. However, relationships between MPC vacuum and SCC at cow level have not yet been reported. Vacuum stability is an indicator of the technical condition of the milking equipment. High frequencies of vacuum drops during milking is associated with increased risk of new intra-mammary infections (Rønningen, 2002). Because MTT data is recorded at cow-level and the vacuum conditions in the teatcup are known to be affected by variations in milk flow rate between individual cows, the vacuum levels recorded in one milking should not be considered representative for the herd (Nørstebø et al., 2018). Adjustments in the milking system will typically affect all cows in a herd or group, but the effect in terms of change in udder health status will become apparent at the individual cow-level. For these reasons, relationships between MTT variables and SCC also need to be studied at cow-level to learn more about strengths and limitations of MTT as a tool for udder health advisors.

Automatic milking systems (AMS) have been adopted by an increasing number of farmers, especially in the Nordic countries, since their introduction in the 1990s. The use of quarter-based milking is an important difference between AMS and conventional milking systems (CMS; milking parlors and pipeline milking systems), leading to a reduction in overmilking in AMS compared with CMS (Hogeveen et al., 2001; Svennersten-Sjaunja and Pettersson, 2008). Furthermore, in the group of CMS, most pipeline milking systems in Norway are high-line while milking parlors are low-line. It is therefore a relevant question whether results from MTT can be interpreted in the same way in the different milking systems.

The overall aim of this study was to increase our knowledge on cow-level associations between CMSCC and MTT results obtained under field conditions in different milking systems. Our first objective was to describe whether, and to what extent, cow-level differences in CMSCC could be explained by MTT results when adjusting for known factors associated with changes in CMSCC. Secondly, we aimed to compare the findings from our first objective across different milking systems.

2. Material and methods

2.1. MTT data collection

We performed a cross-sectional study by collecting results from previously performed MTT from herd advisors in the Norwegian dairy industry. The MTT used in this study were ordered by the farmers and performed by 18 trained advisors located all over Norway, using VaDia vacuum loggers and corresponding software (Biocontrol, Rakkestad, Norway). The activated vacuum loggers were attached to one of the rear teacups and connected as recommended by the manufacturer to the short milk tube, pulsation tube and mouthpiece chamber (Biocontrol, Rakkestad, Norway; Postma, 2012). Vacuum levels in the different compartments were recorded at a rate of 200 Hz and the collected data was processed by the advisors for calculation of result variables (Postma, 2012). Data from the pulsation tube was not used in this study. In CMS, the milking was performed by the herd personnel according to their usual practice. In AMS, the herds' own milking settings were used. The number of cows or milkings tested in each herd was not standardized, and the available data did not contain details on number of clusters or milking stations evaluated per herd. It was also not known whether automatic cluster removers were used in the CMS herds.

2.2. MTT variables

The vacuum measurements used for the calculation of MTT variables were recorded in one of the rear teatcups. Because CMSCC is measured at udder level, we considered the MTT results as being representative for the entire udder in this study. The following cow-level MTT variables were calculated as described by Nørstebø et al. (2018): average vacuum level in the short milk tube during the main milking period (MMVAC) and overmilking period (OMVAC), average vacuum level in the mouthpiece chamber during the main milking period and overmilking period, duration of the main milking period (MMDUR), and duration of the overmilking period (OMDUR). Vacuum stability was evaluated by counting events per milking of sudden irregular vacuum drops with a rate of vacuum change greater than 55 kPa/sec and a magnitude of 14 kPa or more (Postma, 2012).

2.3. Herd- and cow-data collection

We obtained data on type of milking system for the herds, and on breed, lactation stage, parity, test-day milk yield, CMSCC, and mastitis history for cows represented in the MTT results from the Norwegian Dairy Herd Recording System (NDHRS; Østerås et al., 2007). Milk samples for CMSCC had been collected during routine milk recordings, and analyzed using Fossomatic 5000 (Foss, Hillerød, Denmark). Frequency of milk recordings varies between herds, but is typically carried out 6 to 12 times per year in the NDHRS. We used data, including test-day milk yield, from the closest milk recording before the day of MTT for the assessment of relationships between CMSCC and the explanatory variables. Non-matching observations were omitted, i.e., if an animal or a herd in the MTT data could not be identified in the NDHRS. Records with missing data of either CMSCC, lactation stage, parity, yield or milking system were excluded from the analyses. We restricted the analyses to data recorded within a standard 305-day lactation period for each cow. The resulting dataset, containing MTT results and corresponding herd- and animal data for 7069 cows in 1009 herds, was used in the statistical analyses.

2.4. Statistical analyses

Cow was the unit of study. Principal Component Analysis (PCA) was used for descriptive multivariate analysis of the data. PCA is a commonly used method for exploratory analysis of multivariate data. It is used to test data consistency and to find systematic patterns, similarities, and differences in the data (Martin and Morris, 2002). In this method, the many individual input variables in the data are combined into a few so-called principal components (PC), symbolized as latent variables. The relationships of the PC to the observations are called scores, and to the variables called loadings. The method has been applied to MTT data in our previous research (Nørstebø et al., 2018). In this study, we applied PCA on the following variables: MMVAC, OMVAC, MMDUR, OMDUR, average vacuum level in the mouthpiece chamber during the main milking and overmilking periods, parity, DIM, yield and milking system. Dummy variables were used for first, second, and third or later parities, and for AMS herds, herds using milking parlors, and herds using pipeline milking systems. A loading plot and score plots, which reveal the correlation between the variables and observations, respectively, were used for interpretation of the results.

Table 1

Descriptive statistics for automatic milking systems (AMS) milking parlors (Parlor) and pipeline milking systems (Pipeline): Number of herds and cows, number of observations with average vacuum level in the short milk tube during main milking (MMVAC) within, below, and above the guidelines suggested by ISO, and number of cows with mastitis treatment registered prior to milk sampling. Median and mean composite milk somatic cell count (CMSCC) and mean values for the explanatory variables used in this study. Mean values are presented as arithmetic means with their corresponding confidence interval (mean ± 1.96 * SEM).

Descriptive statistics, numbers of observations[a]	AMS	Parlor	Pipeline
Number of herds	421	154	434
Number of cows	2,670	1,134	3,265
MMVAC within ISO guidelines	2,575	1,091	2,943
MMVAC below ISO guidelines	20	32	311
MMVAC above ISO guidelines	75	11	11
Mastitis before milk sampling	79	55	206
Outcome variable	**AMS**	**Parlor**	**Pipeline**
Median CMSCC, 1000 cells/mL	70	70	80
Mean CMSCC, 1000 cells/mL	232 (207–257)	198 (171–224)	221 (204–239)
Explanatory variables, mean values[a]	**AMS**	**Parlor**	**Pipeline**
MMVAC, kPa	38.6 (38.5–38.6)	37.4 (37.2–37.5)	35.6 (35.5–35.7)
OMVAC, kPa	40.6 (40.5–40.7)	39.3 (39.2–39.4)	39.8 (39.7–39.9)
MMDUR, min	3.8 (3.7–3.9)	4.3 (4.2–4.4)	4.4 (4.3–4.5)
OMDUR, min	0.32 (0.31–0.33)	1.0 (0.9–1.1)	1.1 (1.0–1.1)
MPC1, kPa	18.8 (18.4–19.1)	20.7 (20.1–21.2)	19.6 (19.3–20.0)
Irregular vacuum fluctuations per milking	10.7 (9.4–12.0)	1.9 (1.6–2.3)	3.5 (2.9–4.2)

[a] MMVAC = average vacuum level in the short milk tube during main milking; OMVAC = average vacuum level in the short milk tube during overmilking; MMDUR = duration of the main milking; OMDUR = duration of the overmilking period; MPC1 = average mouthpiece chamber vacuum during the main milking period.

We used an advanced chemometrics software, PLS_Toolbox, built within the Matlab (MathWorks, Natick, USA) computational environment for the PCA analysis of the data.

We transformed the CMSCC data to a natural logarithmic scale (lnSCC) as the outcome variable in linear regression models (Schepers et al., 1997; Reksen et al., 2008). The following potential explanatory variables were evaluated: 1) variables obtained by MTT; MMDUR, OMDUR, MMVAC, OMVAC, average vacuum level in the mouthpiece chamber in the main milking and overmilking periods, and 2) variables obtained from NDHRS; DIM, parity, milk yield and mastitis treatment registered between last calving and day of milk sampling for CMSCC. In the regression models, parity was categorized into: first lactation, second lactation, and third or later lactations. The mouthpiece chamber vacuum during the main milking period was evaluated both as a continuous variable and as a dichotomized variable describing whether the vacuum level was considered appropriate (10–30 kPa) or not according to guidelines suggested by Rønningen and Postma (2012). To account for changes in CMSCC throughout the lactation period, we used a lactation curve including DIM and the natural logarithm of DIM, as suggested by Reksen et al. (2008), in all multivariable models. Because differences in degree of udder filling between morning and evening milking might have affected the MTT results, we evaluated the time of milking (morning, evening) as a potential confounding factor (Tančin et al., 2006). The variable describing registered mastitis treatments was forced into all models to adjust for a possible effect of a clinical mastitis on our outcome and explanatory variables (Zecconi et al., 2018). The regression analyses were conducted using STATA (Stata SE/14, Stata Corp., College Station, TX, USA)

The explanatory variables were first evaluated by descriptive statistics. We assessed linearity in the relationship between the outcome and explanatory variables separately for each continuous variable, using locally weighted scatterplot smoothing curves (Stata SE/14, Stata Corp., College Station, TX, USA). In addition to being included in the regression analysis, the variable MMVAC was used to classify all observations according to the ISO guidelines, which recommends a vacuum level between 32 and 42 kPa in the short milk tube during periods of high milk flow (International Organization for Standardization (ISO), 2007a).

Statistical significance was considered with a P-value < 0.05. Relationships between the outcome variable lnSCC and the explanatory variables were initially tested in unconditional univariable linear regression models. We used a backwards variable selection procedure to build multivariable models; variables with a P-value ≤ 0.2 in the univariable analyses were entered into the initial model. Results from the PCA were used to avoid including highly correlated variables in the same model. The model was reduced by excluding the variable showing the highest P-value and re-running the model, and variables with a P-value below 0.15 were retained after backwards selection. This level was chosen to ensure that potential confounders and known explanatory variables of importance for udder health were not excluded although their association with the outcome did not prove significant in our specific models. Quadratic terms and biologically plausible first-order interactions were also tested. We evaluated potential confounding effects by assessing the change in coefficient estimates when a variable was added or removed from the model. As suggested by Dohoo et al. (2009), we regarded a change of 20% or more as evidence of confounding.

Due to lack of independence between measurements from cows within the same herd, we treated herd as a random intercept. Because we wanted to investigate the herd effect independently of milking system, and to compare the relationships between outcomes and explanatory variables across different milking systems, the procedure was run separately for herds using AMS, milking parlors, and pipeline milking systems.

Residual diagnostics were performed by calculating standardized residuals for the 2 levels (herd and cow) as suggested by Rabe-Hesketh and Skrondal (2012), and thereafter evaluating the normality assumption graphically. Intraclass correlation coefficient (ICC) and coefficient of determination (R^2) was calculated based on the final model for each of the milking systems (Rabe-Hesketh and Skrondal, 2012).

3. Results

3.1. Descriptive results

In Table 1 we present data on the number of animals and herds for

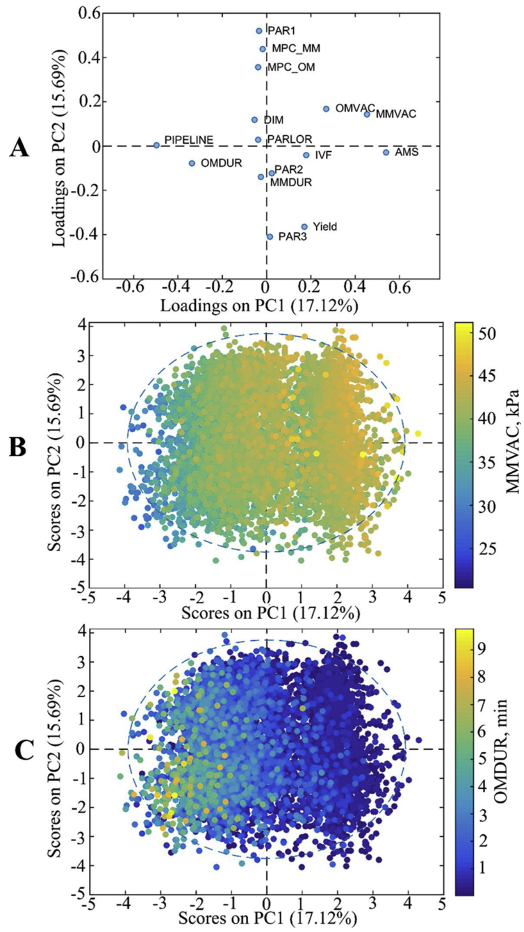

Fig. 1. Results from Principal Component Analysis. Correlation loading plot (A) showing the pattern of relationships between the milking-time test variables and cow variables, and corresponding score plots showing patterns of relationships between the observations in the dataset, colored according to the average vacuum level in the short milk tube during the main milking period (B) and the duration of the overmilking period (C), respectively. PIPELINE = dummy variable for pipeline milking systems; PARLOR = dummy variable for milking parlors; AMS = dummy variable for automatic milking systems; MMVAC = average vacuum level in the short milk tube during main milking; OMVAC = average vacuum level in the short milk tube during overmilking; MMDUR = duration of the main milking; OMDUR = duration of the overmilking period; MPC_MM = average mouthpiece chamber vacuum in the main milking period; MPC_OM = average mouthpiece chamber vacuum in the overmilking period; DIM = days in milk; PAR1 = dummy variable for first parity; PAR2 = dummy variable for second parity; PAR$\geq$3 = dummy variable for third and later lactations; Yield = daily milk yield in kg.

the different milking systems, the average and median CMSCC, the number of cows where a mastitis treatment was recorded before the day of milk sampling in the current lactation, the classification according to ISO guidelines for vacuum level and average values for the explanatory variables. There were small differences in overall levels of CMSCC between the 3 milking systems. The majority of observations were within the ISO guidelines for vacuum level at the teat end during milking. In our data from CMS, 37.44% of the MTT were conducted at morning milking and 62.56% at evening milking. Average number of MTT observations per herd was 7, ranging from 1 to 27.

Median time between milk recording and MTT was 17 days, and the 10 and 90% percentiles were 3 and 56 days, respectively. Norwegian Red was the dominating breed in our material (94.6% of the cows).

3.2. PCA results

The results from the PCA are presented in Fig. 1, where Fig. 1A shows the loading plot. The loading vectors hold the information about the contribution of the respective original variables to the components PC1 and PC2, which together described 32.81% of the total variation in the set of variables. In Fig. 1A, a 2-dimensional loading plot is used to study how the original variables co-vary. If the variables are situated close together geometrically, they co-vary positively. The dummy variables for pipeline milking systems and AMS have negative and positive loadings on PC1, respectively, and only small loadings on PC2. The dummy variable for milking parlor was located close to the center of the plot, hence this variable was not well represented by either PC1 or PC2. The angle between OMDUR and PIPELINE was small, indicating a positive association between these variables. In contrast, the angle between OMDUR and AMS was close to 180°, indicating a rather strong negative association. Similarly, MMVAC and OMVAC were positively associated with AMS, and negatively related to pipeline milking systems. The variable describing irregular vacuum fluctuations had a positive loading on PC1, indicating a positive relationship with AMS. The dummy variables for parity were aligned on a line with low loading on PC1 and higher loading on PC2, where first parity and third or later parities have the highest negative and positive loadings, respectively. The variables describing mouthpiece chamber vacuum in the main milking and overmilking period were positively associated. Based on their allocation on the loading plot, there was a positive association between the variables second parity, MMDUR, third or later parities and yield, and a negative association between these variables and DIM, mouthpiece chamber vacuum levels in main milking and overmilking, and first parity. These results indicate that the MTT variables MMVAC, OMVAC and OMDUR are associated with the milking system, while mouthpiece chamber vacuum in the main milking and overmilking periods, and MMDUR are predominantly associated with cow factors.

Fig. 1B and Fig. 1C show plots of scores on PC1 and PC2 for all observations in our dataset, where the data points are colored according to the value of MMVAC (Fig. 1B) and OMDUR (Fig. 1C). Fig. 1B shows a tendency of higher MMVAC with increasing PC1 score, corresponding to the observations from AMS herds, whereas lower values of MMVAC were found with decreasing PC1 score, corresponding to observations from pipeline milking systems. Fig. 1C illustrates that observations with negative scores on PC1 (corresponding to the alignment of pipeline milking systems in Fig. 1A), had longer OMDUR than those with positive scores on PC1 (corresponding to the alignment of AMS in Fig. 1A).

3.3. Univariable analysis

The results from the univariable analysis of the relationships between lnSCC and the possible explanatory variables, as presented in Table 2, were used in the model building process. The results also illustrate which relationships we can expect to be apparent when not accounting for other causes of fluctuations in SCC. It is notable that the duration of the overmilking period, as recorded by the MTT, had a significant positive relationship with lnSCC in pipeline milking systems, but not in milking parlors and AMS. The continuous variable describing average vacuum level in the mouthpiece chamber during the main milking period was associated with lnSCC in pipeline systems. However, we could not find significant relationships between lnSCC and mouthpiece chamber vacuum when dichotomized according to suggested guidelines (Rønningen and Postma, 2012) in any of the milking systems. Similarly, we could not find significant relationships between lnSCC and the number of irregular vacuum fluctuations per milking. The lactation curve described by DIM and the natural logarithm of DIM was significantly associated with lnSCC in all milking systems, together with average vacuum level in the main milking and the duration of the main milking period. Yield was negatively associated with lnSCC both alone and together with its quadratic term. We found that vacuum level

Table 2
Results from univariable linear regression models describing relationships between ln-transformed SCC and milking-time test variables, parity, mastitis history and yield, and a lactation curve described by DIM and the natural logarithm of DIM (lnDIM) respectively.

Variable[a]	Automatic milking systems				Milking parlors				Pipeline milking systems			
	β	*P*	95 % CI		β	*P*	95 % CI		β	*P*	95 % CI	
			Lower	Upper			Lower	Upper			Lower	Upper
MMVAC, kPa	−0.042	< 0.001	−0.065	−0.018	−0.044	0.008	−0.076	−0.011	−0.045	< 0.001	−0.062	−0.029
OMVAC, kPa	−0.020	0.007	−0.034	−0.006	−0.024	0.106	−0.054	0.005	−0.026	0.001	−0.042	−0.010
MMDUR, min	−0.062	< 0.001	−0.089	−0.036	−0.075	< 0.001	−0.112	−0.038	−0.106	< 0.001	−0.129	−0.083
OMDUR, min	0.059	0.435	−0.089	0.206	0.069	0.061	−0.003	0.141	0.088	< 0.001	0.045	0.131
MPC1, kPa	0.0004	0.876	−0.005	0.005	−0.007	0.064	−0.014	0.0004	−0.013	< 0.001	−0.018	−0.009
MPC2, (1/0)	−0.002	0.965	−0.103	0.099	−0.087	0.249	−0.236	0.061	−0.068	0.130	−0.157	0.020
IVF (count)	0.0004	0.547	−0.001	0.002	−0.008	0.186	−0.021	0.004	0.001	0.248	−0.001	0.004
Mastitis (1/0)	0.498	< 0.001	0.227	0.769	0.049	0.767	−0.277	0.376	0.203	0.019	0.033	0.372
DIM	0.005	< 0.001	0.003	0.006	0.007	< 0.001	0.005	0.009	0.005	< 0.001	0.003	0.006
lnDIM	−0.381	< 0.001	−0.496	−0.266	−0.465	< 0.001	−0.643	−0.287	−0.359	< 0.001	−0.473	−0.244
Parity 1	–				–				–			
Parity 2	0.330	< 0.001	0.208	0.452	0.321	0.001	0.139	0.503	0.269	< 0.001	0.160	0.377
Parity ≥3	0.617	< 0.001	0.501	0.732	0.665	< 0.001	0.494	0.835	0.653	< 0.001	0.552	0.753
Yield (kg/day)	−0.066	< 0.001	−0.097	−0.035	−0.081	0.001	−0.130	−0.032	−0.070	< 0.001	−0.105	−0.036
Yield^2	0.001	< 0.001	0.000	0.001	0.001	0.005	0.000	0.002	0.001	< 0.001	0.000	0.002

[a] MMVAC = average vacuum level in the short milk tube during main milking; OMVAC = average vacuum level in the short milk tube during the overmilking period; MMDUR = duration of the main milking period in min; OMDUR = duration of the overmilking period in minutes; Mastitis = registered mastitis treatment in the current lactation, before milk sampling for SCC analysis; IVF = count of irregular vacuum fluctuations per milking; MPC1 = average mouthpiece chamber during the main milking period; MPC2 = average mouthpiece chamber vacuum in the main milking period between 10 and 30 kPa.

in the overmilking period was associated with lnSCC in AMS and pipeline systems, but not in milking parlors.

3.4. Multivariable models

The final models describing lnSCC showed that MMDUR was significantly and negatively associated with the outcome variable in all milking systems when we had taken into account lactation stage, parity, mastitis history, milk yield and MMVAC. Hence, an increase in the duration of the main milking was associated with a decrease in lnSCC in all milking systems. Similarly, an increasing MMVAC was significantly associated with a decrease in lnSCC in both AMS and milking parlors. This association was not apparent for pipeline milking systems. Only minor changes in parameter estimates were found when comparing models with and without the variable discriminating between morning and evening milking in CMS herds, and therefore the time of milking variable was not included in any of the final models. According to the coefficients of determination, our models described 8%, 10%, and 10% of the overall variability in lnSCC in AMS, milking parlors, and pipeline milking systems, respectively. The difference between herds, as evaluated by ICC, accounted for an additional 7% of the variance in CMSCC for AMS herds, 8% for herds using milking parlors, and 6% for herds using pipeline milking systems. This proportion includes differences in management between herds with the same milking system. The results of the multivariable regression models are presented in Table 3.

We retained the interactions between mastitis treatment and both

Table 3
Final multivariable linear regression models describing the relationship between ln-transformed SCC and milking-time test variables, adjusting for lactation stage, parity and milk yield in automatic milking systems, milking parlors, and pipeline milking systems. The models included a random intercept at herd-level to account for differences between herds.

Variable[a]	Automatic milking systems				Milking parlors				Pipeline milking systems			
	β	*P*	95 % CI		β	*P*	95 % CI		β	*P*	95 % CI	
			Lower	Upper			Lower	Upper			Lower	Upper
Intercept	6.953	< 0.001	5.822	8.085	7.733	< 0.001	6.131	9.335	7.033	< 0.001	6.123	7.943
MMVAC, kPa	−0.026	0.049	−0.051	−0.0001	−0.039	0.034	−0.076	−0.003	−0.015	0.115	−0.034	0.004
MMDUR, min	−0.069	< 0.001	−0.096	−0.0415	−0.064	0.001	−0.104	−0.025	−0.110	< 0.001	−0.137	−0.083
Mastitis (0/1)	−5.708	0.064	−11.736	0.321	0.161	0.335	−0.166	0.488	0.089	0.308	−0.082	0.261
Mastitis x MMVAC	0.141	0.081	−0.018	0.301								
Mastitis x MMDUR	0.132	0.065	−0.008	0.272								
DIM	0.002	0.047	0.00002	0.003	0.003	0.015	0.0006	0.006	0.002	0.012	0.0004	0.003
lnDIM	−0.208	0.001	−0.335	−0.082	−0.284	0.003	−0.472	−0.097	−0.287	< 0.001	−0.402	−0.173
Parity 1												
Parity 2	0.416	< 0.001	0.289	0.542	0.378	< 0.001	0.194	0.562	0.388	< 0.001	0.279	0.497
Parity ≥3	0.757	< 0.001	0.629	0.885	0.810	< 0.001	0.623	0.997	0.798	< 0.001	0.691	0.905
Yield (kg/day)	−0.050	0.002	−0.081	−0.018	−0.061	0.015	−0.109	−0.012	−0.044	0.011	−0.079	−0.010
Yield^2	0.0005	0.038	0.00002	0.001	0.001	0.109	−0.0002	0.002	0.0004	0.147	−0.0002	0.001
Model diagnostics[b]	ICC	R^2			ICC	R^2			ICC	R^2		
	0.069	0.080			0.081	0.104			0.063	0.099		

[a] MMVAC = average vacuum level in the short milk tube during main milking; MMDUR = duration of the main milking period in min; Mastitis = registered mastitis treatment in the current lactation, before milk sampling for SCC analysis.
[b] ICC = Intraclass Correlation Coefficient; R^2 = coefficient of determination.

Table 4
Estimated effects of the milking-time test variables (duration of the main milking period and average vacuum level in the short milk tube during the main milking period) on composite milk SCC according to the final models for automatic milking systems (AMS), milking parlors (Parlor), and pipeline milking systems (Pipeline), respectively. The effects were calculated for a second parity cow with no registered mastitis treatments, while keeping the other variables in the models at their mean values.

Main milking duration, min	AMS	Parlor	Pipeline
Lower quartile	2.45	2.88	3.07
Upper quartile	4.72	5.33	5.43
Effect on CMSCC per min increase[a]	−5.32	−4.83	−9.49
Effect over interquartile range[a]	−12.1	−11.8	−22.39
Average vacuum level in short milk tube during main milking, kPa	**AMS**	**Parlor**	**Pipeline**
Lower quartile	37.55	36.10	33.99
Upper quartile	39.81	38.83	37.34
Effect on CMSCC per kPa increase[a]	−2.00	−2.95	−1.33
Effect over interquartile range[a]	−4.52	−8.05	−4.46

[a] Expressed as 1000 cells/mL.

MMDUR and MMVAC in the final model for AMS. In the group with a mastitis treatment prior to milk sampling, an increase in both MMDUR and MMVAC was associated with increasing lnSCC. These associations were non-significant according to the predefined limit, but had P-values close to the cutoff value.

Based on the multivariable regressions models for the 3 milking systems, we calculated the change in CMSCC per unit increase and over the interquartile range for MMVAC and MMDUR for a second parity cow with no registered mastitis treatments, while keeping the other variables included in the models at their mean value (Table 4). The difference in CMSCC from the upper to the lower quartiles were generally small (i.e. between -4500 cells/ml and -22400 cells/ml).

4. Discussion

To our knowledge, this is the first presentation of a large observational study describing relationships between SCC and MTT variables at cow-level. We also compared results between different milking systems, which is novel in this area of research.

We found that an increase in the duration of the main milking period was associated with a decrease in CMSCC in all milking systems. The duration of the main milking is a result of the milk yield and the milk flow rate. Our multivariable models adjusted for the former, but not the latter. Furthermore, our results showed that an increasing average vacuum level in the short milk tube in the main milking was associated with a decrease in CMSCC in AMS and milking parlors. The vacuum level in the short milk tube is a result of the system vacuum level and the vacuum loss due to milk transport through the same tube (Besier and Bruckmaier, 2016). It seems unlikely that an increasing duration of the main milking and increasing vacuum levels contribute *per se* to a lower CMSCC. A more plausible explanation is that main milking duration and vacuum level in the short milk tube are influenced by underlying factors that are also associated with CMSCC. Researchers in the field of cattle breeding have reported weak, but negative correlations between milking duration and somatic cell count, and average milk flow rate and somatic cell count in various breeds (Berry et al., 2013; Gray et al., 2011; Prendiville et al., 2010). Nørstebø et al. (2018) found a strong relationship between average milk flow and average vacuum level in the short milk tube in AMS, illustrating the importance of milk transport for the vacuum levels measured in the short milk tube during a MTT. Hence, a likely explanation for the observed negative relationship between CMSCC and milking duration and vacuum level, respectively, is that both the outcome and explanatory variables are associated with cow traits related to milking speed and mastitis resistance, such as variations in the anatomy of the teat end. The relationships described in our multivariable models are important when using MTT as a tool in advisory services; unlike previously described relationships between system vacuum levels and udder health at herd level (Langlois et al., 1981; Østerås and Lund, 1988), high vacuum levels in the short milk tube and long main milking durations found in a MTT are not associated with elevated CMSCC.

Our models explained 8–10 % of the total variance in lnSCC, showing that a considerable proportion of the variability in CMSCC is unexplained, even when adjusting for parity, lactation stage, and mastitis history (Schepers et al., 1997). However, the SCC used in our study was a single measurement, and this is likely to show more variation than an average of SCC values over time. The ICC showed that 6–8 % of the variation in CMSCC could be attributed to differences between herds. This is in agreement with Schepers et al. (1997), who reported that herd explained only a small part of the variation in SCC. Furthermore, the estimated effects on CMSCC of the MTT variables that we included in our final models were generally small. Overall, our findings demonstrate that the common MTT variables that we used in our study have limitations for evaluating the effect of the milking machine on CMSCC. The described associations and relatively small effect of the MTT variables on CMSCC are important for the understanding of strengths and limitations of MTT as a tool in herd advisory services.

The interactions between mastitis treatment and MMVAC and MMDUR in the final model for AMS indicated that there was a difference between cows with and without a history of mastitis in the relationship between lnSCC and MMVAC and MMDUR. In the group where the cows had a registered mastitis treatment, increases in MMVAC and MMDUR were associated with an increase in lnSCC compared with that seen in healthy cows. Our data included only a limited number of mastitis cases, and, because P-values were outside the predefined significance limit, we cannot perform detailed analysis of these relationships. However, we hypothesize that pathological changes in the udder after an episode of mastitis may affect milk let down to the extent that the duration of the milking is increased, and that a higher vacuum level is measured due to a decrease in the rate of milk flow. This suggests that advisors using MTT should consider standardizing the selection of cows by excluding cows with recent episodes of clinical mastitis. We aim to explore these findings more closely in future studies.

We found that the duration of the overmilking period, as determined by the MTT, was significantly associated with CMSCC in the univariable analysis for pipeline milking systems, but not for AMS and milking parlors. Although the mean overmilking duration in milking parlors (1.0 min) and pipeline milking systems (1.1 min) was similar, the results from the PCA indicated that the longest overmilking durations were associated with pipeline milking systems. The mean overmilking duration in AMS was only 0.33 min. This is in agreement with previous research indicating that a reduction in overmilking is an advantage of AMS over CMS (Hogeveen et al., 2001; Svennersten-Sjaunja and Pettersson, 2008). Our measurement of overmilking was based on vacuum registrations in the teatcup during milking (Borkhus and Rønningen, 2003), and was therefore not affected by differences between systems in the distance from teat-end to milk meter. Furthermore, because our MTT data were recorded on one of the rear teats, the measured duration of the overmilking period is likely to be conservative for cows in CMS herds. Although our data did not contain information on whether the CMS herds in our study used manual or automatic cluster removal, we must assume that both were represented in our material and that this is a possible explanation for finding a significant association between CMSCC and overmilking duration in pipeline milking systems and not AMS and milking parlors. It is possible that automatic cluster removers are more common in milking parlors than in pipeline systems, and this could explain why the PCA indicated that the longest overmilking durations were associated with pipeline milking systems. The association between overmilking duration and CMSCC was apparent in the univariable analysis, but not in the multivariable analysis when other factors were accounted for.

The descriptive statistics showed that most observations were within the ISO standard for vacuum level at the teat-end during the main milking period. As illustrated by the PCA, we found high vacuum levels more often in AMS herds, but in spite of this, we also found a decreasing CMSCC with increasing vacuum levels in this milking system. However, more teat-end callosity is found in cows with a high vacuum level in the short milk tube during main milking (Nørstebø et al., 2018), and severe degrees of teat-end callosity is a known risk factor for clinical mastitis (Neijenhuis et al., 2001). Thus, high vacuum levels might have negative consequences for udder health, but we could not detect this as an increase in CMSCC in this study.

Mean number of irregular vacuum fluctuations per milking was clearly highest in AMS herds. However, no significant relationships between irregular vacuum fluctuations and CMSCC were found in any milking system, indicating that this variable is of limited value for advisory services. In contrast, Rønningen (2002) found a relationship between milkline vacuum stability and new infection rate at herd level, but also argued that number of vacuum drops during a single milking should be interpreted with care and that other measures are better suited for evaluating vacuum stability.

The mouthpiece chamber vacuum level during the main milking was significantly associated with CMSCC in pipeline milking systems in the univariable analysis. The association pointed towards decreasing CMSCC with increasing MPC. However, the relationship was not apparent in the multivariable models. When the mouthpiece chamber vacuum in the main milking was categorized according to Rønningen and Postma (2012), no relationship with CMSCC was found. Hence, our findings are in contrast with previous research which suggested that the mouthpiece chamber vacuum is an important variable for udder health (Rasmussen, 1997; Rønningen and Postma, 2012). However, mouthpiece chamber vacuum might still be valuable for other purposes, such as minimizing circulatory disturbances in the teat (Penry et al., 2017).

We recognize that our study has some limitations that are important to consider when interpreting the results. Due to limitations in the available data, our study did not include the system vacuum level and pulsation characteristic. Furthermore, the reasons for performing the MTT used in our study are not known in all cases. The MTT are typically performed in herds experiencing problems with milk quality and udder health, or as a routine checkup of the milking system. Compared with previously reported average CMSCC in herds of Norwegian Red cows (Reksen et al., 2008), our average CMSCC values were relatively high, indicating that our data contained an excess of herds experiencing some degree of udder health problems. It is possible that the findings would have been different if the data had been obtained from herds with no udder health problems. However, we attempted to account for differences between herds by including herd as a random intercept in the multivariable models, thereby reducing the likelihood that differences in herd health status would have had a major impact on the results. Pre-milking routines and teat dimensions are known to be important for milking speed and udder emptying, but our data did not contain information on these aspects. We assume that the pre-milking routine was the same within herd, and that the possible effect of this on SCC was accounted for by including herd as a random effect.

Norwegian Red is the dominant breed in the Norwegian dairy production (Østerås et al., 2007), and this was reflected in our data. The Norwegian Red is a crossbred dual-purpose cow, and traits such as reproductive performance, longevity, and health have been allocated higher value than, e.g., milking speed and milk yield. Other cattle breeds might differ in their response to the milking process, and our results should therefore be considered valid for Norwegian Red and extrapolation to other breeds should be done with caution.

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References

Berry, D.P., Coughlan, B., Enright, B., Coughlan, S., Burke, M., 2013. Factors associated with milking characteristics in dairy cows. J. Dairy Sci. 96, 5943–5953. https://doi.org/10.3168/jds.2012-6162.

Besier, J., Bruckmaier, R.M., 2016. Vacuum levels and milk-flow-dependent vacuum drops affect machine milking performance and teat condition in dairy cows. J. Dairy Sci. 99, 3096–3102. https://doi.org/10.3168/jds.2015-10340.

Borkhus, M., Rønningen, O., 2003. Factors affecting mouthpiece chamber vacuum in machine milking. J. Dairy Res. 70, 283–288. https://doi.org/10.1017/S0022029903006253.

Dohoo, I.R., Martin, W., Stryhn, H., 2009. Veterinary Epidemiologic Research, 2nd. edition. VER Inc., Charlottetown, PEI, Canada.

Gray, K.A., Vacirca, F., Bagnato, A., Samoré, A.B., Rossoni, A., Maltecca, C., 2011. Genetic evaluations for measures of the milk-flow curve in the Italian Brown Swiss population. J. Dairy Sci. 94, 960–970. https://doi.org/10.3168/jds.2009-2759.

Heikkilä, A.M., Liski, E., Pyörälä, S., Taponen, S., 2018. Pathogen-specific production losses in bovine mastitis. J. Dairy Sci. 101, 9493–9504. https://doi.org/10.3168/jds.2018-14824.

Hogeveen, H., Ouweltjes, W., de Koning, C.J.A.M., Stelwagen, K., 2001. Milking interval, milk production and milk flow-rate in an automatic milking system. Livest. Prod. Sci. 72, 157–167. https://doi.org/10.1016/S0301-6226(01)00276-7.

International Organization for Standardization (ISO), 2007a. Milking Machine Installations - Construction and Performance. ISO Standard no. 5707. ISO, Geneva, Switzerland.

International Organization for Standardization (ISO), 2007b. Milking Machine Installations - Vocabulary. ISO Standard no. 3918. ISO, Geneva, Switzerland.

Jain, N.C., 1979. Common mammary pathogens and factors in infection and mastitis. J. Dairy Sci. 62, 128–134. https://doi.org/10.3168/jds.S0022-0302(79)83214-2.

Langlois, B.E., Cox, J.S., Hemken Jr, R.H., Nicolai Jr, J., 1981. Milking vacuum influencing indicators of udder health. J. Dairy Sci. 64, 1837–1842. https://doi.org/10.3168/jds.S0022-0302(81)82773-7.

Leonardi, S., Penry, J.F., Tangorra, F.M., Thompson, P.D., Reinemann, D.J., 2015. Methods of estimating liner compression. J. Dairy Sci. 98, 6905–6912. https://doi.org/10.3168/jds.2015-9380.

Martin, E.B., Morris, A.J., 2002. Enhanced bio-manufacturing through advanced multivariate statistical technologies. J. Biotechnol. 99, 223–235. https://doi.org/10.1016/S0168-1656(02)00212-2.

Mein, G.A., 2012. The role of the milking machine in mastitis control. Vet. Clin. North Am. Food Anim. Pract. 28, 307–320. https://doi.org/10.1016/j.cvfa.2012.03.004.

Mein, G.A., Williams, D.M., Thiel, C.C., 1987. Compressive load applied by the teatcup liner to the bovine teat. J. Dairy Res. 54, 327–337. https://doi.org/10.1017/S0022029900025504.

National Mastitis Council (NMC), 2012. Procedures for Evaluating Vacuum Levels and Air Flow in Milking Systems. NMC, New Prague, NM, USA.

Neijenhuis, F., Barkema, H.W., Hogeveen, H., Noordhuizen, J.P.T.M., 2001. Relationship between teat-end callosity and occurrence of clinical mastitis. J. Dairy Sci. 84, 2664–2672. https://doi.org/10.3168/jds.S0022-0302(01)74720-0.

Nørstebø, H., Rachah, A., Dalen, G., Rønningen, O., Whist, A.C., Reksen, O., 2018. Milk-flow data collected routinely in an automatic milking system: an alternative to milking-time testing in the management of teat-end condition? Acta Vet. Scand. 60, 2. https://doi.org/10.1186/s13028-018-0356-x.

Østerås, O., Lund, A., 1988. Epidemiological analysis of the associations between bovine udder health and milking machine and milking management. Prev. Vet. Med. 6, 91–108. https://doi.org/10.1016/0167-5877(88)90047-5.

Østerås, O., Solbu, H., Refsdal, A.O., Roalkvam, T., Filseth, O., Minsaas, A., 2007. Results and evaluation of thirty years of health recordings in the Norwegian dairy cattle population. J. Dairy Sci. 90, 4483–4497. https://doi.org/10.3168/jds.2007-0030.

Penry, J.F., Upton, J., Mein, G.A., Rasmussen, M.D., Ohnstad, I., Thompson, P.D., Reinemann, D.J., 2017. Estimating teat canal cross-sectional area to determine the effects of teat-end and mouthpiece chamber vacuum on teat congestion. J. Dairy Sci. 100, 821–827. https://doi.org/10.3168/jds.2016-11533.

Postma, E., 2012. New technology for milking vacuum diagnostics helps veterinarians better understand and manage udder health problems. Proceedings of the British Mastitis Conference, Sixways, Worcester 87–88.

Prendiville, R., Pierce, K.M., Buckley, F., 2010. A comparison between Holstein-Friesian and Jersey dairy cows and their F1 cross with regard to milk yield, somatic cell score, mastitis, and milking characteristics under grazing conditions. J. Dairy Sci. 93, 2741–2750. https://doi.org/10.3168/jds.2009-2791.

Rabe-Hesketh, S., Skrondal, A., 2012. Multilevel and Longitudinal Modeling Using Stata. Volume 1: Continous Responses, Third edition. STATA press, Texas, USA.

Rasmussen, M.D., 1997. The relationship between mouthpiece vacuum, teat condition and udder health. 36th Natl. Mastitis Counc. Ann. Mtg. Proc. 91–96.

Reksen, O., Sølverød, L., Østerås, O., 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.

Rønningen, O., 2002. Milkline vacuum stability in milking machine installations. J. Dairy Res. 69, 501–509. https://doi.org/10.1017/S0022029902005812.

Rønningen, O., Postma, E., 2012. Classification of mouthpiece chamber vacuum records in milking-time tests. 51st Natl. Mastitis Counc. Ann. Mtg. Proc. 219–220.

Schepers, A.J., Lam, T.J.G.M., Schukken, Y.H., Wilmink, J.B.M., Hanekamp, W.J.A., 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.

Schukken, Y.H., Wilson, D.J., Welcome, F., Garrison-Tikofsky, L., Gonzalez, R.N., 2003. Monitoring udder health and milk quality using somatic cell counts. Vet. Res. 34, 579–596. https://doi.org/10.1051/vetres:2003028.

Svennersten-Sjaunja, K.M., Pettersson, G., 2008. Pros and cons of automatic milking in Europe. J. Anim. Sci. 86, 37–46. https://doi.org/10.2527/jas.2007-0527.

Tančin, V., Ipema, B., Hogewerf, P., Mačuhovà, J., 2006. Sources of variation in milk flow characteristics at udder and quarter levels. J. Dairy Sci. 89, 978–988. https://doi.org/10.3168/jds.S0022-0302(06)72163-4.

Zecconi, A., Frosi, S., Cipolla, M., Gusmara, C., 2018. Effects of chronic mastitis and its treatment with ketoprofen on the milk ejection curve. J. Dairy Res. 85, 50–52. https://doi.org/10.1017/S0022029917000863.