Norwegian University of Life Sciences
Department of Production Animal Clinical Sciences
Faculty of Veterinary Medicine

Philosophiae Doctor (PhD)
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The applicability of milking-time testing in automatic milking systems

Milking-time testing: anvendbarhet i automatiske melkingssystemer

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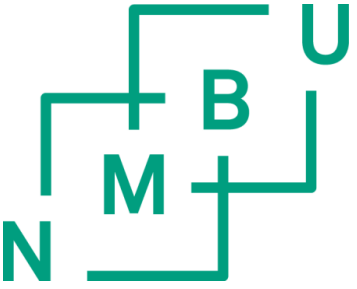

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Table of contents

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Abbreviations

AMS	Automatic milking system
BIC	Bayesian information criterion
CI	Confidence interval
CMT	California mastitis test
CV	Coefficient of variation
DHI	Dairy herd improvement
DIM	Days in milk
EMR	Elevated mastitis risk (OCC-based mastitis indicator)
ICAR	International Committee for Animal Recording
IDF	International Dairy Federation
IMI	Intramammary infection
ISO	International Organization for Standardization
MPC	Mouthpiece chamber
MTT	Milking-time test
NDHRS	Norwegian dairy herd recording system
NMSM	Nordic Dairy Associations' Committee for Milk Quality Issues (Nordiske meieriorganisasjoners samarbeidsutvalg for melkekvalitetsarbeid)
OCC	On-line cell count; SCC measured by DeLaval On-line cell counter
Pat-1	Pathogens from which a high cell count would be expected during an IMI
Pat-2	Other known mastitis pathogens not included in Pat-1
PCA	Principal component analysis
QMS	Quarter milk sample
ROC	Receiver operating characteristic
SCC	Somatic cell count
Se	Sensitivity of a diagnostic test
Sp	Specificity of a diagnostic test

List of papers

Paper I

Nørstebø, H., Rachah, A., Dalen, G., Østerås, O., Whist, A.C., Nødtvedt, A., Reksen, O. 2019. Large-scale cross-sectional study of relationships between somatic cell count and milking-time test results in different milking systems. Preventive Veterinary Medicine. DOI: https://doi.org/10.1016/j.prevetmed.2019.02.007

Paper II

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 Veterinaria Scandinavica. DOI: https://doi.org/10.1186/s13028-018-0356-x

Paper III

Nørstebø, H., Dalen, G., Rachah, A., Heringstad, B., Whist, A.C., Nødtvedt, A., Reksen, O. 2019. Cow level factors associated with milking-to-milking variability in somatic cell counts in an automatic milking system. Preventive Veterinary Medicine. DOI: https://doi.org/10.1016/j.prevetmed.2019.104786

Paper IV

Dalen, G., Rachah, A., Nørstebø, H., Schukken, Y.H., Reksen, O. 2019. The detection of intramammary infections using online somatic cell counts. Journal of Dairy Science DOI: https://doi.org/10.3168/jds.2018-15295

Summary

Norwegian dairy production is changing towards larger production units, increasing use of automatic milking systems (AMS), and decreasing economic margins. Bovine mastitis remains a challenge in Norwegian dairy production, with an estimated annual cost of 120 million Norwegian kroner. The disease is not only important from an economic perspective, but also because it affects the general health and wellbeing of the cow and because mastitis is the main reason for using antimicrobial drugs in dairy production. Preventing mastitis is therefore essential for maintaining efficient and sustainable production on Norwegian dairy farms, and there is a need for knowledge to improve udder health in an environment that is changing rapidly as the number of farms with AMS continues to grow in Norway.

It is recognized that the milking process can be involved in the pathogenesis of mastitis. One part of the Norwegian mastitis control program is, therefore, to provide services in which the milking equipment is evaluated as a possible contributor to impaired udder health. The Milking-Time Test (MTT) is frequently used as a tool in these advisory services. However, the MTT was developed for conventional milking, and the method is cumbersome to use in AMS herds. In addition, various improvements in the milking equipment have made it necessary to update our knowledge on how to interpret the results from MTT in general, and in AMS herds specifically, to ensure that the advisory services retain their high quality.

Only one cow is milked at a time in AMS, and hence only one set of sensors is required to collect data during milking. This makes the AMS very suitable for implementing sensors that continually monitor udder health status. Monthly or bimonthly measurements of Somatic Cell Count (SCC) have been used in traditional Dairy Herd Improvement systems. In AMS, auxiliary equipment also allows for SCC measurements at every milking, resulting in a substantial increase in the amount of data collected per cow. However, to improve the use of frequent SCC data for mastitis prevention, knowledge is needed on how to distinguish physiological fluctuations from fluctuations that are due to pathological processes.

The overall aim of this thesis was to provide new knowledge on how to characterize udder health in clinically healthy cows using frequently measured SCC, and to improve udder health in AMS herds by evaluating the applicability of MTT, in combination with other sources of auxiliary sensor data.

For an MTT to be useful for udder health advisory services, it is necessary that there are robust relationships between udder health and MTT result variables. The work presented in this thesis shows that interpretation of MTT results is challenging because differences between cows have a major impact on the interpretation of the results. For example, a negative relationship between SCC and vacuum level in the short milk tube was detected. However, this relationship is likely to be due to differences in milk flow rates between cows, where cows with more patent teat canals will have higher milk flow rates and hence lower vacuum levels. Vacuum levels recorded in the short milk tube may therefore be more closely related to cow characteristics than to the milking system. Consequently, MTT will have limited value as a stand-alone tool for evaluating possible negative impacts of the milking machine on udder health in AMS as well as conventional milking systems.

The teat-end is the first line of defense against mastitis pathogens, and poor teat-end condition is associated with an increased mastitis risk. This work showed that the likelihood of a teat-end being roughened or thickened increases with decreasing milk flow rate. Furthermore, a strong negative relationship between vacuum level in the short milk tube and quarter milk flow rate was confirmed. These findings have two main implications: 1) milk flow data can be used in combination with MTT results to provide better advice applicable to a herd, and 2) in herds experiencing poor teat-end condition, data on milk flow rate could be used to determine whether the milking system may be a contributory factor in the pathogenesis of the problem.

This thesis also contributed to a better understanding of the possibilities and limitations of using frequently measured SCC values as an indicator of intramammary infection (IMI) in clinically healthy cows. Close monitoring of IMI status based on culture results from monthly quarter milk samples made it possible to investigate the distribution in SCC in periods of differing udder-health status. Although elevated SCC values were seen in periods of IMI compared with healthy periods, there was a considerable degree of overlap between SCC values in periods of different IMI status. The variability in SCC was further investigated in linear mixed models, showing that there is high physiological variability in frequently measured SCC. Only a relatively modest proportion of the SCC variability was accounted for by IMI, cow-specific factors, variables derived from sensors in the AMS, and other explanatory variables included in the model. Furthermore, diagnostic test properties of three different SCC-based IMI indicators were investigated. In addition to single SCC values and rolling average SCC, the Elevated Mastitis Risk (EMR) indicator, using the level

and trend of smoothed SCC values to indicate the mastitis risk on a standardized scale, was evaluated. Due to the large degree of normal fluctuations and overlap in SCC values in groups of different IMI status, decreasing sensitivity was observed when greater specificity was demanded. In order to adapt the detection system to situations where the tolerance towards false-negative results and false-positive results differs, threshold values may be adjusted to achieve sensitivity and specificity levels suitable for the intended use.

Sammendrag (Summary in Norwegian)

Norsk mjølkeproduksjon forandrer seg i retning større produksjonsenheter, økende bruk av automatiske mjølkingssystemer (AMS) og reduserte økonomiske marginer. Til tross for at situasjonen har forbedret seg kraftig de siste to tiårene er mastitt fortsatt en utfordring i norsk mjølkeproduksjon. Det årlige tapet på grunn av mastitt er beregna til 120 millioner kroner. Mastitt er ikke bare viktig i et økonomisk perspektiv, men også fordi sjukdommen påvirker dyrevelferden og fordi det er den viktigste årsaken til bruk av antibiotika i mjølkekubesetninger. Dette gjør det viktig å forebygge mastitt for å opprettholde en effektiv og bærekraftig mjølkeproduksjon. De raske endringene i norsk mjølkeproduksjon med stadig økende bruk av AMS gjør det nødvendig med ny kunnskap for å forbedre jurhelsa.

Det er kjent at mjølkingsprosessen kan være involvert i patogenesen til mastitt. Derfor omfatter det norske mastittarbeidet tjenester hvor mjølkingsutstyret vurderes som en potensiell bidragsyter til dårlig jurhelse. Milking-time testing (MTT) er et mye brukt rådgivingsverktøy i disse tjenestene, men metoden ble utviklet for konvensjonell mjølking og er upraktisk til bruk i AMS-besetninger. Det har i tillegg skjedd forbedringer innen mjølkingsutstyr som gjør det nødvendig å oppdatere kunnskapen om hvordan resultatene fra MTT bør tolkes for å sikre at rådgivingstjenestene som baserer seg på MTT holder en høg kvalitet. Med stadig økende bruk av AMS er denne kunnskapen særlig relevant for denne besetningstypen.

I AMS mjølkes en ku om gangen, noe som gjør at kun ett sett med sensorer er nødvendig for å samle data under mjølking. Dette gjør AMS velegnet for bruk av sensorer for kontinuerlig overvåking av jurhelsa. Måling av somatisk celletall (SCC; somatic cell count) annenhver eller hver måned brukes i tradisjonelle husdyrkontroller. I besetninger med AMS gjør tilleggsutstyr det mulig å måle SCC for hver mjølking, noe som gir en kraftig økning i datamengden per ku. Det er behov for mer kunnskap om hvordan en kan skille mellom fysiologiske og patologiske svingninger for bedre utnyttelse av hyppige celletallsmålinger i forebyggende mastittarbeid.

Målet med denne avhandlingen var å skaffe ny kunnskap for å beskrive jurhelse i kyr uten klinisk mastitt ved bruk av hyppige celletallsmålinger, og å forbedre jurhelsa i AMS-besetninger ved å vurdere anvendbarheten av MTT i kombinasjon med data fra andre kilder.

For at resultater fra MTT skal være nyttig for arbeid med jurhelse må det finnes sammenhenger mellom jurhelse og resultatvariabler fra MTT. Arbeidet som presenteres i

denne avhandlingen viste at tolkning av resultater fra MTT er krevende fordi forskjeller mellom kyr har stor betydning for tolkning av resultatene. For eksempel ble det funnet en negativ sammenheng mellom SCC og vakuumnivå i kort mjøleslange under mjølking. Denne sammenhengen er sannsynligvis et resultat av forskjeller i mjølkestrøm mellom kyr, hvor kyr med høg mjølkestrøm vil ha en mer åpen spenekanal som gir høgere mjølkestrøm og dermed lavere vakuumnivåer. Vakuumnivåer målt i kort mjølkeslange med MTT kan derfor sees på som et resultat av kua som mjølkes heller enn av mjølkeanlegget. En konsekvens av dette er at en MTT alene vil ha liten verdi som en vurdering av hvorvidt mjølkeanlegget har en negativ påvirkning på jurhelsa.

Spenespissen er kuas førstelinjeforsvar mot mastittpatogener, og spenespisser i dårlig forfatning er assosiert med økt risiko for mastitt. Dette arbeidet viste at forekomsten av ru og fortykka spenespisser økte med synkende mjølkestrøm. Videre ble det funnet en sterk negative sammenheng mellom vakuumnivået i kort mjølkeslange og mjølkestrøm fra den enkelte spene. Disse sammenhengene kan være nyttige på følgende måter: 1) informasjon om mjølkestrøm kan brukes i kombinasjon med resultater fra en MTT for å gi bedre råd om besetningen, og 2) dersom en besetning opplever problemer med dårlige spenespisser kan informasjon om mjølkestrømmen brukes til å undersøke om mjølkeanlegget er en medvirkende årsak.

Denne avhandlinga har også bidratt til en bedre forståelse av muligheter og begrensninger ved bruk av hyppige celletallsmålinger som en indikator for intramammære infeksjoner (IMI) i kyr uten klinisk mastitt. Kartlegging av IMI med månedlige speneprøver gjorde det mulig å undersøke fordelinga i SCC i perioder med ulik jurhelsestatus. Selv om forhøyet SCC ble observer i perioder med IMI var det stor grad av overlapp i SCC-verdier mellom perioder med og uten IMI. Variasjonen i SCC ble videre undersøkt i lineære regresjonsmodeller. Disse viste at det er store fysiologiske svingninger i SCC. Bare en beskjeden andel av den totale variasjonen kunne forklares av IMI, ku-spesifikke faktorer og variabler fra AMS. Videre ble egenskapene til fire ulike SCC-baserte IMI-indikatorer undersøkt. På grunn av den store graden av fysiologisk variasjon og overlapp i SCC-verdier mellom perioder med ulik IMI-status ga indikatorene lavere sensitivitet når det ble krevd høgere spesifisitet. Justering av terskelverdier kan brukes for å tilpasse sensitivitet og spesifisitet til situasjoner med ulik toleranse for falske negative og positive testresultater.

Introduction

Background

Over the past decades, herd health management in dairy production has shifted focus from treatment of diseases in individual animals to preventive measures advocated by advisory services and veterinary practitioners (LeBlanc et al., 2006). Bovine mastitis is considered the most costly disease in dairy production worldwide (Bradley, 2002). Mastitis prevention is not only important for avoiding production losses, but also to improve animal welfare, to maintain good milk quality, and to minimize the usage of antimicrobials and thereby limit the development of antimicrobial resistance (LeBlanc et al., 2006). The outcome of an infection with a mastitis pathogen can range from a subclinical state to fatal disease. Subclinically infected cows might transmit bacteria to healthy cows, possibly resulting in more severe manifestations in the newly infected cows, and existing subclinical infections may also develop into clinical disease. Preventive udder health work should therefore emphasize identifying and reducing the number of subclinical mastitis cases.

In practice, there are many different approaches to mastitis prevention in dairy herds. In Norway, the Norwegian Dairy Herd Recording System (NDHRS) has been a cornerstone in the Norwegian mastitis control program (Østerås & Sølverød, 2009), not only enabling farmers to target specific areas of improvement in their own herds, together with a herd veterinarian or advisor, but also providing data for breeding programs for simultaneous improvement of disease resistance and increments in milk yields (Østerås et al., 2007; Heringstad & Østeras, 2013). A central part in the Norwegian mastitis control program has been to advise farmers on good milking routines and ensuring a properly functioning milking machine.

Reliable identification of animals by electronic ID collars or tags has been available since the 1980s, and this technology has provided opportunities for new management approaches in dairy production, exemplified by the implementation of individual concentrate feeding in free-stall systems and identification of animals at milking (Hogeveen et al., 2010). When an animal can be identified at milking, then auxiliary sensors can be placed in the milk line, and the recorded data can be linked to the individual animal. Recently, there has been an increasing interest in developing sensor-based decision-support tools, where sensors continually monitor the animals and alert the farmer when signs of disease or other events of interest (e.g., estrus) are detected (e.g., Norberg et al., 2004; Kamphuis et al., 2008b;

Mollenhorst et al., 2010; Løvendahl & Chagunda, 2010). Rutten et al. (2013) described the development of sensor systems in dairy health management, using four levels of increasing degrees of information provided to the farmers: 1) information solely from the sensor, 2) interpretation of the sensor data to provide information about the health status, 3) integrating sensor data and other information to form advice, and 4) the farmer or sensor system making a decision. However, Rutten et al. (2013) observed that no sensor system for mastitis at that time had reached higher than level 2, which indicates that the full potential of sensor-based mastitis detection is not yet reached.

Increasing the use of sensor technologies in dairy production presents the possibility of improving disease prevention, either directly, through decision-support tools, or indirectly, through integration with existing advisory services and dairy herd improvement (DHI) systems. From the dairy farmer's perspective, it is highly important that investing in new technologies pays off in terms of improved production or reduced (e.g., disease related) costs. The sensors, however, only provide indirect measurements of the biological outcomes of interest, and therefore evaluation of the output from sensor systems against biological observations is necessary. In this context, it is also important that both the normal variation of the biological observations and the variability arising from the sensor itself are considered.

Norwegian dairy production

Norwegian dairy farmers have a strong tradition of participating in the national dairy herd recording system, the NDHRS, which has been available for Norwegian farmers since 1975 and currently includes 98 % of Norwegian dairy herds (TINE SA, 2019a). The NDHRS has formed the basis for the development and improvement of the Norwegian Red breed, the dominant breed in Norwegian dairy production, encompassing more than 90 % of the dairy cattle population. The Norwegian Red is a dual-purpose breed, well suited to the traditional Norwegian system where bulls are kept on the farm and fattened. Data from the NDHRS made it possible to include clinical mastitis in the breeding program for Norwegian Red at an early stage (Heringstad & Østeras, 2013; Østerås et al., 2007).

Norwegian dairy production has undergone substantial structural changes during the last 20 years. While the average number of cows in Norwegian dairy herds was 13.7 in 1998, the number had increased to 27.6 in 2018 (Statistics Norway, 2018). According to NDHRS, during the same period of time, milk production per cow year increased from 6,200 kg to

7,987 kg (TINE SA, 2019b). As total milk production in Norway has remained stable, at a level of around 1,500 million L per year, a corresponding reduction in the total number of dairy cows has occurred during the same period of time. This shows that Norwegian dairy production is moving towards fewer, but larger herds with more high-yielding cows. It is expected that this trend will continue the coming years.

Out of 8,074 dairy herds registered in the NDHRS in 2016, 5,358 used a tie-stall system and 2,716 used a free-stall system. Among the free-stall herds, 1,659 used Automatic Milking Systems (AMS), whereas 1,057 used conventional milking (TINE SA, 2017). Average herd sizes differ substantially between AMS herds (48 cows), free-stall herds with conventional milking (29 cows), and tie-stall herds (18 cows) (TINE SA, 2017). Because free-stall herds are generally larger, 53 % of enrolled cows were housed in free-stall systems in 2016 (TINE SA, 2017). Impending changes in animal welfare legislation, in which tie-stall barns will be banned from year 2034, are expected to result in substantial changes in these figures. Although Norwegian herds are still small compared with those of our neighboring countries, the recent increase in herd size, production levels, and the use of automatic milking makes our production systems more similar to those of other Nordic herds.

Norwegian dairy farmers receive subsidies based on the number of cows in the herd. In the current agreement between the Norwegian State and the farmers' unions, the subsidy rate per cow decreases with increasing cow numbers (Anon, 2018). Consequently, larger herds are more dependent on income from the production output than smaller herds. Although larger herds might benefit from more efficient production, the same herds have often made bigger investments in buildings, equipment (e.g., AMS), and other production factors. Maintaining efficient production is therefore highly important for the growing group of dairy herds that are considered large under Norwegian conditions. In addition to optimized feeding and reproduction, mastitis prevention is an essential part of maintaining the efficiency in a dairy herd due to the requirement to avoid production losses, treatment costs, withdrawn milk, and, potentially, a lower milk price (due to poor milk quality) associated with this disease (Halasa et al., 2007; Halasa et al., 2009; Bradley, 2002). The ongoing changes in the Norwegian dairy production, together with the decreasing economic margins, mean that it is necessary for the dairy advisory services to develop their services and knowledgebase to keep in pace with the farmers, and to evaluate continually whether the services that they provide are likely to result in improvements for the farmers.

Automatic milking systems in Norway

The first AMS was introduced on commercial farms in the 1990s, and the first AMS was installed in Norway in 2000. Since then, the use of AMS has increased rapidly in Norway, and, by the end of 2018, approximately 50 % of the overall milk volume in Norway was produced in these systems (TINE SA, 2019b). Statistics from the Nordic Dairy Associations' Committee for Milk Quality Issues (NMSM) show that Norway has more herds using AMS than our neighboring countries, as illustrated in Figure 1. High labor costs, relatively high and stable milk prices, and a desire to combine family life with dairy farming have motivated many Norwegian farmers to invest in an AMS (Hansen, 2015). Furthermore, it can be expected that many farmers will reconstruct their animal-housing systems due to the ban of tie-stalls from 2034, and will take this opportunity to install AMS when converting to free-stall systems. With the rapid increase in the number of farms using AMS in Norway, there is both an opportunity and need for studies on various aspects of dairy production in these systems, including mastitis prevention. With comprehensive cow-level and herd-level data available in the NDHRS, Norway is well suited for research on milk production in AMS herds.

Because most AMS can only milk one cow at a time, production in each AMS is limited by the efficiency of the system (including handling time and washing routines), and the milking speed of the cows. Minimizing handling time and maximizing milking speed are especially important for herds with a targeted production (quota) close to that which can be achieved by each AMS. Milking-machine settings for maximized milking efficiency have been studied in the light of this challenge (Ferneborg & Svennersten-Sjaunja, 2015). However, it is a concern that some milking-machine settings, especially high vacuum levels, may result in damage to the teats, and therefore have a negative impact on udder health (Langlois et al., 1981; Mein et al., 2003). Furthermore, selection of fast-milking, high-yielding cows is another possible way of increasing the total milk production in each AMS. It is known that increased milking speed is associated with more patent teat canals, which is a known risk factor for mastitis (Grindal & Hillerton, 1991; Grindal et al., 1991). Hence, by breeding for increased milking speed, one possible side-effect is that the cows will be more susceptible to infection with mastitis pathogens. There is a need for more knowledge on the interaction between milking-machine settings, milking speed, and udder health, such that farmers can be provided with high-quality, evidence-based advice on these important aspects.

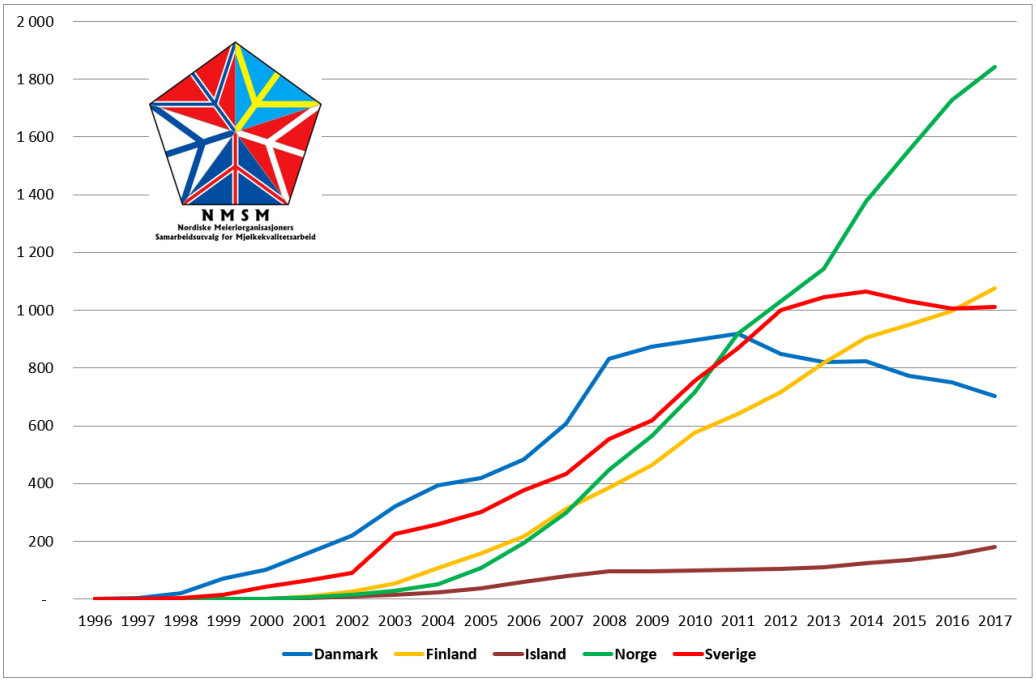

Figure 1. Number of dairy herds using AMS in the Nordic countries. Presented with permission from the NMSM.

Mastitis and mastitis prevention

Bovine mastitis is the most economically important disease in dairy production worldwide (Bradley, 2002), with financial losses due to decreased production in affected quarters for prolonged periods, veterinary treatment costs, and, under some circumstances, reduced product quality (Halasa et al., 2007). Preventing mastitis (as well as other diseases) is also important from an animal welfare perspective, as the disease can be painful and affect the general wellbeing of the cows. With increasing focus on animal welfare among consumers, it is important for the industry to continue their efforts to prevent diseases such as mastitis. Although it is documented through the NDHRS that the frequency of mastitis treatments has decreased substantially during the last two decades, mastitis is still an important disease in Norwegian dairy production, with an estimated annual economic loss of 120 million Norwegian kroner in 2018 (TINE SA, 2019a). Furthermore, an increase in somatic cell count (SCC) values in bulk tank milk are seen with increasing herd size, and the new infection rate also increased slightly from 2016 to 2018 (TINE SA, 2019b). This shows that

subclinical mastitis remains a challenge, and is possibly more pronounced in larger herds in which AMS milking is often used.

Antimicrobial resistance is an increasing concern worldwide. In line with the aim of the Norwegian government for a 10 % reduction in the use of antimicrobials in terrestrial food-producing animals by 2020 (Anon, 2015), Norwegian dairy production is continuing their efforts to reduce the use of antimicrobials further (Anon, 2017). Mastitis is the disease responsible for most antibiotic treatments in Norwegian dairy production, and therefore prevention of this disease is important for success in reducing antimicrobial use within the dairy sector (Anon, 2017).

Bovine mastitis is defined as an "inflammation in one or more quarters of the mammary gland, almost always caused by infectious microorganisms" (IDF, 2011). It is common to distinguish between clinical and subclinical mastitis, based on the clinical manifestation of the disease. When a cow shows any local or systemic signs of disease (redness, swelling, increased temperature in the affected quarter, pyrexia, pain, or abnormal milk), the case is classified as clinical, whereas subclinical cases can only be detected by using diagnostic tools.

In udder-health research, it is important to distinguish between mastitis and intramammary infection (IMI). Whereas, mastitis is defined as an inflammation in the udder, an IMI is diagnosed by the presence of mastitis pathogens in milk. Hence, an IMI may cause subclinical or clinical mastitis, but signs of inflammation are not always present. Mastitis in dairy cows is usually the result of an IMI, but inflammation may also arise from non-infectious causes, such as trauma to the udder. Different definitions of IMI can be found in the literature. For example, Zadoks et al. (2002) defined IMI on the basis of a combination of bacterial counts and persistence, whereas Berry and Meaney (2006) used the presence of pathogens as the single criterion in their definition.

Mastitis is a multifactorial disease, where infectious agents, the environment, and the immune system of the cow are all important components. Preventive measures may focus on one or more of these components; for example reducing herd prevalence or transmission of mastitis pathogens, to improve the hygienic conditions in the barn, or minimizing stress in cows to maintain adequate function of the immune system. In order to reduce the prevalence of mastitis pathogens, it is essential that cows with IMI are identified, because these cows might shed and transmit bacteria in large numbers, without showing signs of

disease. Hence, detection of IMI enables farmers to reduce the underlying infectious pressure in the herd by acting before an IMI turns into clinical mastitis. While an IMI diagnosis requires microbiological analysis, indicators of subclinical mastitis, such as SCC, are often used as proxies for IMI under field conditions.

Most mastitis cases are caused by pathogens accessing the udder through the teat orifice and the teat canal. Therefore, the ability of the cow to resist invasion of mastitis pathogens starts with the ability of the teat-end to form an efficient barrier between the udder and the environment (Paulrud, 2005). Sphincter muscles around the teat orifice and keratin-producing cells lining the teat canal form an anatomical barrier that prevent pathogens from entering the udder (Sordillo et al., 1997). The sphincter muscle contributes to closing the teat canal between milkings, and the keratin layer acts as a barrier by entrapping bacteria in the teat canal, which are then removed, together with the outermost part of the keratin layer, at the next milking (Capuco et al., 1992; Paulrud, 2005). An in-depth review of the teat-canal as a mastitis barrier can be found in Paulrud et al. (2005). It is recognized that milking-induced changes in the teat tissue may have a negative impact on these mechanisms (IDF, 1987). Systems for evaluating changes in the tissue around the teat orifice (i.e., the teat-end condition) have been developed (Neijenhuis et al., 2000), and a relationship between poor teat-end condition and clinical and subclinical mastitis has been described (Neijenhuis et al., 2001; Breen et al., 2009; Guarín et al., 2017). The mechanism behind an increased mastitis risk is likely a combination of a higher bacterial load on hyperkeratotic teat skin and impaired closing mechanisms in teats with poor teat-end condition. These, in sum, increase the likelihood of bacteria penetrating the teat canal and accessing the udder.

When mastitis pathogens overcome the anatomical barriers and gain access to the udder, the immune system will try to combat them by initiating an inflammatory response that is characterized by elevated blood flow to the udder, increased capillary permeability, and an influx of white blood cells to the affected quarter. A detailed review of the inflammatory processes in the udder with mastitis is outside the scope of this thesis, and may be found elsewhere (Ezzat Alnakip et al., 2014).

Mastitis detection

Clinical mastitis

In conventional milking (i.e., without automatic milking), teat stripping before attachment of the clusters may reveal abnormalities in the milk, and further inspection by, for example, the California Mastitis Test (CMT) or similar can be conducted when necessary. Such tests are often performed at the quarter level and are based on adding a reagent causing gel formation, where the viscosity of the gel is proportional to the DNA content of the milk, hence reflecting the cell content (Carroll & Schalm, 1962). Conventional milking often also includes manual palpation of the udder, and this reveals most clinical signs associated with cases of acute of mastitis. The cow can then be separated from the herd for appropriate treatment, and the milk is prevented from reaching the bulk tank, as is required by EU legislation (Regulation (EC) No. 853/2004), which is also implemented in Norwegian law. An AMS, however, must rely on automatic sensor-based detection of abnormal milk to fulfil the same purposes. For this reason, detection of clinical mastitis in AMS received considerable attention after the introduction of these systems (Kamphuis et al., 2008a; Kamphuis et al., 2008b; Kamphuis et al., 2010; Khatun et al., 2018). A decision on whether or not to discard the milk must be reached automatically at every milking by the AMS. However, management of (possible) cases of clinical mastitis, such as initiating treatment, isolation, or other measures, is performed by herd personnel when the cows appear on a "mastitis alert list" that is created by the AMS software (Hogeveen et al., 2010). Depending on the performance of the clinical mastitis indicators and algorithms, cows on these lists will be classified, either correctly or incorrectly, with clinical mastitis. Some cows might also be incorrectly classified as healthy, such that milk that should have been discarded reaches the bulk tank and these cows do not appear on the "mastitis alert list".

Using clinical mastitis as an example, data obtained from electrical conductivity sensors and color sensors may represent level 1 in the framework of Rutten et al. (2013). In this first level, data are presented, but have not yet been interpreted to classify the cows as healthy or diseased. At level 2, data from the sensors are used in detection algorithms based on established relationships and these are used to distinguish between healthy and diseased animals. The output from level 2 can, for example, be presented as a "mastitis alert list" as mentioned above. Rutten et al. (2013) observed that no sensor system at that time had reached level 3 (integrating sensor data and other information to form advice) or 4 (the farmer or sensor system making a decision). Although level 2 provides a list of (possible)

mastitis cases, more information might be needed to determine the appropriate action (e.g., culling, treatment, drying off) for the individual cows on the list. In this example, step 3 could include a review of disease history, pregnancy status, lactation stage, etc. to inform the farmer about which actions would be most economically viable. Level 4 is where the actual decision is made, either by the farmer or, autonomously, by the sensor system, and is based on information presented in step 3. In systems where the decision is made autonomously, there is also a possibility of actions being automatically initiated. In this example, this could be, for example, a call being placed to the veterinarian for prescribing treatment.

Subclinical mastitis

Although improvements in the detection of clinical mastitis have received attention, the potential for detecting subclinical mastitis by sensor systems available in AMS has been the subject of fewer investigations. As indicated earlier, detection of subclinical mastitis is also highly relevant because this information may be used in surveillance of the underlying infectious pressure in the herd, enabling targeted preventive measures against new cases of infectious mastitis. Hence, the sensor data collected in the AMS can be considered to represent an opportunity for further improvements in udder health, by potentially providing approaches for targeting subclinical mastitis.

SCC data have been used for decades as an indicator of inflammatory processes in the udder and for quality assessment of bulk tank milk. Composite-milk SCC is a widely used indicator of udder health (Schukken et al., 2003), and SCC data are commonly used in DHI programs worldwide, including the NDHRS since 1978 (Østerås et al., 2007). SCC are typically measured monthly or bimonthly, and the results are used for management purposes. In conventional systems, updated SCC results can, for example, be used to establish a milking order, in which healthy udders are milked before those that are suspected to be subclinically infected. Alternatively, the milking cluster can be disinfected after milking a cow with high SCC.

When information about a cow's SCC is accumulated over time, the data can be used for selecting candidates for selective dry-cow therapy or for culling. In the case of selective dry-cow therapy, confirmation of the diagnosis by culture results from aseptic quarter milk samples (QMS) is recommended (Whist et al., 2007). The current national guidelines for selective dry-cow therapy recommend that QMS are analyzed from cows with a geometric

mean SCC above 100.000 cells/mL on the last three samples from the monthly or bimonthly dairy herd recordings before drying off. Antibiotic dry-cow treatment is mostly advised if *Staphylococcus aureus*, *Streptococcus dysgalactiae,* or *Streptococcus agalactiae* are found in the QMS. If the geometric mean SCC is above 500.000 cells/mL, the prognosis is generally regarded as poor, and culling is often recommended (Norwegian Medicines Agency, 2012).

Following the development of AMS, auxiliary equipment for on-farm measurement of SCC has also been introduced. The technology varies between systems: whereas the Lely on-line SCC sensor operates at quarter level and, similar to the CMT, is based on measuring the viscosity in a milk sample after mixing it with a reagent (Mollenhorst et al., 2010), the DeLaval On-line Cell Counter operates at the cow-level and uses a staining technique and optical cell counting by digital image processing (Lusis et al., 2010), providing on-line cell count (OCC) data in the management software. Studies have demonstrated that detection of clinical mastitis is possible by using data from on-line SCC sensors in AMS (Sørensen et al., 2016), and that current methods for detection of clinical mastitis based on electrical conductivity are improved by including SCC data (Kamphuis et al., 2008b).

In current AMS systems, SCC may be monitored at every milking, thereby substantially increasing the amount of data collected per cow. The reason for measuring SCC is, in the first place, to monitor milk quality and udder health, such that the equipment investment and running costs can be compensated for by improved udder health and better milk quality. However, little is known about basic characteristics of frequent OCC measurements, and how such data may be processed for effective implementation in dairy herd management. Researchers in the field of precision dairy farming have already started exploring the possibility of utilizing frequently monitored SCC data for the detection of clinical mastitis (Kamphuis et al., 2008b; Sørensen et al., 2016; Khatun et al., 2018). However, reports are lacking about physiological fluctuations in frequently measured SCC and the extent of variability both within and between cows. This knowledge is highly relevant for future development of decision-support tools based on OCC data. Whereas clinical mastitis is often associated with a marked increase in SCC (Shuster et al., 1991), more subtle changes are expected in subclinical cases. Therefore, knowledge on physiological fluctuations in SCC becomes increasingly important when the intention is to use the OCC data for the detection of subclinical mastitis.

Improved udder health is an aim that is included in the breeding programs for many cattle breeds. Treatments against clinical mastitis and SCC data are both used in the evaluation of Norwegian Red cows. Although SCC is a frequently used indicator of udder health, fluctuations may be found without other signs of disturbances in udder health (Forsbäck et al., 2010; Dohoo & Meek, 1982). With more detailed data on SCC throughout lactation by using OCC, it might be possible to develop new and improved udder health traits for breeding programs. An initial step is to increase the knowledge on basic characteristics of frequent SCC measurements, such that physiological fluctuations can be distinguished from those arising from pathological processes in the udder.

Milking equipment and mastitis

Today's principle for milking cows, with vacuum suction and a pulsating two-chambered teatcup, has been used for more than 100 years (Reinemann et al., 2003), and the main components of the milking system remain the same, even in AMS; a vacuum pump, vacuum-regulating mechanisms, a pulsation system, a collection jar separating milk from air, and teatcups with a liner. In addition, automatic cluster removers, a necessity in AMS, are also in common use in conventional milking. A detailed description of the milking machine and its individual components is outside the scope of this introduction and can be found elsewhere (Mein & Reinemann, 2014).

The current theories on how the milking equipment contributes to increasing the mastitis incidence in dairy herds are, to a large extent, based on an International Dairy Federation (IDF) publication from 1987 (IDF, 1987), which has been updated and discussed more recently (Mein, 2012; Mein et al., 2004). These papers present five main mechanisms linking the milking machine to an increased mastitis risk: 1) changing the number of pathogens on the teat skin or teat orifice, 2) altering the resistance of the teat canal to invasion by mastitis pathogens as a result of mechanical forces applied on the teat tissue during milking, 3) reversed milk flow events, in which bacteria might enter the udder through the teat canal, 4) dispersal of pathogens within the udder, and 5) frequency and degree of udder evacuation, with special attention to residual milk in the udder after milking (IDF, 1987).

The relative importance of the milking equipment for udder health, as compared with other management factors, is an ongoing discussion. However, it is generally accepted that the milking process contributes to the overall new-infection risk by the mechanisms mentioned

above (Mein, 2012). In an epidemiological study among herds in northern Norway, Østerås and Lund (1988) found that 15 – 45 % of the variation in udder health at the herd level could be attributed to differences in milking-machine and milking-management variables. However, since this study was conducted, international standards for milking equipment have been systematically implemented through the Norwegian mastitis control program to improve milking equipment on Norwegian farms (Østerås & Sølverød, 2009). Furthermore, when Østerås and Lund (1988) collected their data, free-stall housing was still uncommon in Norwegian dairy production and AMS was not yet introduced. Thus, these data may be considered outdated, and it is necessary to revisit the potential impact of the milking machine on udder health under current conditions in Norwegian dairy herds.

Although AMS is based on the same principles for milking as conventional systems, there are some important differences that are related to the mechanisms linking the milking procedure to increased mastitis risk. Whereas most conventional milking systems have a milking cluster, where the four teatcups are connected through the claw, the teatcups of the AMS are connected directly to the milk receiver jar and operate individually. This enables the AMS to attach and detach the teatcups separately, making it possible to remove a teatcup as soon as the milk flow drops below a certain limit in one of the four quarters. As a result, the overmilking duration is reduced to a minimum (Hogeveen et al., 2001), possibly counteracting mechanism number 2) described above. Since the teatcups are not connected by a claw in an AMS, transport of milk (possibly containing mastitis pathogens) from one teatcup to another, which is a part of mechanism number 3), is not possible (IDF, 1987). Concerning mechanism number 5), compared with two milkings per day in most herds using conventional milking, the milking frequency is often increased in AMS herds. Furthermore, the increased milking frequency is often accompanied by early detachment of the teatcups and hence incomplete emptying of the udder. These differences make it relevant to study relationships between udder health and milking machine characteristics in AMS herds specifically.

Testing the milking system

There are three main approaches for testing milking-machine performance in AMS, as well as in conventional milking systems (Rasmussen et al., 2003): dry tests, wet tests, and milking-time tests (MTT). All three methods were established before AMS had been introduced, but because AMS uses the same main principles as conventional milking, the three methods are used in all systems. In a dry test, the technical properties of the system are

tested with the machine running, but without any involvement of cows or liquids. The results from dry tests are compared with reference values or guidelines to assess whether the system is working as intended. This approach is well suited for detection of errors, such as deficits in the vacuum regulatory mechanism, air leaks, and pulsator failure (Reinemann et al., 2001). In wet tests, the milking machine is operated as when milking, but an artificial udder or milk-flow simulator is used instead of live cows. In general, wet tests are not frequently used, but have the advantage that the response of the milking system to a standardized artificial udder can be measured and compared with reference values (Rasmussen et al., 2003). Finally, the MTT is based on observations and measurements made during milking of live cows. Whereas early MTT were based on vacuum measurements in the milkline, technical developments in the late 1990s made it possible to measure vacuum levels in the teatcup while the milking cluster was being operated (Rønningen, 2017). Measuring equipment and software for analyzing the recorded data are commercially available (Biocontrol, Rakkestad, Norway). The equipment consists of a portable, battery-powered vacuum logging unit that is attached to the teatcup. Steel or plastic tubes are inserted through the rubber of the teatcup to gain access to the different compartments (pulsation tube, short milk tube, and mouthpiece chamber), and silicone tubes are used to connect the vacuum logger to the measurement points. The vacuum logger records data from milking of one or more cows before the data are uploaded to a computer for analysis.

The MTT is now used worldwide and has partly replaced dry tests as the method of choice in the Nordic countries (Rønningen, 2017). With increasing technical complexity in AMS compared with conventional milking systems, dairy advisors tend to specialize in MTT, and leave dry testing in AMS to service personnel with in-depth knowledge about the specific AMS type. The methodology for MTT was initially developed for milking clusters in conventional milking. In conventional milking, MTT are often combined with recording of the milking routines, milking hygiene, and the general workflow during milking (Rønningen, 2017). However, this aspect becomes less important in AMS, where udder preparation is according to the settings in the AMS software. Furthermore, MTT are more time demanding and impractical in AMS herds because only one cow is milked at a time. This means it would be useful to investigate the possibilities of improving the methodology for use in AMS herds.

Previous research has shown that milking speed may be increased by increasing the system vacuum level (Rasmussen & Madsen, 2000; Spencer et al., 2007). Adjusting milking-machine settings for increased milking speed is a possibility in AMS herds aiming to increase production in an existing system. The International Organization for Standardization (ISO) recommends that the vacuum level at the teat end should be between 32 and 42 kPa in periods of high milk flow, and that the system vacuum level is set to achieve this (ISO, 2007). Exposing the teat to high vacuum levels may cause congestion in the teat tissue, and this may interfere with the closing mechanisms of the teat canal, thereby compromising its function as a barrier to mastitis pathogens (Hamann et al., 1993; Paulrud, 2005). The pulsation system counteracts this by allowing the liner to exert pressure on the teat during the massage phase of the pulsation cycle. The intensity of the massage depends on three factors: the characteristics of the liner (Gleeson et al., 2004b), the pressure difference across the liner wall that causes the liner to collapse (Gleeson et al., 2004b), and the duration of the massage phase of the pulsation cycle (Upton et al., 2016). Penry et al. (2016) investigated how different combinations of liners and vacuum settings affected milk flow rate, and reported that the highest milking speed was achieved at high system vacuum and with liners exerting high massage pressure. However, at the settings resulting in the highest milk flow rates, the authors reported signs of discomfort (stepping and kicking) in the cows prior to cluster removal (Penry et al., 2016). As noted earlier, impaired teat-end condition has been identified as a risk factor for clinical mastitis (Neijenhuis et al., 2001; Breen et al., 2009). One study has also reported a relationship between teat-end hyperkeratosis and subclinical mastitis, as evaluated by CMT (Lewis et al., 2000). Mein et al. (2003) investigated different liner types, and found more hyperkeratosis when using liners characterized by forceful massage. This brief overview shows that the milking system and its impact on the teat has been investigated in detail in previous studies. However, field studies of relationships between udder health and vacuum levels at the teat end during milking (as recorded by MTT) are lacking.

Vacuum levels recorded in the mouthpiece chamber (MPC) are known to be affected by teat dimensions relative to liner dimensions, and are therefore considered a measure of liner fit (Borkhus & Rønningen, 2003; Newman et al., 1991). High MPC vacuum levels have been shown to cause congestion in the teat tissue, leading to narrowing of the teat canal (Penry et al., 2017). Maintaining a low MPC vacuum is therefore important to facilitate efficient, yet gentle, milking. Rønningen (2017) reported that an increasing proportion of cows within a

herd with an MPC vacuum level between 10 and 30 kPa was associated with lower economic losses due to mastitis.

In order to continue improving udder health in Norwegian dairy production, it is important that all services provided to the farmers are critically reviewed to ensure that the time and money are directed towards activities that are likely to result in progress. Therefore, updated and increased knowledge on MTT as a tool for dairy advisors is necessary. Furthermore, the increasing use of AMS in Norwegian dairy herds makes it relevant to investigate whether knowledge from conventional systems can be used in AMS herds, or whether differences between the systems requires that interpretation of the results is modified.

Knowledge gaps

To summarize the knowledge gaps identified, three main research questions were formulated:

Can relationships be detected between biological outcomes of importance for udder health at the cow level and MTT variables?

Although MTT is frequently used in advisory services, knowledge on how to interpret MTT results is limited. Interactions between the milking machine and teat tissue have been studied in detail in experimental studies, but few studies have evaluated result variables from MTT against indicators of udder health under field conditions. The current situation is that advisors largely rely on their own experience when evaluating whether the results from an MTT indicate that the milking machine is contributing toward an udder health problem in a herd. It is therefore necessary to study relationships between biological outcomes of importance for udder health and results from MTT, such that the quality of advice given based on results from an MTT can be improved. The MTT equipment and methodology were originally developed for conventional milking systems, but AMS is now the predominant milking method in Norway. Therefore, it is relevant to investigate whether interpretations of MTT variables in conventional milking systems can be extrapolated to AMS.

Can MTT methodology be improved by using data from sensors in the AMS?

Performing MTT in AMS herds can be time consuming and impractical, because only one cow is milked at a time. However, advisors should be careful to collect enough data when suggesting changes to the milking system that will affect the entire herd. There is a need for new approaches to strengthen the basis for advice, but without spending too much time

recording data. For example, the AMS records a wide range of data from every milking, and the possibilities of utilizing these data to improve advisory services based on MTT, has not yet been fully investigated.

To what extent do frequently measured SCC data reflect udder health status?

Sensor systems in the AMS continually monitor parameters relevant for udder health management. It is, however, important that output from the sensors are evaluated thoroughly against known biological outcomes to ensure their relevance for the intended use. With the introduction of on-farm measurement equipment, SCC can now be recorded at every milking, resulting in a substantial increase in the amount of data available per cow. Whereas clinical mastitis is often associated with drastic changes in milk composition, including a marked increase in SCC, more subtle changes occur in subclinical cases. Fluctuations in SCC from milking to milking mean that it is challenging to turn these data into useful information, and more knowledge is needed to improve the use of frequent SCC recordings for distinguishing between physiological and pathological fluctuations in clinically healthy udders. This is especially important for quarters with subclinical mastitis, which can be important reservoirs for infectious udder pathogens.

Although frequent SCC measurements have been evaluated for the detection of clinical mastitis, basic characteristics of the variability in frequently measured SCC within and between cows have not yet been reported. Automatic detection of clinical mastitis is important in AMS herds, but detecting subclinical mastitis is probably more important for mastitis prevention. There is a need for more work on the ability of frequently measured SCC in on-line measurement systems to detect IMI.

Aims and objectives

The overall aim of this thesis was to provide new knowledge for characterizing udder health in clinically healthy cows by frequently measured SCC, and to improve udder health in AMS herds by evaluating the applicability of MTT, in combination with other sources of auxiliary sensor data. The specific objectives were to:

- Describe relationships between composite milk SCC and MTT result variables in AMS, milking parlors, and pipeline milking systems (Paper I)
- Describe relationships between MTT result variables and teat-end condition in an AMS herd (Paper II).
- Investigate whether data recorded routinely by sensors in AMS could be a substitute for, or supplement to, outputs from MTT in the management of teat-end condition and SCC (Papers II and III).
- Study the threshold between physiological and elevated SCC in cows free from clinical mastitis by explaining the overall variability in frequently measured SCC using bacteriological culture results, sensor information from AMS, and other cow data (Paper III).
- Evaluate the diagnostic test properties of OCC-based indicators for the detection of IMI, as detected by bacteriological cultures, and clinical mastitis (Paper IV).

Materials and methods

Four different studies were used in this thesis. One study combined results from previously performed MTT in Norwegian dairy herds throughout the country with SCC data obtained from the NDHRS (Paper I). The other three studies were based on data collected at the Animal Research Centre, Norwegian University of Life Sciences; one study was of relationships between teat-end condition and MTT results (Paper II), one study was of the variability in frequently measured OCC (Paper III), and one study was of the diagnostic properties of OCC for the detection of IMI (Paper IV).

Longitudinal study of frequently measured OCC

A longitudinal study was conducted at the Animal Research Centre at the Norwegian Univerisity of Life Sciences over a 17-month period from January 5th 2016 to May 22nd 2017, forming the basis for Papers III and IV of the current thesis. The research herd consisted of approximately 100 lactating cows housed in two lactation pens, each equipped with one AMS (DeLaval VMS, DeLaval International AB, Tumba, Sweden).

Most cows in the study herd belonged to one of two genetic groups of Norwegian Red, selected for high milk yield (1) or low incidence of clinical mastitis (2) (Heringstad et al., 2007). Genetic differences have previously been reported between the groups, also with respect to SCC (Heringstad et al., 2008).

The cows were housed on slatted floors, cubicles were equipped with rubber mattresses, and a thin layer of sawdust was used for keeping the cubicle surface dry. The floors were cleaned with a robotic scraper. Concentrate was provided in both the milking stations and the concentrate feeders in the free-stall area. The housing conditions and management practices in the herd were comparable to commercial dairy farms with AMS in Norway.

The two AMS in the research herd were equipped with a DeLaval Online Cell Counter that provided OCC measurements at every milking, in addition to the sensors installed as standard equipment. This resulted in a comprehensive dataset containing detailed registrations for every milking conducted during the study period. A summary of the data utilized in this thesis can be found in Table 1.

Table 1. Sensor data from the AMS used in Papers II-IV in this thesis. The variables were recorded at every milking.

Sensor	Variable	Cow-level	Quarter-level
Milk meter	Milk yield (kg)	X	X
	Average milk flow rate (kg/min)	X	X
	Peak milk flow rate (kg/min)	X	X
Conductivity sensor	Conductivity (average during milking)		X
On-line somatic cell counter	On-line cell count (OCC)	X	
	Cell count from the previous cow milked in the same AMS	X	
Other data	Milking interval	X	

Aseptic QMS were collected monthly during the study period. The samples were frozen shortly after collection and transported to the laboratory before being thawed. Bacterial culture was performed according to standard procedures (Hogan et al., 1999). Species identification was performed by matrix-assisted laser desorption ionization-time of flight mass spectrometry microflex LT (Bruker Corporation, Billerica, USA) (Cheuzeville, 2015).

Culture results from the QMS were used to assign an IMI status to each cow throughout the study period. The definition of IMI used in this study was adapted from that of Zadoks et al. (2002): a high concentration of a mastitis pathogen and/or persistence over time was required for a period to be defined as IMI. Positive culture results not associated with an IMI were classified as transient colonization. The mastitis pathogens were divided in two groups: Pat-1, consisting of pathogens from which a high cell count would be expected during an IMI, and Pat-2, consisting of other known mastitis pathogens. Pat-1 included *Staphylococcus aureus*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Enterococcus faecalis*, *Enterococcus faecium*, *Lactococcus lactis*, *Staphylococcus epidermidis*, and *Staphylococcus simulans* (Djabri et al., 2002; Reksen et al., 2008; Simojoki et al., 2009; Fry et al., 2014). Pat-2 included *Corynebacterium bovis*, *Staphylococcus chromogenes*, *Staphylococcus haemolyticus*, *Aerococcus viridans*, *Staphylococcus hominis*, *Staphylococcus xylosus*, and other bacteria cultured. Because the OCC data were recorded at the cow-level, the quarter IMI diagnoses were transformed to cow-level diagnoses. The

cows were thus assigned one of the following udder-health status groups at every milking: Healthy, Pat-1 IMI, Pat-2 IMI, or transient colonization.

Records of clinical mastitis treatments were collected retrospectively. According to the standard operating procedures in the research herd, cows receiving antimicrobial treatment were kept in a separate pen and milked with a separate milking system during the treatment period and withdrawal period. Consequently, for cows that were treated, OCC data were collected until the detection of clinical mastitis, but no OCC records were available during treatment and withdrawal periods.

In order to evaluate the agreement between OCC values and SCC measured in a DHI laboratory, additional composite milk samples were collected for a subset of milkings (n = 1661) in one of the two milking stations. The samples were conserved with bronopol and shipped to a central laboratory used in NDHRS for analysis. Furthermore, the repeatability of the OCC sensors used in the study was evaluated by repeated OCC measurements in one bulk milk sample.

Teat-end condition and milking-time testing

At the first QMS sampling occasion in the longitudinal study, an additional cross-sectional study was initiated, aiming to investigate whether data from the AMS could be useful in the management of teat-end condition. Immediately after collecting the QMS, the teat-end condition was evaluated using the classification system of Neijenhuis et al. (2000). In this system, the teat skin around the teat orifice (the callosity ring) is classified according to its thickness and surface roughness (Neijenhuis et al., 2000). In addition, the length of the teats, and the width at basis and apex were measured, and the teat shape (cylindrical, conical) and teat-end conformation (flat, round, pointed, or inverted) were registered.

To obtain MTT results from milking of all teats in the herd, MTT were performed using VaDia vacuum loggers and corresponding software (Biocontrol, Rakkestad, Norway) as described in a separate section. We collected data on quarter milk yield, and average and peak milk flow rates from the AMS software (DelPro, DeLaval, Tumba, Sweden). The data formed a cross-sectional dataset, where each teat had a corresponding teat-end score, a set of MTT results, teat dimensions, and information on milk flow from the milking in which the MTT data were recorded.

Two dichotomous outcome variables (THICKNESS and ROUGHNESS) were established based on the results from the teat-end scoring according to Table 2.

Table 2. Transformation of results from the scoring system by Neijenhuis et al. (2000) to the dichotomous outcome variables THICKNESS and ROUGHNESS.

Callosity ring thickness	**Callosity ring roughness**		THICKNESS
	Smooth	Rough	
No callosity ring	N	-	0
Thin	1A	1B	
Intermediate	2A	2B	1
Thick	3A	3B	
Extreme	-	4	
ROUGHNESS	0	1	

Somatic cell count and milking-time testing

Data for the study of relationships between SCC and MTT results were collected by inviting advisors in the TINE advisory service to upload a copy of their locally stored databases containing results from MTT that had been performed previously. These advisors regularly perform MTT as a part of their services to the farmers, either as a routine check of the milking system or in cases of problems with udder health or milk quality. The advisors are equipped with VaDia vacuum loggers and corresponding software for conducting the test and had been trained to conduct MTT according to standardized routines. The database contained a set of MTT result variables per animal, including farm ID and animal ID.

Production and health data for the cows represented in the MTT data were collected from the NDHRS, and SCC data, measured as close as possible before the day of the MTT, were used as an indicator of udder health status. The dataset also contained records of clinical mastitis treatments.

Three different milking systems were represented in the data: AMS, milking parlors, and pipeline milking systems. The statistical analyses were performed separately for each of the three milking systems to allow for comparisons between them.

Milking-time testing methodology

Papers I and II both include data from MTT conducted using VaDia vacuum loggers and corresponding software (Biocontrol, Rakkestad, Norway). Schematically, an MTT consists of two distinct phases: 1) data collection (vacuum measurements), and 2) data processing

for calculation of result parameters. For Paper II, the data collection was performed by attaching one VaDia vacuum logger to each of the four teatcups of the two AMS used in the research herd. The activated vacuum loggers were firmly taped to the teatcup, ensuring that the equipment did not interfere with the robotic arm. Thereafter, stainless steel tubes were inserted through the rubber in the pulsation tube, short milk tube, and MPC, and connected to the vacuum loggers using silicone tubes. The AMS operated in automatic mode after attachment of the vacuum loggers, and the cows were allowed to enter the milking station voluntarily. The vacuum loggers were subsequently disconnected from the teatcups, and the data files were uploaded to a computer with the appropriate software for analysis. In Paper I, we collected results from previously performed MTT. The procedure used by the field advisors is the same as that described for Paper II, except that vacuum measurements under field conditions are routinely performed on one rear teat only.

The data analysis is a semi-automatic process. The operator identifies a milking based on a graphical presentation of the recorded vacuum levels and selects the relevant time period (one milking at a time) for further analysis. The individual milking is then divided into four phases based on characteristics of the vacuum curves: 1) the let-down period where the teatcup is attached, but the vacuum levels are not yet stable, 2) the main milking period, 3) the overmilking period, and 4) takeoff. The software is programmed to identify the start of milking, the transition between the different phases, and the end of milking, based on a set of criteria summarized in Table 3. The standard operating procedures for the MTT service also contains a written description of these definitions, and the automatic detection is reviewed by the operator who may adjust the transitions based on their best judgement. After dividing the milking into four phases, result variables are calculated, including: 1) duration of the different phases, 2) average vacuum level in the short milk tube during a) the main milking period, and b) the overmilking period, 3) average vacuum level in the MPC during a) the main milking period, and b) the overmilking period, and 4) counts of sudden vacuum drops (irregular vacuum fluctuations). The data for Paper II also included a variable, “teat-compression intensity”, in which the forces applied to the teat by the liner during the closed phase of the pulsation cycle have been estimated. In brief, the pressure difference across the liner wall was calculated and the duration of the massage phase was identified to express the combined effect of mechanical forces and time.

Table 3. Criteria used by VaDia software to determine transitions between different phases of milking, as used in Papers I and II.

Transition point	Description
Start	First registered vacuum level above 25 kPa in the short milk tube
Start of main milking	Average vacuum level in the short milk tube is calculated in 10 second intervals from the defined start of milking. When the difference between two 10-second periods is less than 0.3 kPa, the start of the main milking is set to the midpoint of the first of the two 10-second periods.
Start of overmilking	The transition is based on vacuum measurements in the MPC, and is set to the time when there is a marked increase in the variation in the MPC vacuum.
Start takeoff	The start of takeoff is set to when the vacuum drops lower than 5 kPa from the maximum level recorded during milking.
End of milking	The maximum vacuum level in the short milk tube is calculated in 1-second intervals throughout the milking, and the end of the milking is set as when the maximum value is less than 5 kPa.

Statistical analyses

Multilevel linear and logistic regression

Linear regression was chosen as the method for evaluating relationships between the outcome variables and potential explanatory variables in Papers I and III. SCC-values were used in Paper I, whereas Paper III used OCC-values as the outcome. The original values were transformed to a natural logarithmic scale before analysis to approach a normal distribution.

The linear regression model comes with a set of assumptions: 1) the values of the outcome variable are statistically independent, 2) the variance of the outcome variable is constant at all levels of the predictor variables, 3) the residuals follow a normal distribution, and 4) the relationships between the outcome variable and continuous explanatory variables are linear (Dohoo et al., 2009). The hierarchical structure, with OCC values clustered in cows and lactations within cow (Paper III) and SCC values clustered within herd (Paper I), represents a situation where the first assumption is likely to be violated. Multilevel linear regression

with random intercepts was therefore chosen to model these data. In this framework, the intercepts are allowed to vary between groups, and the coefficients describing the effect of an explanatory variable on the outcome are assumed to be the same for all groups. In Paper I, a two-level linear regression model, with random intercept at herd level, was chosen to account for the lack of independence between cows from the same herd. This approach allowed the overall SCC to vary between herds, such that, for example, differences between herds in management practices affecting SCC were accounted for. The variability residing at herd level was evaluated using the intraclass correlation coefficient. In Paper III, a three-level model, with random intercepts at cow-level and lactation-level within cow, was selected. Because the data contained repeated measurements of OCC over time at the lowest grouping level, it was also necessary to apply a correlation structure to account for correlations between residual errors from adjacent observations within cow and lactation.

In Paper II, the outcome of interest was the teat-end condition, which was classified in one of eight different categories based on the thickness and roughness of the callosity ring. This was transformed into two dichotomous outcome variables describing whether the teat ends were classified as thickened (1/0) or roughened (1/0), respectively. Logistic regression models were used to describe relationships between the outcomes and explanatory variables. Due to lack of independence between the four teats in a single cow, we used multilevel logistic regression models, with a random intercept at the cow level.

To evaluate the goodness of fit for the linear mixed model used in Paper II, the method of Nakagawa and Schielzeth (2013) was used. While the goodness of fit in simple linear models is often reported as the coefficient of determination, R^2, a standard method has not been established for describing the proportion of variance explained in linear mixed models. Nakagawa and Schielzeth (2013) presented the marginal and conditional coefficient of determination for the purpose, which quantifies the variance explained by the fixed effects, and by the fixed and random effects combined, respectively. The approximate contributions of the individual variables to the overall coefficient of determination were estimated by observing the change in the marginal coefficient of determination when one independent variable was removed at a time from the model.

Principal component analysis

The MTT data used in Papers I and II contained several intercorrelated variables. To avoid collinearity in the regression models, it was necessary to apply a variable selection

procedure. To help guiding the variable selection, and as a descriptive statistic, we applied Principal Component Analysis (PCA). Briefly, this is a technique that transforms the original set of (correlated) variables to uncorrelated variables, called principal components, describing the variability in the data. The first principal component will, by definition, describe more of the variability than the second, the second more than the third, and so on. The contribution of the original variables to the principal components are called loadings, whereas the contribution of the individual observation is called the score. The two-dimensional plot of loadings on principal components 1 and 2 can be used for a graphical assessment of relationships between potential explanatory variables. The technique is described as being useful in variable selection procedures in epidemiological research (Dohoo et al., 2009). In Paper II, PCA was also used to assess relationships between teat dimensions, MTT result variables, and milk-flow data from the AMS software.

Diagnostic test evaluation

Paper IV evaluated the performance of OCC as an indicator of clinical and subclinical mastitis, and diagnostic test evaluation was used for the purpose. A diagnostic test can be a laboratory method, a clinical sign, or any other measurable factors that indicates the presence or absence of a certain disease. A perfect test will classify all healthy test subjects as being healthy and all sick subjects as being sick. However, due to biological variation and the characteristics of the methods used, this level of classification accuracy is usually out of reach, and the performance of a diagnostic test must therefore be evaluated against a known gold standard. The sensitivity (Se) of a diagnostic test describes the test's ability to classify sick animals correctly, whereas the specificity (Sp) describes the test's ability to classify healthy animals correctly. When a gold standard is used in the diagnostic test evaluation, the Se and Sp can be calculated as illustrated in Table 4.

Table 4. Calculation of diagnostic test properties using a gold standard.

Test result	**Gold standard**		
	Diseased	Healthy	
Positive	a	b	a + b
Negative	c	d	c + d
Total	a + c	b + d	n

Sensitivity = a / (a + c)

Specificity = b / (b + d)

For tests where the result is reported on a continuous scale, such as SCC, it is necessary to use threshold values to enable the results to be used to classify the cows as either healthy or sick. However, if the distributions of the test variable for the sick and healthy groups overlap, some misclassification will occur when threshold values are applied, resulting in suboptimal performance of the diagnostic test. In this situation, the threshold value can be adjusted to include all sick individuals in a population, but this will be accompanied by a misclassification of some healthy animals as sick. In many situations the user of the diagnostic tests must decide which is more important: to detect all sick individuals or to avoid classifying some healthy animals as sick.

The diagnostic properties of OCC for detection of episodes of IMI and new cases of clinical mastitis were investigated in Paper IV. For subclinical mastitis, three different patterns of OCC were evaluated: a single OCC value, a 7-day rolling average OCC, and Elevated Mastitis Risk (EMR; Sørensen et al. 2016). The EMR uses the level and trend of smoothed OCC values to form a mastitis indicator ranging from 0 to 1, where values close to 0 indicate low mastitis risk, and higher values indicate increased mastitis risk. For clinical mastitis, a 48-h rolling average OCC was used instead of the 7-d average. Four levels of specificity (80%, 85%, 90%, and 99%) were selected, and the sensitivity was calculated at each of these levels.

Agreement and repeatability

The agreement between OCC values and SCC measured in a DHI laboratory was evaluated using the concordance correlation coefficient. Repeatability was evaluated by calculating the coefficient of variation for each of the two sensors.

Summary of results

Relationships between udder-health related outcomes and MTT results

Papers I and II included studies of relationships between udder-health related outcomes and MTT results. The same MTT equipment and methodology were used in Papers I and II. In Paper I, SCC was the outcome and used as an indicator of udder health. In Paper II, teat-end condition was chosen as the outcome variable due to its previously described relevance for udder health.

In Paper I, relationships between lnSCC and MTT variables were evaluated in linear regression models for each milking system. An initial assessment of the variability by intraclass correlation coefficient showed that differences between herds accounted for 7 %, 8 %, and 6 % of the variation in lnSCC in herds using AMS, milking parlor, and pipeline milking systems, respectively. Descriptive statistics showed that most observations had vacuum levels within the ISO recommendations (ISO, 2007). Adjusting for the herd effect (random intercept at herd level), lactation stage, parity, and milk yield, we found that an increase in the duration of the main milking period was associated with a decrease in lnSCC in all milking systems investigated (AMS, milking parlors, and pipeline milking systems). A negative relationship between average vacuum level in the short milk tube during the main milking period and lnSCC was found in both AMS and milking parlors. Hence, increasing vacuum levels were associated with decreasing lnSCC. No significant relationships were found between lnSCC and MPC vacuum or irregular vacuum fluctuations in the multivariable models. The descriptive results showed that the duration of the overmilking period was shorter in AMS than in milking parlors and pipeline systems. The overmilking duration had a significant relationship with lnSCC in the univariable analysis for pipeline milking systems, but the effect was not significant when adjusting for other variables. Furthermore, based on the final models for each of the three milking systems, estimated differences in SCC between the 25th and 75th percentiles (interquartile range) were calculated for the explanatory variables; main milking duration and average vacuum level in the short milk tube during main milking. The results showed that the estimated differences across the interquartile range were small, ranging from -11,800 cells/mL to -22,390 cells/mL for main milking duration, and -4,520 cells/mL and -8,050 cells/mL for average vacuum level in the short milk tube.

Relationships between teat-end condition and MTT results were investigated in Paper II, which included results from 251 teats in 65 cows. The univariable analysis indicated that relationships existed between the outcome variables (thickened and roughened callosity ring, respectively) and four of the potential explanatory variables: vacuum level in the short milk tube during the main milking, teat compression intensity, average milk flow rate, and peak milk flow rate. Due to evidence of correlations between the explanatory variables found in the PCA, relationships between the outcomes and sets of explanatory variables were evaluated in separate models, and selection of the final model was based on the Bayesian information criterion (BIC). The results from the multivariable models showed that both alterations of the teat end were significantly associated with all four explanatory variables, also when adjusting for lactation stage, parity, and milk yield. The models using average milk flow rate had the lowest BIC value (better fit) for both outcomes (thickness and roughness), although the differences in BIC were generally small between the four models.

Sensor data from the AMS as a substitute or supplement to MTT

As a part of the model-building process in Paper II, PCA was used to evaluate relationships between the potential explanatory variables. A two-dimensional loading plot clearly showed that the data contained groups of correlated variables: the variables based on vacuum levels in the short milk tube were positively correlated with each other, and negatively correlated with average and peak milk flow rate. Furthermore, the teat-dimension variables were positively correlated, and negatively correlated with vacuum levels in the MPC in the main milking and overmilking phases.

A strong relationship between average vacuum level in the short milk tube during the main milking and average milk flow rate was confirmed in a linear regression model (Paper II). The model had a coefficient of determination (R-squared) of 0.71, and, for the system used in this study, the relationship could be described by the following equation:

Average vacuum level in the short milk tube = 42.9 – 0.38 x Average milk flow.

The average milk flow rates per quarter is useful information for comparison with other studies, but were not reported in Paper II. Average values were calculated for the purpose of this thesis, and were found to be 0.7 kg/min (standard deviation; SD = 0.29) for front quarters and 0.8 kg/min (SD = 0.25) for rear quarters.

Data on milk flow rate per quarter were obtained during the longitudinal study in the NMBU research herd. Results from Paper III showed that increasing milk flow rate was associated with increasing SCC.

Somatic cell count as an indicator of udder health status

Variability in frequently measured OCC

Basic characteristics of fluctuations in OCC were presented in Paper III, which included data from a total of 62,471 milkings. Lactations where a case of clinical mastitis had been registered were omitted from the analysis. The data were modeled in three-level linear regression models (milkings within lactations within cows).

In the final model, the following fixed effects explained 15.0 % of the variability in ln-transfomed OCC values: a lactation curve described by days in milk (DIM) and lnDIM, yield at the current milking, lnOCC from the previous cow milked in the same AMS (carry-over effect), parity, udder health status, conductivity (inter-quarter difference), genetic lineage, average milk flow rate, and season. The degree of explanation increased to 55.2 % when also accounting for differences between cows and lactations within cows (random intercepts at cow level and lactation level within cow). The individual fixed effects explained only small parts of the variability. With the selected calculation method, the IMI status, as determined by culture results from monthly QMS, explained 2.9 % of the variability. This is, however, a conservative estimate because the model includes other variables (e.g., conductivity) that also account for changes in udder health.

The distribution in lnOCC within the four different IMI status groups (No IMI, Pat-1 IMI, Pat-2 IMI, and transient colonization) is presented graphically in Figure 2. Observations classified as No IMI generally had lower lnOCC than Pat-1 IMI and Pat-2 IMI.

The study of agreement between results from one of the DeLaval OCC sensors used in our study and SCC results from an International Committee for Animal Recording (ICAR)-accredited lab resulted in a concordance correlation coefficient of 0.82 (95 % CI: 0.78 – 0.85), showing that the concordance was reasonably good. Furthermore, the repeatability of the two OCC sensors was evaluated by repeated analyses from the same milk sample. The coefficient of variation (CV) was 0.11 for both sensors, with a mean OCC value of 115,000 cells/mL.

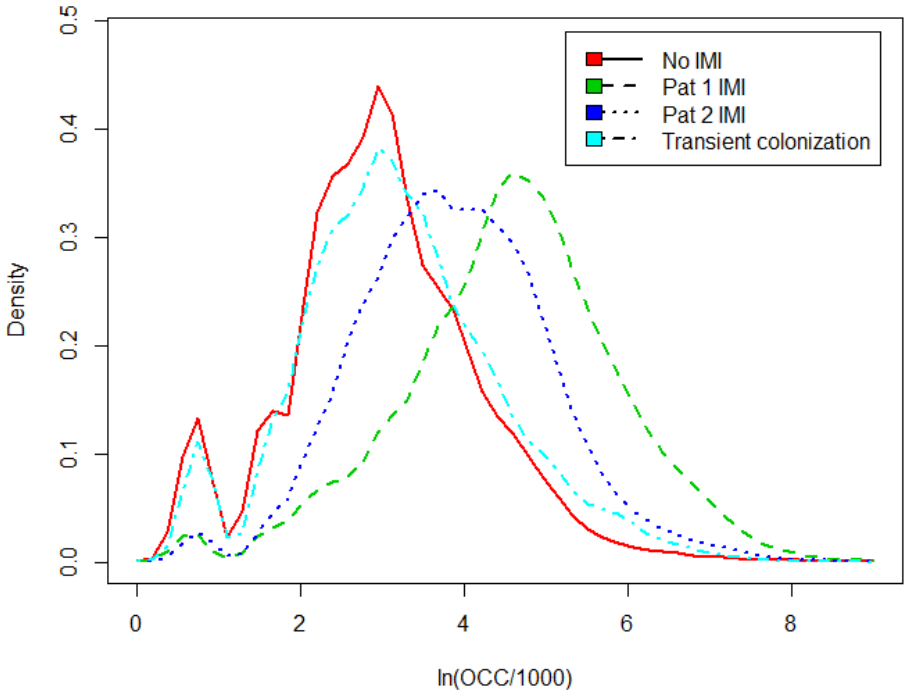

Figure 2. Distribution of lnOCC values in periods of different IMI status. Figure from Nørstebø et al. (2019).

OCC as an IMI indicator

Bacteria were cultured in 1,222 out of 5,330 quarter milk samples (QMS) collected during the study period. A detailed summary of the microbiological diagnoses can be found in Paper IV, Table 1. In summary, the most commonly cultured pathogens were: *Staphylococcus epidermidis* (n = 234), *Corynebacterium bovis* (n = 225), *Staphylococcus chromogenes* (n = 167), *Staphylococcus aureus* (n = 119), and *Staphylococcus haemolyticus* (n = 116). Based on these results, 106 episodes of Pat-1 IMI and 117 episodes of Pat-2 IMI not coinciding with a Pat-1 IMI in the same cow were identified.

At the time of QMS collection, 7-day average OCC values in 1000 cells/mL (95 % CI) were 260 (224-298), 83 (70-96), 124 (56-192), and 53 (44-61) for Pat-1 IMI, Pat 2-IMI, transient colonization, and no IMI, respectively.

The IMI status based on the microbiological diagnoses or new cases of clinical mastitis were used as the gold standard in the diagnostic test evaluation. The ability of the different OCC-based indicators (single OCC value, EMR, rolling average) to classify IMI or new cases of clinical mastitis correctly were evaluated at four levels of specificity (80 %, 85 %, 90 %, and 99 %). In general, higher sensitivities were found for Pat-1 IMI than for Pat-2 IMI at the defined specificity levels. Furthermore, a decrease in sensitivity was observed when a higher specificity was set. For example, the EMR detected Pat-1 IMI with a sensitivity of 69 %, 59 %, 48 %, and 8 % at the specificity levels of 80 %, 85 %, 90 %, and 99 %, respectively. Complete results are reported in Paper IV, Table 4.

Receiver operating characteristic (ROC) curves for the detection of Pat-I IMI using the different indicators are presented in Figure 3. The results show that in this herd, the EMR and rolling 7-day average of OCC performed better than a single OCC for the detection of Pat-1 IMI. Detailed results are reported in Paper IV.

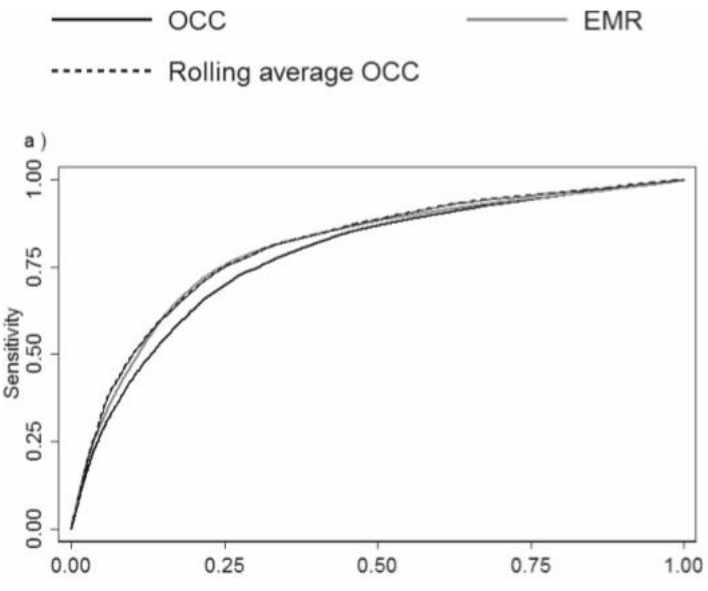

Figure 3. ROC curves for the detection of Pat-1 IMI using a single OCC value, 7-d rolling average OCC, and the EMR indicator. Figure from Dalen et al. (2019a).

Discussion

The MTT can be considered a sensor system that measures the interaction between the cow and the milking machine, where the results partly reflect the milking machine and partly reflect physiological aspects of the cow. As underlined by Rutten et al. (2013), sensor data need validation against a known biological standard or state to ensure that the output from the sensor system is relevant for its intended purpose. The concept of MTT in dairy advisory services relies on a sparsely documented assumption that the MTT can detect factors associated with poor udder health. The studies of relationships between udder-health related outcomes and MTT results presented in this thesis have contributed to a better understanding of the strengths and limitations of the MTT as a tool for udder health advisory services. Furthermore, using sensor data from the AMS in combination with MTT results is proposed as a way of improving advisory services.

While monthly or bimonthly SCC data have been successfully used for decades as an indicator of subclinical mastitis for the individual cow, the introduction of equipment that measures OCC at every milking provides a new situation, in which the amount of data is dramatically increased. Two of the studies presented in this thesis contribute to our understanding of the basic characteristics of these frequent OCC measurements in cows that are free from clinical mastitis, and indicate how the data might be utilized to contribute to improved udder health by detecting cases of subclinical IMI during lactation.

This section will elaborate further on how the results contribute to the overall and specific aims of the thesis, followed by methodological considerations, a validity discussion, and some thoughts on future perspectives.

Relationships between udder-health related outcomes and MTT results

An essential step in evaluating the applicability of MTT in AMS herds was to investigate whether relationships between MTT result variables and outcomes related to udder health could be documented. The results presented in this thesis show that interpreting results from an MTT is challenging because differences between cows have a large effect on the results. Most observations in the data used in Paper I had vacuum levels within the ISO recommendations (32 – 42 kPa during periods of high milk flow) (ISO, 2007). Both increasing vacuum levels in the short milk tube and increasing duration of the main milking period were associated with decreasing SCC. This finding is highly relevant for advisors using MTT, because it shows that high vacuum levels in the short milk tube (within the

values recommended by ISO) should not be interpreted as a risk factor for elevated SCC at the cow level. The relationships between lnSCC and MTT result variables were consistent across the different milking systems, indicating that knowledge from conventional systems can be extrapolated to AMS despite differences between the systems.

A likely explanation for the decreasing SCC with increasing vacuum levels and milking duration is that the outcome is related to the anatomical characteristics of the teat canal in the individual cow; with the same system vacuum settings, more patent teat canals will produce a higher milk flow rate, which results in a lower vacuum level in the short milk tube, and, at the same time, makes the teat more susceptible to the introduction of mastitis pathogens. This suggested interpretation is supported by Grindal and Hillerton (1991) who reported significantly higher new infection rates in cows with high milk flow rates (Grindal & Hillerton, 1991), and also by reports of negative genetic correlations between milking duration and SCC, and average milk flow and SCC (Berry et al., 2013; Gray et al., 2011; Prendiville et al., 2010). Finding a positive relationship between lnOCC and average milk flow rate when investigating the variability in frequently measured OCC also indicates the same explanation.

In contrast, there was an increased likelihood of finding roughened or thickened teat-end callosity rings with increasing vacuum levels in the short milk tube. Several previous studies have shown a relationship between severely impaired teat-end condition and occurrence of clinical mastitis (Neijenhuis et al., 2001; Breen et al., 2009) or subclinical mastitis (Gleeson et al., 2004a). However, one study reported that they could not detect any effect of teat condition on udder health (Zoche-Golob et al., 2015). Nevertheless, it can be argued that poor teat-end condition should be avoided, because this is a clear sign of unnecessary stress on the teat tissue. The study of relationships between teat-end condition and MTT results was conducted in one herd in which the same milking-machine settings were used for all cows. Hence, although vacuum levels in the short milk tube varied between cows, the variation in teat-end condition in this study must reflect differences between cows. The strong relationship between the average milk flow rate and the vacuum level in the short milk tube indicates that differences in milk flow rate between cows explain the variation in vacuum levels in the short milk tube. Furthermore, models using average milk flow rate performed better (as evaluated by BIC) than models using average vacuum level in the short milk tube in predicting the presence of a thickened or roughened teat-end callosity ring.

The negative relationship between SCC and vacuum level in the short milk tube, and the increased likelihood of impaired teat-end condition with increasing vacuum levels in the short milk tube might seem contradictory. However, most observations in our field study had vacuum levels within the guidelines suggested by ISO, and it is possible that negative effects on udder health caused by impaired teat-end condition do not occur frequently when vacuum levels lie within these guideline boundaries. Although moderate degrees of teat-end callosity can be considered as physiological adaptations to the forces applied to the teat tissue during milking (Sieber & Farnsworth, 1981), severe degrees of teat-end callosity are associated with an increased mastitis risk (Breen et al., 2009). The classification of thickened and roughened teat-ends used in this thesis include tissue changes of lesser severity than those associated with an increased mastitis risk. Hence, the relationship between teat-end condition and vacuum level in the short milk tube described in this thesis probably represents a combination of physiological adaptation and alterations that are possibly involved in an increased mastitis risk. Furthermore, factors other than the milking machine, such as environmental factors and genetics, are involved in the development of teat-end callosity (Mein et al., 2001). It is therefore necessary to combine an evaluation of teat-end condition with an MTT before a conclusion can be reached on whether the milking machine is likely to have a negative impact on udder health.

Breeding for higher milk flow rates is one possible way of increasing the production capacity in AMS herds. However, because differences in milk flow rate between cows was a likely explanation for the relationship described between lnSCC and vacuum level in the short milk tube, the downside of breeding for increased milk flow rate is likely to be an increase in SCC. To compensate for this and to maintain a stable udder health status, herds selecting cows for increased milking speed should also implement more effective preventive measures against IMI.

A relationship between MPC vacuum and udder health was first described by Rasmussen (1997), who reported that a difference in the MPC vacuum of 10 kPa was associated with a 3.4 % difference in the mastitis incidence rate at the quarter level. However, the author reports that one of two study herds had an outbreak of pseudocowpox during the study period, and this might have affected the results (Rasmussen, 1997). Rønningen (2017) (results first presented in 2002) reported an association between herd-level economic losses due to mastitis and the percentage of cows with either low (< 10 kPa) or high (> 30 kPa) MPC vacuum levels (Rønningen, 2017). In contrast to both Rasmussen (1997) and

Rønningen (2017), no significant relationship between SCC and MPC vacuum levels was found in the work described in this thesis, perhaps indicating that the relevance of MPC vacuum levels from an udder-health perspective is of little importance under the current conditions in Norwegian dairy herds. However, experimental studies have shown that a high vacuum level in the MPC of the teatcup causes congestion in the teat tissue, possibly affecting the milking speed (Penry et al., 2017). The results from Paper II showed that the MPC vacuum was associated with the dimensions of the teats, with lower MPC vacuum levels measured in teats with larger dimensions. In Paper I, the PCA results indicated that high MPC vacuum levels were more often found in first parity cows than in cows of third and later parities, probably reflecting differences in teat dimensions between parities (Zwertvaegher et al., 2012). The MPC vacuum level might therefore be of value when assessing liners for optimized milking speed and gentleness.

Sensor data from the AMS as a substitute or supplement to MTT

The results presented in this thesis show that vacuum levels in the short milk tube recorded by MTT should be interpreted with care because the recorded vacuum level is not only an effect of the settings of the milking system, but is also influenced by differences between cows. An MTT is often performed on a limited number of animals per herd. In our field data, an average of seven milkings were evaluated per herd. Because adjustments of the milking-machine settings will typically affect the whole herd, an advisor must be careful not to make inferences for the whole herd based on a non-representative number of samples in the herd. This may be more important in AMS herds, because, under Norwegian conditions, the herds are generally larger, and because the procedure is more time consuming when only one cow is milked at a time (which may lead to fewer observations).

One of the objectives in this thesis was to investigate the possibilities of using the data that are routinely recorded by sensors in the AMS to evaluate whether the milking procedure affects the teats. The strong relationship between the vacuum level in the short milk tube (as recorded by MTT) and average milk flow rate recorded by the AMS can be utilized for this purpose. Mein and Reinemann (2014) reported a relationship between claw vacuum and milk flow rate in conventional systems, and proposed that this could be used for selecting the appropriate vacuum level. Furthermore, it was underlined that the relationship differs substantially between systems, and that it must be established for the individual herd (Mein & Reinemann, 2014). The results presented in this thesis show that the ideas proposed by Mein and Reinemann (2014) can be extended to AMS herds and can contribute towards

more efficient and better advice: The herd-specific relationship between milk flow rate and short milk tube vacuum can be established based on a relatively small number of MTT recordings. This relationship, expressed as a predictive equation, can be used to estimate the short milk tube vacuum levels for all quarters in the herd, based on the milk flow rates recorded in the AMS software. The estimated vacuum levels can be evaluated against the ISO guidelines by showing, for example, the proportion of cows with estimated short milk tube vacuum levels that lie within or outside the reference values. In this way, an advisor will obtain an overview of how the current vacuum settings apply for the entire herd, and the basis for giving advice is thereby strengthened. Although the same principles will apply to any milking system, the availability of milk flow data in the AMS software makes this highly relevant as a way of improving MTT advisory services for this group of herds.

Furthermore, in herds experiencing poor teat-end condition, the relationship between average milk flow rate and vacuum levels in the short milk tube might be used when investigating possible causes for the problem. If poor teat-end condition is mainly identified in cows with low milk flow rates, then this might suggest that the milking-machine settings are not optimal for this group of cows. However, finding poor teat-end condition in cows across the range of milk flow rates might suggest that environmental or genetic factors are the predisposing factor, or that the milking system has major defects or suboptimal settings that affect all cows.

This thesis did not detect relationships between SCC and MTT-result variables that could be used directly when interpreting whether the milking equipment is a component associated with poor udder health. However, the results showed that milk flow data recorded by the AMS, together with MTT results, can be used to efficiently evaluate how the settings in a specific AMS concur with the ISO guidelines for vacuum level at the teat end. Conducting MTT in AMS is time consuming because only one cow is milked at a time, and evaluation of the entire herd by MTT is most often not possible. This problem can be addressed by using the suggested method, making the MTT more applicable to AMS herds.

Somatic cell count as an indicator of udder health status

SCC has been used as an indicator of ongoing inflammatory processes in the udder for decades (Schukken et al., 2003; Dohoo & Leslie, 1991). In traditional dairy herd recording systems, SCC are analyzed monthly or bimonthly. However, automated equipment for SCC measurements are now available for AMS, as with the DeLaval OCC. This enables farmers

to analyze SCC at every milking if desired. SCC as an indicator of udder health status can be considered a sensor system, where the output from the analysis may reflect an underlying biological state. In the current work, SCC was used as an indicator of udder health status when evaluating relationships between udder health and MTT result variables, and the final models only explained a small part of the overall variability in SCC values.

Variability in frequently measured OCC

The variability in frequently measured OCC values may arise from different sources. Firstly, fluctuations of a physiological nature are likely to occur over time within cows (Forsbäck et al., 2010). Secondly, fluctuations may arise from an immune response towards microbes present in the udder. In addition, the sensor system, including sampling and analysis procedures, might be imperfect. The results presented in this section contribute to a better understanding of fluctuations in OCC in cows that are free from clinical mastitis.

For OCC to be a relevant tool in the detection of IMI during lactation in cows where the IMI has not developed into a clinical mastitis, there must be a difference in OCC values between healthy udders and udders with IMI. In the longitudinal study presented in this thesis, the IMI status of the cows was closely monitored over 17 months. These data, combined with the OCC values recorded at every milking, meant that it was possible to investigate differences in OCC between periods of different udder-health status in cows without clinical mastitis. The distribution of lnOCC values within periods of different IMI status (Figure 2) showed that in the No-IMI group, the most frequently observed lnOCC value was approximately 3 (i.e., 20,000 cells/mL), and the majority (95 %) of observations in this group had lnOCC values between 0 (1000 cells/mL) and 5 (148,000 cells/mL). This range may be interpreted as the physiological OCC level when the udder is not affected by mastitis pathogens. The transient-colonization group of cows had a similar distribution in lnOCC values as the No-IMI group, which shows that the bacterial findings in this group were not associated with an inflammatory process, and likely represent the presence of bacteria in the teat canal or teat cistern without colonization of the udder parenchyma (Persson et al., 1995). Although there was an overlap between the distributions for the different IMI statuses, the Pat-1 and Pat-2 groups were both characterized by higher lnOCC values than the No-IMI group. However, as would be expected with the classifications used, the difference was most pronounced between the Pat-1 IMI group and the No-IMI group. The most frequently observed lnOCC value in the Pat-1 IMI group was 5 (148,000 cells/mL), and most observations (97 %) were between 2 (7,400 cells/mL) and 8 (2,980,000

cells/mL). The Pat-2 IMI group had a distribution approximately midway between that of No IMI and Pat-1 IMI, and there was a large degree of overlap between Pat-2 IMI and No IMI and Pat-1 IMI.

The fluctuations in lnOCC in the longitudinal study were modeled in a linear mixed model with the following fixed effects: a lactation curve described by DIM and lnDIM, yield in the current milking, lnOCC from the previous cow milked in the same AMS (carry-over effect), parity, udder health status, conductivity (inter-quarter difference), genetic lineage, average milk flow rate, and season. Using the method of Nakagawa and Shielzeth (2013), the fixed effects explained 15.0 % of the total variability in lnOCC. The degree of explanation increased to 55.2 % when random intercepts were included. The large proportion of unexplained variation shows that physiological fluctuations over time are likely to be the main source of variation in OCC values in cows free from clinical mastitis. Milking-to-milking variability in SCC has been investigated in previous studies (Quist et al., 2008; Forsbäck et al., 2010). However, these studies were of relatively short duration, and data on udder health status were not included in the study of Forsbäck et al. (2008). Schepers et al. (1997) studied SCC at quarter-level in seven herds over 20 months, using 5-week sampling intervals, and included results from microbiological analyses. The authors report that their final model described 50 % of the variability in ln-transformed SCC values (Schepers et al., 1997). Unlike the data from the longitudinal study in this thesis, the data of Schepers et al. (1997) contained records from cows with signs of clinical mastitis, and the analyses were performed at the quarter level. Nevertheless, the findings of Schepers et al. (1997) support the high degree of unexplained variability reported in this thesis.

The large degree of unexplained variability may be interpreted as the physiological variability in SCC. When SCC is used in research as an udder health indicator, as was done in Paper I, the physiological fluctuations must be considered when interpreting the results. One implication is that low degrees of explanation may be expected in studies using SCC as an indicator of udder health. Nevertheless, as Figure 2 illustrates, udders without IMI generally have low SCC, whereas IMI are associated with higher SCC values. Therefore, a relationship between SCC and an outcome variable is likely to be relevant, even if a low degree of explanation is reported.

As mentioned, some of the variability in OCC measurements may also arise from the sensor system. Sørensen et al. (2016) investigated the accuracy of the DeLaval OCC on seven

commercial farms (Sørensen et al., 2016), and, although they concluded that the agreement was reasonably good, the results differed between herds. With a concordance correlation coefficient of 0.82, the results from the agreement study presented in this thesis are in line with those of Sørensen et al. (2016). Another finding, however, is that the agreement seems better for high values than low values. This might be of relevance when low threshold values are used in the detection of IMI.

The multivariable linear mixed model showed a significant relationship between OCC and average milk flow rate. This strengthens the suggested interpretation of the relationships between SCC and MTT result variables, namely that differences in milk flow rate were an underlying factor responsible for changes in average vacuum level in the short milk tube, as measured by MTT, and also for changes in the outcome variable SCC.

OCC as an IMI indicator

This thesis investigated the performance of frequent OCC measurements for the detection of IMI and clinical mastitis. The results showed that detection of Pat-1 subclinical mastitis episodes was improved by using a 7-d rolling average or the EMR indicator rather than a single OCC measurement; for Pat-2 subclinical mastitis, the EMR performed better than the 7-d average and a single OCC. Both the 7-d rolling average and the EMR indicator place less weight on the individual OCC measurements, and are therefore more robust regarding the large physiological variability in OCC. For clinical mastitis, however, similar performance was achieved using a single OCC value, a 48-h average, and EMR. Clinical mastitis is often associated with a dramatic increase in SCC (Shuster et al., 1991), which was also observed in the cases of clinical mastitis in the longitudinal study in this thesis. The acute increase in SCC to levels that are not often observed in subclinical mastitis explains why a single OCC can be a useful indicator of clinical mastitis. Detection of clinical mastitis is important for initiating the correct treatment at an early stage. However, detecting and treating clinical mastitis is, in itself, insufficient for preventing future cases. The focus in this thesis was therefore to detect IMI with mastitis pathogens, but that have not yet manifested as clinical disease in the infected cows. By monitoring subclinical mastitis and taking actions to reduce the occurrence of these infections, farmers are likely to experience fewer cases of clinical mastitis in their herds and have a better likelihood of avoiding production losses associated with subclinical cases.

In the diagnostic test evaluation, the specificity was adjusted by applying different threshold values for the different IMI indicators (single OCC, 7-d average OCC, and EMR). For a single OCC value, this can be illustrated using Figure 1, where increasing specificity may be achieved by moving the threshold value to the right on the x-axis to include a higher proportion of the observations in the No-IMI group. By doing this, it becomes clear that a larger proportion of observations in the Pat-1 and Pat-2 groups will be incorrectly classified as healthy, resulting in a lower sensitivity.

The focus in this thesis was primarily fluctuations in SCC in clinically healthy cows; these are more subtle than the large increase in SCC often seen in clinical mastitis. The broad range of physiological fluctuations and the considerable degree of overlap in SCC between periods of IMI and No IMI are probably the main limitations to using SCC as an indicator of udder health in cows free from clinical mastitis, and hence the reason why single OCC values were outperformed by EMR in the detection of Pat-1 and Pat-2 IMI. Nevertheless, the Pat-1 group, containing those pathogens often considered most important for bovine mastitis, had a SCC distribution that was clearly distinct from that of the No-IMI group and the transient colonization group.

The four levels of specificity used in this thesis may represent different situations, with varying tolerances towards incorrect classification of healthy animals. For example, when the aim is to identify candidates for antibiotic dry-cow treatment, those selected on the basis of SCC are further diagnosed by QMS, according to recommended procedures in Norway, and this process happens once per lactation, close to drying off. In this case, the farmer might choose to lower the specificity and thereby to increase the sensitivity. In this way, there will be more QMS to be collected, but the risk of missing some cows requiring dry-cow treatment will be reduced. On the other hand, when the purpose is to detect IMI during lactation based on OCC, this may happen at every milking. Demanding a high specificity in this situation may result in a large number of false “alerts” due to the combination of a low sensitivity (many cows erroneously classified as infected) and a large number of “decision moments” (milkings) every day. These examples illustrate that using different OCC threshold values for classifying IMI in different situations is one way of increasing the potential usefulness of OCC as an indicator of IMI in clinically healthy cows. Detecting a case of subclinical mastitis does not require immediate attention, but surveillance of the occurrence of this condition over time may inform the farmer whether udder health is being controlled or whether it is improving or worsening. A recent study demonstrated that OCC

data can be used as a proxy for IMI in dynamic transmission models to forecast the herd prevalence of subclinical mastitis (Dalen et al., 2019b). With this approach, farmers can act at an early stage to prevent the transmission of mastitis pathogens to healthy cows.

Methodological considerations

The studies in this thesis are based on observational studies, and one crucial point is to distinguish between causal and non-causal relationships. Cross-sectional studies, which we have used in Papers I and II, are less suitable for detecting causal relationships than longitudinal studies. This is because, as the subjects are not followed over time, it is impossible to evaluate the effects of exposure variables over time. The outputs from cross-sectional studies must therefore be interpreted as associations, and any causal inferences must rely on existing knowledge. However, cross-sectional studies are generally easy to perform, and are often appropriate as a first step, before refining the research questions and pinpointing central hypotheses for subsequent studies. In the data collected for Paper I, most herds had been visited once, while SCC data for the individual animals were available both before and after the visit. It was unknown whether any adjustments were done in the milking system as a consequence of the MTT. Hence, instead of assuming that the MTT results were valid for periods before or after the herd visit, we chose to focus on the SCC values recorded as close as possible before the day of MTT in order to avoid changes implemented in response to the herd visit affecting the results.

The SCC records in Paper I could have been transformed to a dichotomous indicator of subclinical mastitis by applying a threshold value. This approach was explored as an alternative, by creating a dichotomous outcome variable based on the threshold values from Paper IV and applying the dichotomous outcome in the final models for each milking system. It was found that increasing vacuum level and longer duration were associated with a decreased likelihood of subclinical mastitis (results not shown). The advantage of modeling the SCC on a continuous scale in Papers I and III, was that this approach is independent of threshold values and uncertainties regarding whether the chosen cutoff values were able to classify subclinical mastitis correctly.

Research in the field of mastitis, including Paper I in this thesis, often relies on indicators of mastitis rather than an IMI diagnosis based on microbiological analyses. An IMI can, to various extents, cause inflammatory responses that we are able to observe or measure, such as an increase in SCC, increased conductivity, or other signs. With this in mind, it becomes

clear that: 1) the state that we are interested in modeling is sometimes not directly measurable, and 2) that the indicators that we are able to measure are all outcomes of IMI. Therefore, methods modeling a single outcome variable, such as the linear and logistic regression models used in this thesis, have some limitations. It is possible that multivariate techniques and models utilizing latent factors might provide a framework for mastitis modeling in the future. However, the current techniques are not well suited for modeling longitudinal and multilevel data, both of which are important in mastitis research based on observational studies.

Monthly QMS were used when defining the IMI status throughout lactation in Papers III and IV, and a shift from one IMI status to another was assumed to occur midway between two sampling occasions. Because cows might have been infected or cured at any time between the two sampling occasions, it is possible that this has led to some misclassification, both in the direction of classifying a period as No IMI when the true status was Pat-1 or Pat-2 IMI, and vice versa. It is therefore possible that the overlap between the OCC distributions in periods of different IMI status and the unexplained variation in lnOCC would have been reduced by more frequent QMS analyses.

The definition of IMI used in this thesis was based on concentrations of bacteria in the QMS and persistency over time; this is conservative compared with only using the presence of mastitis pathogens. With the implemented method, there were probably few periods that were incorrectly classified as IMI, whereas some periods might have been wrongly classified as healthy. The result is a possible negative bias in specificity.

Validity

Data for Papers II, III, and IV were collected in one research herd with Norwegian Red cows. The milking system used settings recommended by the manufacturer, and average milk flow rates in the herd were comparable to figures reported for Brown Swiss x German Braunvieh (Weiss et al., 2004) and Holstein cows (Tančin et al., 2006). Although the housing conditions and management routines are similar to those used in commercial Norwegian farms of the same size, generalization to other herds should be done with some caution. However, Papers II and IV focused on exploring novel approaches for using data from the AMS for teat-end condition management and detection of subclinical mastitis, respectively, and the results should be considered proof of concept rather than being directly applicable to other herds. Furthermore, it is known that differences between cows account

for most of the variability in SCC, whereas differences between herds is less important in the overall picture (Emanuelson & Persson, 1984). According to the intraclass correlation coefficients in Paper I, this was also the case for the field data used in this thesis. Paper III aimed to describe the variability and factors associated with variability in OCC. The relative importance of the different factors associated with variability in OCC is likely to vary between herds.

Paper I used data from the NDHRS. The system is accredited by ICAR and contained records from 96 % of Norwegian dairy herds in 2018. Milk recording and sampling are performed according to membership instructions with ICAR-approved milk meters and sampling equipment (TINE SA, 2018). Veterinary treatments are reported directly to the database by the veterinarian. The NDHRS database has been validated for its completeness compared with on-farm registrations (Espetvedt et al., 2013), and is frequently used in research (e.g., Toftaker et al., 2016; Martin et al., 2015; Ruud et al., 2010; Andersen et al., 2011). For Paper I, we extracted data on calving dates, mastitis treatments, SCC, milk yield, and type of milking system.

The data used in Paper I were collected from dairy herds across Norway using different types of milking system. With data from 1009 herds, more than 10 % of all Norwegian herds were represented in the data. The reasons why the herds in our data had ordered an MTT were not known, and the data are likely to contain: 1) herds that primarily required an MMT as a routine check of the milking system, and 2) herds for which the MTT was conducted as tool to investigate a mastitis problem. Ødegård et al. (2004) reported a mean ln-transformed SCC value of 4.11 (61,000 cells/mL) in their data, with more than 1.3 million records from NDHRS; this is lower than the median SCC values reported in Paper I. This comparison assumes a normal distribution of the ln-transformed SCC values of Ødegård et al. (2004), where the mean value equals the median value. Hence the sample of herds used in Paper I is probably slightly biased towards herds with somewhat poorer udder health than average. In order to strengthen the internal validity, the data were analyzed at cow level and the models accounted for differences between herds by including a random intercept at herd level. Analyses were run separately for each milking system, and consistent results across the three groups strengthen the internal validity. The Norwegian Red breed is by far the most common breed in Norwegian dairy herds and was also dominant in our study, hence strengthening the validity for Norwegian Red herds. On the other hand, if the

target population is dairy cows in general, the large proportion of Norwegian Red cows in this study represents a possible limitation in the external validity.

Conclusions

- The applicability of MTT as a tool for preventive approaches to maintaining good udder health in AMS herds is limited because differences between cows have a large impact on the results. Interpretation of MTT results should therefore be complemented by other investigations, such as evaluating teat-end condition.
- Combining milk flow data obtained from the AMS software with MTT results is proposed as an efficient way of evaluating the vacuum level in the short milk tube for all teats in an AMS herd.
- Milk flow data from the AMS might also be utilized when investigating causes of teat-end condition problems.
- By employing smoothing techniques, frequent OCC measurements can be used for detecting of IMI in clinically unapparent cows during lactation, enabling farmers and advisors to improve mastitis prevention in their herds. By adjusting threshold values for the chosen mastitis indicator, end users may adapt the detection system to their needs.
- The OCC levels for cows without IMI were mostly between 1,000 cells/mL and 148,000 cells/mL (95 % of observations), with 20,000 cells/mL being the most commonly observed value. This may be interpreted as the physiological OCC level in healthy udders. Infected udders had higher OCC values, but there was a large degree of overlap between values from infected and healthy udders.
- The physiological variation in OCC between milkings in cows without clinical mastitis is likely much greater than the variation due to extraneous factors, including IMI.

Future perspectives

This thesis indicates various challenges in the use of MTT as a tool for dairy advisors in AMS herds. Future research projects aiming to investigate the influence of the milking machine on udder health may consider using methods (or parameters) that are less influenced by differences between cows, such as techniques from the dry-test category. Alternatively, detailed data should be collected to adjust for these differences.

Udder health was the focus in this thesis. However, efficient milking is also an important aspect for dairy farmers. Further research is needed to increase our knowledge on how milking-machine settings may be adjusted to maximize milking efficiency, while not compromising good udder health. AMS herds are well suited for studies in this field of research, because the system records detailed data during milking, and because it is technically possible to use different settings (e.g., pulsation characteristics or takeoff level) between cows or even within cows.

Increased use of milk flow data in the evaluation of milking equipment and for management of teat-end condition might contribute to better and more efficient advisory services, and the proposed methodology should be further investigated in a larger number of herds.

Detection of IMI with high sensitivity and specificity remains a challenge. One possible way of improving the performance of OCC for IMI detection is to implement more advanced methods from the field of data science, such as machine-learning algorithms, to data that are already available from sensors in the AMS. Machine-learning algorithms, combining data from various sensors, should therefore be investigated further. It is also possible that the SCC (or OCC) data have limitations (e.g., large physiological variations) as an indicator of IMI and that these are detrimental to the performance. Developing new sensors, or implementing other commercially available sensor technologies, is therefore relevant for research projects aiming to improve udder health surveillance and mastitis prevention. One example is olfactory sensing (“electronic nose”) that can be used for the detection of volatile organic compounds in milk that are associated with inflammatory processes or bacterial metabolism. Another example is infrared spectroscopy techniques, where recent examples have demonstrated that such sensors can be implemented on-line for analysis of milk composition (Melfsen et al., 2012).

References

Andersen F., Østerås O., Reksen O., Toft N., Gröhn Y.T., 2011. Associations between the time of conception and the shape of the lactation curve in early lactation in norwegian dairy cattle. Acta Vet. Scand. 53, 5. https://doi.org/10.1186/1751-0147-53-5

Anon, 2015. Nasjonal strategi mot antibiotikaresistens 2015-2020. The norwegian government. Https://www.Regjeringen.No/no/dokumenter/nasjonal-strategi-mot-antibiotikaresistens-2015-2020/id2424598/, accessed 05.09.2019.

Anon, 2017. Husdyrnæringas felles handlingsplan mot antibiotikarestistente bakterier. Https://www.Animalia.No/contentassets/d554e650401e4381a0ec83908136403a/husdyrnaringas-felles-hplan-amr-endelig-enkeltsider_190617.Pdf, accessed 30.09.2019.

Anon, 2018. Jordbruksavtalen 2018-2019. Inngått mellom staten, norges bondelag og norsk bonde- og småbrukarlag. Https://www.Regjeringen.No/contentassets/f52d24b151c64b8a96a073438b977bc5/revidert-jordbruksavtale-2018-2019.Pdf, accessed 30.09.2019.

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

Berry D.P., Meaney W.J., 2006. Interdependence and distribution of subclinical mastitis and intramammary infection among udder quarters in dairy cattle. Prev. Vet. Med. 75, 81-91. https://doi.org/10.1016/j.prevetmed.2006.02.001

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

Bradley A.J., 2002. Bovine mastitis: An evolving disease. The Veterinary Journal 164, 116-128. https://doi.org/10.1053/tvjl.2002.0724

Breen J.E., Green M.J., Bradley A.J., 2009. Quarter and cow risk factors associated with the occurrence of clinical mastitis in dairy cows in the united kingdom. J. Dairy Sci. 92, 2551-2561. http://dx.doi.org/10.3168/jds.2008-1369

Capuco A.V., Bright S.A., Pankey J.W., Wood D.L., Miller R.H., Bitman J., 1992. Increased susceptibility to lntramammary infection following removal of teat canal keratin. J. Dairy Sci. 75, 2126-2130. https://doi.org/10.3168/jds.S0022-0302(92)77972-7

Carroll E.J., Schalm O.W., 1962. Effect of deoxyribonuclease on the california test for mastitis. J. Dairy Sci. 45, 1094-1097. https://doi.org/10.3168/jds.S0022-0302(62)89565-4

Dalen G., Rachah A., Nørstebø H., Schukken Y.H., Reksen O., 2019a. The detection of intramammary infections using online somatic cell counts. J. Dairy Sci. https://doi.org/10.3168/jds.2018-15295

Dalen G., Rachah A., Nørstebø H., Schukken Y.H., Reksen O., 2019b. Dynamics of somatic cell count patterns as a proxy for transmission of mastitis pathogens. J. Dairy Sci. https://doi.org/10.3168/jds.2019-16847

Djabri B., Bareille N., Beaudeau F., Seegers H., 2002. Quarter milk somatic cell count in infected dairy cows: A meta-analysis. Vet. Res. 33, 335-357. 10.1051/vetres:2002021

Dohoo I.R., Leslie K.E., 1991. Evaluation of changes in somatic cell counts as indicators of new intramammary infections. Prev. Vet. Med. 10, 225-237. https://doi.org/10.1016/0167-5877(91)90006-N

Dohoo I.R., Martin W., Stryhn H., 2009. Veterinary epidemiologic research, 2nd edition. Avc inc. University of prince edward island, charlottetown, prince edward island, canada.

Dohoo I.R., Meek A.H., 1982. Somatic cell counts in bovine milk. The Canadian Veterinary Journal 23, 119-125.

Emanuelson U., Persson E., 1984. Studies on somatic cell counts in milk from swedish dairy cows. Acta Agric. Scand. 34, 33-44. https://doi.org/10.1080/00015128409436179

Espetvedt M.N., Reksen O., Rintakoski S., Østerås O., 2013. Data quality in the norwegian dairy herd recording system: Agreement between the national database and disease recording on farm. Journal of Dairy Science 96, 2271-2282. https://doi.org/10.3168/jds.2012-6143

Ezzat Alnakip M., Quintela-Baluja M., Böhme K., Fernández-No I., Caamaño-Antelo S., Calo-Mata P., Barros-Velázquez J., 2014. The immunology of mammary gland of dairy ruminants between healthy and inflammatory conditions. J. Vet. Med. http://dx.doi.org/10.1155/2014/659801

Ferneborg S., Svennersten-Sjaunja K., 2015. The effect of pulsation ratio on teat condition, milk somatic cell count and productivity in dairy cows in automatic milking. J Dairy Res 82, 453-459. https://doi.org/10.1017/S0022029915000515

Forsbäck L., Lindmark-Månsson H., Andrén A., Åkerstedt M., Andrée L., Svennersten-Sjaunja K., 2010. Day-to-day variation in milk yield and milk composition at the

udder-quarter level. J. Dairy Sci. 93, 3569-3577. https://doi.org/10.3168/jds.2009-3015

Fry P.R., Middleton J.R., Dufour S., Perry J., Scholl D., Dohoo I., 2014. Association of coagulase-negative staphylococcal species, mammary quarter milk somatic cell count, and persistence of intramammary infection in dairy cattle. J. Dairy Sci. 97, 4876-4885. https://doi.org/10.3168/jds.2013-7657

Gleeson D.E., Meaney W., O'Callaghan E., Rath M., 2004a. Effect of teat hyperkeratosis on somatic cell counts of dairy cows. Intern. J. Appl. Res. Vet. Med 2, 115-122.

Gleeson D.E., O'Callaghan E.J., Rath M.V., 2004b. Effect of liner design, pulsator setting, and vacuum level on bovine teat tissue changes and milking characteristics as measured by ultrasonography. Ir Vet J 57, 289-296. https://doi.org/10.1186/2046-0481-57-5-289

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

Grindal R.J., Hillerton J.E., 1991. Influence of milk flow rate on new intramammary infection in dairy cows. J. Dairy Res. 58, 263-268. https://doi.org/10.1017/S0022029900029848

Grindal R.J., Walton A.W., Hillerton J.E., 1991. Influence of milk flow rate and streak canal length on new intramammary infection in dairy cows. J. Dairy Res. 58, 383-388. https://doi.org/10.1017/S0022029900029988

Guarín J.F., Paixão M.G., Ruegg P.L., 2017. Association of anatomical characteristics of teats with quarter-level somatic cell count. J. Dairy Sci. 100, 643-652. https://doi.org/10.3168/jds.2016-11459

Halasa T., Huijps K., Østerås O., Hogeveen H., 2007. Economic effects of bovine mastitis and mastitis management: A review. Veterinary Quarterly 29, 18-31. https://doi.org/10.1080/01652176.2007.9695224

Halasa T., Nielen M., De Roos A.P.W., Van Hoorne R., de Jong G., Lam T.J.G.M., van Werven T., Hogeveen H., 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

Hamann J., Mein G.A., Wetzel S., 1993. Teat tissue reactions to milking: Effects of vacuum level. J. Dairy Sci. 76, 1040-1046. http://dx.doi.org/10.3168/jds.S0022-0302(93)77432-9

Hansen B.G., 2015. Robotic milking-farmer experiences and adoption rate in jæren, norway. J. Rural Stud. 41, 109-117. http://dx.doi.org/10.1016/j.jrurstud.2015.08.004

Heringstad B., Klemetsdal G., Steine T., 2007. Selection responses for disease resistance in two selection experiments with norwegian red cows. J. Dairy Sci. 90, 2419-2426. https://doi.org/10.3168/jds.2006-805

Heringstad B., Sehested E., Steine T., 2008. Short communication: Correlated selection responses in somatic cell count from selection against clinical mastitis. Journal of Dairy Science 91, 4437-4439. https://doi.org/10.3168/jds.2008-1330

Heringstad B., Østeras O., 2013. More than 30 years of health recording in norway. Icar 2013 health data conference: Challenges and benefits of health data recording in the context of food chain quality, management and breeding. May 30-31, 2013, århus, denmark. Pages 39-45. Http://www.Icar.Org/documents/technical_series/tec_series_17_aarhus.Pdf.

Hogan J., Gonzalez R., Harmon R., Nickerson S., Oliver S., Pankey J., Smith K.L.J.N.M.C., Madison, WI, 1999. Laboratory handbook on bovine mastitis. 6-10.

Hogeveen H., Kamphuis C., Steeneveld W., Mollenhorst H., 2010. Sensors and clinical mastitis—the quest for the perfect alert. Sensors 10, 7991. https://doi.org/10.3390/s100907991

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. http://dx.doi.org/10.1016/S0301-6226(01)00276-7

IDF, 1987. Milking machine factors affecting mastitis: A literature review. Bulletin 215, machine milking ans mastitis. Pp 2-32.

IDF, 2011. Suggested interpretation of mastitis terminology. Bull. Int. Dairy Fed. 448. Int Dairy Fed., Brussels, Belgium.

ISO (2007: Milking machine installations - construction and performance. Iso 5707:2007. International Organization for Standardization, Geneva, Switzerland.

Kamphuis C., Mollenhorst H., Feelders A., Pietersma D., Hogeveen H., 2010. Decision-tree induction to detect clinical mastitis with automatic milking. Computers and Electronics in Agriculture 70, 60-68. https://doi.org/10.1016/j.compag.2009.08.012

Kamphuis C., Pietersma D., van der Tol R., Wiedemann M., Hogeveen H., 2008a. Using sensor data patterns from an automatic milking system to develop predictive variables for classifying clinical mastitis and abnormal milk. Computers and Electronics in Agriculture 62, 169-181. https://doi.org/10.1016/j.compag.2007.12.009

Kamphuis C., Sherlock R., Jago J., Mein G., Hogeveen H., 2008b. Automatic detection of clinical mastitis is improved by in-line monitoring of somatic cell count. J. Dairy Sci. 91, 4560-4570. https://doi.org/10.3168/jds.2008-1160

Khatun M., Thomson P.C., Kerrisk K.L., Lyons N.A., Clark C.E.F., Molfino J., García S.C., 2018. Development of a new clinical mastitis detection method for automatic milking systems. J. Dairy Sci. 101, 9385-9395. https://doi.org/10.3168/jds.2017-14310

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

LeBlanc S.J., Lissemore K.D., Kelton D.F., Duffield T.F., Leslie K.E., 2006. Major advances in disease prevention in dairy cattle. J. Dairy Sci. 89, 1267-1279. https://doi.org/10.3168/jds.S0022-0302(06)72195-6

Lewis S., Cockcroft P., Bramley R., Jackson P., 2000. The likelihood of subclinical mastitis in quarters with different types of teat lesions in the dairy cow. Cattle Practice 8, 293-299.

Lusis I., Antane V., Laurs A. Year. Effectiveness of somatic cell count determination in the milking robots. In Proceedings of the Proceedings of the Conference Engineering for Rural Development, pp. 112-116.

Løvendahl P., Chagunda M.G.G., 2010. On the use of physical activity monitoring for estrus detection in dairy cows. J. Dairy Sci. 93, 249-259. https://doi.org/10.3168/jds.2008-1721

Martin A.D., Kielland C., Nelson S.T., Østerås O., 2015. The effects of building design on hazard of first service in norwegian dairy cows. J. Dairy Sci. 98, 8655-8663. https://doi.org/10.3168/jds.2015-9464

Mein G., Reinemann D.J., Schuring N., Ohnstad I., 2004. Milking machines and mastitis risk: A stom in a teatcup. Proc. National matitis council annual meeting (2004), pp. 176–188.

Mein G.A., 2012. The role of the milking machine in mastitis control. Veterinary Clinics of North America: Food Animal Practice 28, 307-320. https://doi.org/10.1016/j.cvfa.2012.03.004

Mein G.A., Neijenhuis F., Morgan W.F., Reinemann D.J., Hillerton J.E., Baines J.R., Ohnstad I., Rasmussen M.D., Timms L., Britt J.S., Farnsworth R., Cook N., Hemling T., 2001. Evaluation of bovine teat condition in commercial dairy herds: 1. Non-

infectious factors. In: Proceedings of the 2nd international symposium on mastitis and milk quality.

Mein G.A., Reinemann D.J. 2014. *Machine milking: Volume one.*

Mein G.A., Williams D.M., Reinemann D.J., 2003. Effects of milking on teat-end hyperkeratosis: 1. Mechanical forces applied by the teatcup liner and response of the teat. Proc. 43rd annual meeting of the national mastitis council, fort worth, tx, USA, pp 114-123.

Melfsen A., Hartung E., Haeussermann A., 2012. Accuracy of milk composition analysis with near infrared spectroscopy in diffuse reflection mode. Biosystems Engineering 112, 210-217. https://doi.org/10.1016/j.biosystemseng.2012.04.003

Mollenhorst H., van der Tol P.P.J., Hogeveen H., 2010. Somatic cell count assessment at the quarter or cow milking level. J. Dairy Sci. 93, 3358-3364. https://doi.org/10.3168/jds.2009-2842

Nakagawa S., Schielzeth H., 2013. A general and simple method for obtaining r2 from generalized linear mixed-effects models. Methods Ecol. Evol. 4, 133-142. 10.1111/j.2041-210x.2012.00261.x

Neijenhuis F., Barkema H.W., Hogeveen H., Noordhuizen J.P.T.M., 2000. Classification and longitudinal examination of callused teat ends in dairy cows. J. Dairy Sci. 83, 2795-2804. http://dx.doi.org/10.3168/jds.S0022-0302(00)75177-0

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. http://dx.doi.org/10.3168/jds.S0022-0302(01)74720-0

Newman J.A., Grindal R.J., Butler M.C., 1991. Influence of liner design on mouthpiece chamber vacuum during milking. J. Dairy Res. 58, 21-27. 10.1017/S0022029900033471

Norberg E., Hogeveen H., Korsgaard I.R., Friggens N.C., Sloth K.H.M.N., Løvendahl P., 2004. Electrical conductivity of milk: Ability to predict mastitis status. J. Dairy Sci. 87, 1099-1107. https://doi.org/10.3168/jds.S0022-0302(04)73256-7

Norwegian Medicines Agency, 2012. Terapianbefaling: Bruk av antibakterielle midler til produksjonsdyr. (therapy recommendataions: Use of antimicrobial drugs for food producing animals).

Nørstebø H., Dalen G., Rachah A., Heringstad B., Whist A.C., Nødtvedt A., Reksen O., 2019. Factors associated with milking-to-milking variability in somatic cell counts

from healthy cows in an automatic milking system. Prev. Vet. Med. 172, 104786. https://doi.org/10.1016/j.prevetmed.2019.104786

Paulrud C.O., 2005. Basic concepts of the bovine teat canal. Vet. Res. Commun. 29, 215-245. https://doi.org/10.1023/B:VERC.0000047496.47571.41

Penry J.F., Leonardi S., Upton J., Thompson P.D., Reinemann D.J., 2016. Assessing liner performance using on-farm milk meters. J. Dairy Sci. 99, 6609-6618. http://dx.doi.org/10.3168/jds.2015-10310

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. http://dx.doi.org/10.3168/jds.2016-11533

Persson K., Amolina B., Jonsson P., 1995. Inflammation in the bovine teat cistern induced by staphylococcus aureus. J. Vet. Med. Series B 42, 435-442. https://doi.org/10.1111/j.1439-0450.1995.tb00733.x

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. http://dx.doi.org/10.3168/jds.2009-2791

Quist M.A., LeBlanc S.J., Hand K.J., Lazenby D., Miglior F., Kelton D.F., 2008. Milking-to-milking variability for milk yield, fat and protein percentage, and somatic cell count. J. Dairy Sci. 91, 3412-3423. https://doi.org/10.3168/jds.2007-0184

Rasmussen M.D., 1997. The realtionship between mouthpiece vacuum, teat condition, and udder health. Proceedings 36th Annual Meeting, National Mastitis Council, Wisconsin, US, p. 91-96

Rasmussen M.D., Madsen N.P., 2000. Effects of milkline vacuum, pulsator airline vacuum, and cluster weight on milk yield, teat condition, and udder health. J. Dairy Sci. 83, 77-84. http://dx.doi.org/10.3168/jds.S0022-0302(00)74858-2

Rasmussen M.D., Reinemann D.J., Mein G.A., 2003. Measuring vacuum in milking machines. FIL-IDF Bull 381:19-32

Reinemann D., Mein G., Ruegg P., 2001. Evaluating milking machine performance. Proc. VII International Congress on Bovine Medicine, Oviedo, Asturias, Spain, June 29-July 1, 2001

Reinemann D., Mein G.A., Johnson M. Year. Milking machine research: Past, present, and future. In Proceedings of the Annual Meeting - National Mastitis Council Incorporated, pp. 110-113.

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. http://dx.doi.org/10.3168/jds.2008-1006

Rutten C.J., Velthuis A.G.J., Steeneveld W., Hogeveen H., 2013. Invited review: Sensors to support health management on dairy farms. J. Dairy Sci. 96, 1928-1952. https://doi.org/10.3168/jds.2012-6107

Ruud L.E., Bøe K.E., Østerås O., 2010. Associations of soft flooring materials in free stalls with milk yield, clinical mastitis, teat lesions, and removal of dairy cows. J. Dairy Sci. 93, 1578-1586. https://doi.org/10.3168/jds.2009-2752

Rønningen O., 2017. Milking time tests - a tool for milking advisory services. In: 6. Tänikoner melktechniktagung, aspekte zur optimierung der maschinellen milchgewinnung. Ettenhausen: Forschungsanstalt agroscope. 5-15.

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

Shuster D.E., Harmon R.J., Jackson J.A., Hemken R.W., 1991. Suppression of milk production during endotoxin-induced mastitis1. Journal of Dairy Science 74, 3763-3774. https://doi.org/10.3168/jds.S0022-0302(91)78568-8

Sieber R.L., Farnsworth R.J., 1981. Prevalence of chronic teat-end lesions and their relationship to intramammary infection in 22 herds of dairy cattle. J. Am. Vet. Med. Assoc. 178, 1263-1267.

Simojoki H., Orro T., Taponen S., Pyörälä S., 2009. Host response in bovine mastitis experimentally induced with staphylococcus chromogenes. Vet. Microbiol. 134, 95-99. https://doi.org/10.1016/j.vetmic.2008.09.003

Sordillo L.M., Shafer-Weaver K., DeRosa D., 1997. Immunobiology of the mammary gland. J. Dairy Sci. 80, 1851-1865. https://doi.org/10.3168/jds.S0022-0302(97)76121-6

Spencer S.B., Shin J.W., Rogers G.W., Cooper J.B., 2007. Short communication: Effect of vacuum and ratio on the performance of a monoblock silicone milking liner. J. Dairy Sci. 90, 1725-1728. https://doi.org/10.3168/jds.2006-493

Statistics_Norway, 2018. Livestock husbandry. Https://www.Ssb.No/jord-skog-jakt-og-fiskeri/statistikker/jordhus/aar, accessed 18.09.2019.

Sørensen L.P., Bjerring M., Løvendahl P., 2016. Monitoring individual cow udder health in automated milking systems using online somatic cell counts. J. Dairy Sci. 99, 608-620. https://doi.org/10.3168/jds.2014-8823

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

TINE SA, 2017. Statistikksamling fra ku- og geitekontrollen 2016.

TINE SA, 2018. Regler for kukontrollen. https://medlem.tine.no/fagprat/husdyrkontrollen/_attachment/456225?_ts=164600b06c4, accessed 08.05.2019

TINE SA, 2019a. Helsekortordningen, storfe 2018 - statistikksamling.

TINE SA, 2019b. Statistikksamling fra ku- og geitekontrollen 2018.

Toftaker I., Sanchez J., Stokstad M., Nødtvedt A., 2016. Bovine respiratory syncytial virus and bovine coronavirus antibodies in bulk tank milk – risk factors and spatial analysis. Prev. Vet. Med. 133, 73-83. https://doi.org/10.1016/j.prevetmed.2016.09.003

Upton J., Penry J.F., Rasmussen M.D., Thompson P.D., Reinemann D.J., 2016. Effect of pulsation rest phase duration on teat end congestion. J. Dairy Sci. 99, 3958-3965. https://doi.org/10.3168/jds.2015-10466

Weiss D., Weinfurtner M., Bruckmaier R.M., 2004. Teat anatomy and its relationship with quarter and udder milk flow characteristics in dairy cows. J. Dairy Sci. 87, 3280-3289. http://dx.doi.org/10.3168/jds.S0022-0302(04)73464-5

Whist A.C., Østerås O., Sølverød L., 2007. Staphylococcus aureus and streptococcus dysgalactiae in norwegian herds after introduction of selective dry cow therapy and teat dipping. J. Dairy Res. 74, 1-8. https://doi.org/10.1017/S0022029906002135

Zadoks R.N., Allore H.G., Hagenaars T.J., Barkema H.W., Schukken Y.H., 2002. A mathematical model of staphylococcus aureus control in dairy herds. Epidemiol. Infect. 129, 397-416. https://doi.org/10.1017/S0950268802007483

Zoche-Golob V., Haverkamp H., Paduch J.H., Klocke D., Zinke C., Hoedemaker M., Heuwieser W., Krömker V., 2015. Longitudinal study of the effects of teat condition

on the risk of new intramammary infections in dairy cows. J. Dairy Sci. 98, 910-917. http://dx.doi.org/10.3168/jds.2014-8446

Zwertvaegher I., Van Weyenberg S., Piepers S., Baert J., De Vliegher S., 2012. Variance components of teat dimensions in dairy cows and associated factors. J. Dairy Sci. 95, 4978-4988. http://dx.doi.org/10.3168/jds.2011-5296

Østerås O., Lund A., 1988. Epidemiological analyses of the associations between bovine udder health and milking machine and milking management. Prev. Vet. Med. 6, 91-108. http://dx.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. http://dx.doi.org/10.3168/jds.2007-0030

Østerås O., Sølverød L., 2009. Norwegian mastitis control programme. Ir. Vet. J. 62, S26. https://doi.org/10.1186/2046-0481-62-S4-S26

Papers I-IV

I

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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

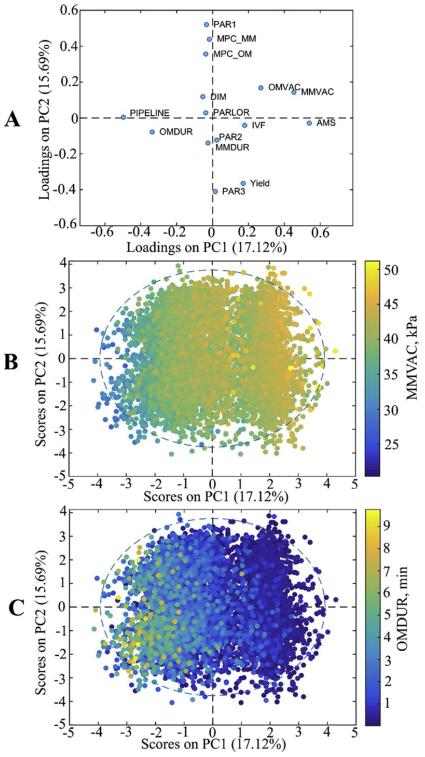

Fig. 1. Results from Principal Component Analysis. Correlation loading plot (A) showing the pattern of relationships between the milking-time test variables and cow variables, and corresponding score plots showing patterns of relationships between the observations in the dataset, colored according to 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≥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. Premilking 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.

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RESEARCH

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Milk-flow data collected routinely in an automatic milking system: an alternative to milking-time testing in the management of teat-end condition?

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Abstract

Background: Having a poor teat-end condition is associated with increased mastitis risk, hence avoiding milking machine settings that have a negative effect on teat-end condition is important for successful dairy production. Milking-time testing (MTT) can be used in the evaluation of vacuum conditions during milking, but the method is less suited for herds using automatic milking systems (AMS) and relationships with teat end condition is poorly described. This study aimed to increase knowledge on interpretation of MTT in AMS and to assess whether milk-flow data obtained routinely by an AMS can be useful for the management of teat-end health. A cross-sectional study, including 251 teats of 79 Norwegian Red cows milked by AMS was performed in the research herd of the Norwegian University of Life Sciences. The following MTT variables were obtained at teat level: Average vacuum level in the short milk tube during main milking (MTVAC), average vacuum in the mouthpiece chamber during main milking and overmilking, teat compression intensity (COMPR) and overmilking time. Average and peak milk flow rates were obtained at quarter level from the AMS software. Teat-end callosity thickness and roughness was registered, and teat dimensions; length, and width at apex and base, were measured. Interrelationships among variables obtained by MTT, quarter milk flow variables, and teat dimensions were described. Associations between these variables and teat-end callosity thickness and roughness, were investigated.

Results: Principal component analysis showed clusters of strongly related variables. There was a strong negative relationship between MTVAC and average milk flow rate. The variables MTVAC, COMPR and average and peak milk flow rate were associated with both thickness and roughness of the callosity ring.

Conclusions: Quarter milk flow rate obtained directly from the AMS software was useful in assessing associations between milking machine function and teat-end condition; low average milk flow rates were associated with a higher likelihood of the teat having a thickened or roughened teat-end callosity ring. Since information on milk flow rate is readily available from the herd management system, this information might be used when evaluating causes for impaired teat-end condition in AMS.

Keywords: Automatic milking, Milk flow, Teat-end callosity

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Background

Changes in condition of the teat-end of dairy cattle, as evaluated by thickness and roughness of the callosity ring, have been associated with increased mastitis risk in previous studies from conventional milking systems (CMS) [1, 2]. Factors related to milking machine function, environment such as housing and climate, and general management have been identified as risk factors for alterations of the integrity of teat tissue [3]. One major advantage of automatic milking systems (AMS) over CMS is a reduction of overmilking by individual attachment and detachment of the four teat cups [4, 5]. However, thickening of the skin surrounding the teat orifice, teat-end callosity, is still found also in AMS herds, necessitating further studies on how to manage the problem. AMS was first introduced in Norwegian dairy herds in 2000 [6], and in 2017 more than 30% of Norwegian dairy farms had adopted this technology [7]. AMS continuously records large amounts of data from the milking process. Using such data in decision-support systems enabling the farmer to improve herd health has been subject to extensive research the last decade [8]. This approach has motivated us to explore whether improved utilization of data from the AMS might be useful when investigating causes for impaired teat end condition in AMS herds.

Milking-time tests (MTT) are used under field conditions to assess the potential negative effects of the milking equipment on the teat. MTT is defined as a "test made on a milking machine during milking of live animals" [9]. However, documentation on how MTT variables relate to each other, and to teat-end condition, is limited in AMS herds because most research on the topic has been performed in CMS [10]. Performing MTT in AMS herds is also time demanding because only one cow is milked at a time, making the method less practical.

Several parameters have been established to evaluate the forces applied on the teat by the collapsing liner [11–13]. Spohr and Uhlenbruck [12] described a variable called "drucksumme" (German; "sum of pressure"), estimating the forces acting on the teat-end during the closed phase of the pulsation cycle by using milking-time vacuum recordings from the short milk tube and pulsation tube, and found a strong positive correlation between "drucksumme" and percentage of cows with high degree of teat-end callosity [12]. However, an association between "drucksumme" and teat-end condition on quarter level has not yet been reported.

The vacuum level in the short milk tube, and hence at the teat-end, is one of many parameters used in a standard MTT. This vacuum level is influenced mainly by the system vacuum level and the milk transport through the short and long milk tube [14]. Quarter milk flow rate is related to system vacuum level [15, 16], teat anatomy [17] and pulsation settings [18]. It seems reasonable to assume that for cows milked by the same milking system (i.e. same system vacuum, pulsation settings and liner), individual cow- or teat factors are responsible for variation in vacuum level in the short milk tube, and hence the forces applied on the teat.

The vacuum level in the mouthpiece chamber (MPC) is another parameter often obtained by MTT. MPC vacuum is a proposed measurement for how well the teat fits the liner [19]. A recent study showed that a high MPC vacuum was associated with congestion at the teat-end [20]. However, a possible association between MPC vacuum and long-term changes in teat-end condition, such as teat-end callosity, has not been evaluated so far.

The overall aim of this study was to assess whether data obtained routinely by the AMS can be used as a proxy for MTT variables in the management of teat-end condition in AMS herds. Our first objective was to describe interrelationships between variables from MTT, the AMS and teat dimension measurements. Secondly, we wanted to assess relationships between these variables and teat-end callosity thickness and roughness, and finally to compare the fit of these models to conclude on the overall aim.

Methods

Herd description and milking machine settings

Our study was performed in the research herd at the Norwegian University of Life Sciences. The herd consisted of 91 Norwegian Red cows divided in two groups, each milked by one AMS (DeLaval VMS, Tumba, Sweden) with identical settings. These 91 cows formed the source population. The two groups were situated in immediate proximity and had identical housing conditions. The teatcups were equipped with DeLaval 20 M VMS liner (Product No. 92725901). The system vacuum was set to 45 kPa, the pulsator ratio was 65% and the pulsation rate was 60 cycles/min. Quarter take-off limit (switch-level) was set to 0.24 kg/min. The system was set with a low-vacuum period of 6 s and a delay from initiation of detachment to detachment of 4 s. The herd, including housing conditions, management and equipment, is comparable to commercial AMS farms in Norway. Data on parity and days in milk (DIM) in the study herd were obtained from the Norwegian Dairy Herd Recording System [21]. Except from a period of 3–5 days immediately after calving, the included cows had not been milked by CMS in the current lactation. Cows in second or later lactations had been milked by CMS in previous lactations.

Teat-end scoring and teat dimensions

Teats on all 91 lactating cows in the herd were evaluated using a scoring system where the thickness and roughness

of the callous ring of the teat orifice was categorized into one of eight classes [22]. Teat length (Length) and width 0.5 cm from apex (Apex) and base (Base) was measured by placing the teat between a white background and a transparent 0.5 cm grid (DeLaval, Tumba, Sweden). In addition, the shape of the teat-end was classified in the following groups; pointed, round, flat or inverted, and the teat position was registered. All registrations were performed once per cow during a 2-day period. The udder and teats were cleaned and stripped prior to the evaluation. The same person performed all scoring and registrations. The time from last milking to teat evaluation was not standardized.

Two outcome variables for logistic regression models were established by transforming the results from the teat-end scoring. The variable THICKNESS was given the value 0 if the thickness of the callosity ring was thin or not visible, and the value 1 if it was classified as medium, thick or extreme according to the scoring system by Neijenhuis et al. [22]. The variable ROUGHNESS was given the value 0 if a smooth callosity ring was registered, and the value 1 if a rough callosity ring was registered. This approach was chosen because previous research has shown that severe degrees of teat-end callosity is significantly related to mastitis risk [2, 23].

Vacuum recordings

VaDia vacuum recorders (BioControl, Rakkestad, Norway) were used to record vacuum at a rate of 200 Hz in the short milk tube, pulsation tube, and MPC in the four individual teatcups during milking in both milking stations. The data were collected during three herd visits, between 1 and 4 weeks after the initial teat-end scoring was performed. We used data from a convenience sample of cows that entered the milking stations voluntarily without interference from the herdsmen. For cases of duplicate vacuum registrations of the same quarter, the recording taken closest to the day of teat-end scoring was used. Cows not entering the milking stations during our visits were excluded from the study. Teats that had been dried-off prior to our observational period and teats where MTT variables could not be calculated due to missing vacuum recordings were also excluded.

Calculation of MTT variables per quarter

In accordance with common procedures for MTT under field conditions, the different periods of the milking were found by evaluating the vacuum recordings [24]. For each milking two main periods were identified, based on the vacuum registrations from the short milk tube and the MPC: (a) the main milking period and (b) the overmilking period. The main milking period was characterized by high milk flow and stable vacuum conditions in the short milk tube. The start of the main milking period was identified by monitoring the average short milk tube vacuum in 10 s periods, until the decline from one period to the next was less than 0.3 kPa. The end of the main milking period, which coincides with the start of the overmilking period, was defined as the point where a marked change in MPC vacuum became apparent as described by Borkhus and Rønningen [19]. Automatic detection of these MPC vacuum changes was set at the point where the MPC vacuum increased at least 30% plus 2 kPa above a weighted running average. The result of the automated procedure was controlled manually and if necessary adjusted taking changes in short milk tube vacuum into account as described previously [19]. The end of the overmilking period was set to the initiation of detachment, i.e. the point where the short milk tube vacuum started to fall markedly ($\geq$ 5 kPa) shortly before the end of milking.

The average vacuum in the short milk tube during the main milking period (MTVAC) was calculated as the mean of all vacuum recordings from the short milk tube within the main milking period. The average vacuum levels in the MPC during the main (MPCVAC) and the overmilking periods (MPCOM) were calculated accordingly.

To estimate the forces applied on the teat-end by the liner during the closed-phase of the pulsation cycle, the variable teat compression intensity (COMPR) was calculated for each teat. This variable is comparable with "drucksumme", as described by Spohr and Uhlenbruck [12]. Vacuum records from the pulsation tube and short milk tube of 10 consecutive pulsation cycles from 60 to 70 s into the main milking period were used for the calculation of COMPR. Differential pressure across the liner wall, i.e. the difference between the short milk tube vacuum and the pulsation tube vacuum, was calculated throughout the 10 pulsation cycles. Touchpoint pressure difference (TPPD) is the pressure difference across the liner wall when two sides of the liner achieves or loses contact during closing and opening respectively [9]. TPPD is traditionally measured without a teat in the teatcup [25]. In this study, however, approximated values for TPPD was derived from the pulsation tube vacuum curves at the points of fastest liner wall movement during opening and closing, respectively [26]. The average of the approximated TPPD at opening and closing was used in further calculations. The closed-phase of the pulsation cycles were defined as the period when the differential pressure across the liner wall was higher than the approximated TPPD. For each of the 10 pulsation cycles, an integral of the differential pressure across the liner wall minus approximated TPPD as a function of time over the liner closed period was calculated. The average of the integrals found in the 10 pulsation cycles represented teat compression intensity, COMPR (kPa s).

Quarter average milk flow rate (AVGFLOW) and quarter peak milk flow rate (PEAKFLOW), for the same milkings in which the vacuum measurements were performed, were obtained from the AMS software (DeLaval, Tumba, Sweden). The milking stations were equipped with ICAR-approved milk meters using near-infrared technology providing in-line data on milk flow and milk yield used in the calculation of these variables.

Statistical analyses

Principal component analysis (PCA) is a multivariate technique that can be used to explore multi-dimensionality of data and to reduce a large set of variables to a small number of latent variables, principal components, which nevertheless retain most of the information in the dataset. PCA is also useful for the analysis of intercorrelation of variables. We applied PCA to study the relationships between the variables obtained from the different registrations, and as suggested by Dohoo et al. [27], we used PCA as a complementary technique in the subsequent model-building process. We used the 2-dimensional scatter plot of loadings for two specified components from PCA, which is most useful for interpreting principal component 1 versus principal component 2, as they contain the most important information in the data. In our application of PCA, we focused on the geometric interpretation of the relationships between variables, plotted as points in the component space using their loadings as coordinates on the "circle of correlations". In addition to PCA, we also used a linear regression model to describe the mathematical relationship between MTVAC and AVGFLOW.

The following variables were evaluated as potential explanatory variables in logistic regression models describing the likelihood of a teat-end having a rough or thickened callosity ring, respectively: DIM, Parity, MTVAC, COMPR, AVGFLOW, PEAKFLOW, MPCVAC, MPCOM, Length, Apex, Base, teat position and overmilking time. Parity was categorized as; first lactation, second lactation, and greater than second lactation.

Linearity between the outcome variables and the explanatory variables was assessed by inspecting lowess smoothing curves obtained with a logit transformation of the outcome variables; THICKNESS and ROUGHNESS (Stata SE/14, Stata Corp., College Station, TX, USA).

To establish logistic regression models for teat-end callosity roughness and thickness, we initially tested each of the explanatory variables in univariable logistic regression models to detect associations with the two outcome variables, THICKNESS and ROUGHNESS. Variables with a P-value less than 0.2 were further evaluated for inclusion in multivariable models. In order to avoid including highly correlated variables in the same model, variables identified by PCA as belonging either to the same cluster or in clusters aligned on the opposite side of the circle of correlation, were evaluated in separate models. We repeated a backwards selection procedure to build multiple multivariable models for each outcome, and variables with P-value $\geq$ 0.05 were excluded from the final multivariable models. Because teat-end callosity thickness and roughness has been shown to vary between parities and lactation stages, parity and DIM were forced into all multivariable models [1].

We expected registrations between teats within the same cow to be correlated, and to account for this, we included a random intercept at cow level in both the univariable and multivariable models. Because the multivariable models were non-nested, i.e. the predictors in one model could not be considered as subsets of the predictors in other models, Bayesian Information Criterion (BIC) was calculated to compare model fit [27].

Data from different sources were assembled in SAS 9.4 (SAS Institute Inc., Cary, NC, USA) to form a final dataset. We used the STATA meqrlogit procedure for the logistic regression analysis and the regress procedure for the linear regression analysis (Stata SE/14, Stata Corp., College Station, TX, USA). The PCA was conducted using the statistical software JMP Pro version 12 (SAS Institute Inc., Cary, NC, USA).

Results

Out of 91 cows that were assigned teat-end scores, eight cows did not enter the milking stations at any of the visits for MTT. Of the remaining 83 cows, complete vacuum registrations from at least one teat were obtained in 79 cows, while four cows were excluded due to complete or partial loss of vacuum data for all teats. From 79 cows, four teats were excluded because they were dried off, while 61 teats were excluded due to complete or partial loss of vacuum data. This resulted in a study sample including 251 teats in 79 cows.

The parities in the study sample were distributed as follows; 34 cows in first lactation, 13 in second, and 32 in third or higher lactations. Table 1 shows the distribution of teat-end callosity scores in the included teats. The data included 123 front teats and 128 rear teats. Concerning teat-end shape, 145 teat-ends were classified as round, 78 flat, 12 pointed and 16 inverted. The number of roughened teat-ends in the same groups were 54, 15, 9 and 0, respectively. Due to the small number of observations with inverted and pointed teats, the teat-end shape was not included in statistical models. The average DIM at the time of teat-end scoring was 76, ranging from 4 to 167. The average overmilking time was 32 s, and ranged from 16 s to 3 min 17 s. However, 90% of the observations had an overmilking time shorter than 48 s.

Table 1 Frequency of teat-end callosity scores [22] in the study herd

Callosity ring thickness	Callosity ring roughness		Total
	Smooth	Rough	
Not visible	53	–	53
Thin	80	37	117
Intermediate	30	31	61
Thick	10	8	18
Extremely thick	–	2	2
Total	173	78	251

Relationships among milk flow-variables, MTT-variables and teat dimensions

Figure 1 shows the plot of the loadings of the explanatory variables MTVAC, COMPR, MPCVAC, MPCOM, PEAKFLOW AVGFLOW, Length, Apex and Base on the components. Each variable is a point whose coordinates are given by the loadings on the principal components. The first and second principal components described 67.4% of the total variation of these explanatory variables. From this loading plot we distinguished 4 clusters of variables: Cluster 1, consisting of MPCVAC and MPCOM; cluster 2, consisting of Length, Apex, and Base; cluster 3, including AVGFLOW and PEAKFLOW; cluster 4, consisting of MTVAC and COMPR. Cluster 1 loaded opposite to cluster 2, showing a negative relationship between teat dimensions and vacuum levels in the MPC. MTVAC and COMPR in cluster 4 were negatively correlated with PEAKFLOW and AVGFLOW of cluster 3.

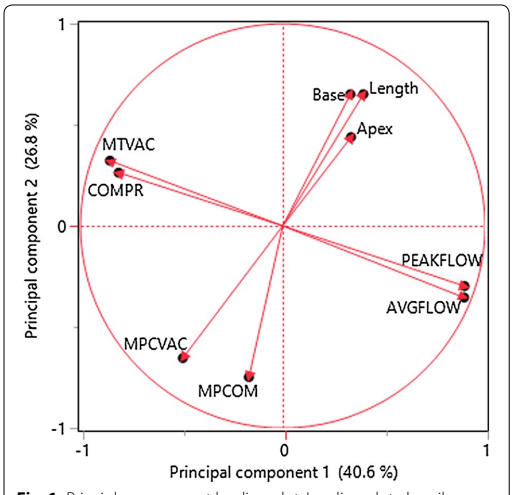

Fig. 1 Principle component loading plot. Loading plot, describing the relationship between milking-time test variables and teat characteristics, derived from principal component analysis. From this loading plot, we distinguished 4 clusters of variables: cluster 1, consisting of MPCVAC and MPCOM; cluster 2, consisting of Length, Apex, and Base; cluster 3, including AVGFLOW and PEAKFLOW; cluster 4, consisting of MTVAC and COMPR

A linear regression model showed a strong linear relationship between MTVAC and AVGFLOW with a coefficient of determination (R^2) of 0.71. The relationship was described mathematically by the following equation:

$$MTVAC = 42.9 - 0.38 \times AVGFLOW$$

The R^2 increased to 0.84 when we omitted the 3 observations with the largest residuals.

Relationships between teat-end callosity and milk flow- and MTT-variables

In the univariable analysis, the outcome variable ROUGHNESS was significantly associated with AVGFLOW, PEAKFLOW, COMPR and MTVAC ($P < 0.05$). No significant association was found between ROUGHNESS and teat position. The results of the univariable analyses are presented as odds ratios in Table 2. Cows with teat-ends classified as normal were the designated comparison group and were assigned the odds ratio (OR) value of 1. OR are multiplicative measures of risk that range from 0 to infinity. OR > 1 is predisposing and implies an increased risk. OR < 1 is preventive and implies an inverse association.

The results from the PCA indicated that the variables AVGFLOW, PEAKFLOW, COMPR and MTVAC were strongly related. To avoid collinearity in the multivariable models, separate model building procedures were performed by including one of these variables in addition to remaining variables from the univariable analyses meeting the inclusion criteria.

The results of the multivariable models showed that MTVAC, COMPR, AVGFLOW and PEAKFLOW were all associated with ROUGHNESS ($P < 0.01$). The model with AVGFLOW as explanatory variable (Table 3) had the lowest BIC (290.59). The models using MTVAC, COMPR and PEAKFLOW had a BIC of 290.70, 297.95 and 291.88, respectively.

For the outcome variable THICKNESS, the univariable analysis showed a significant association with AVGFLOW, PEAKFLOW, COMPR, MTVAC and MPCOM ($P < 0.05$). The results are presented in Table 2. A significant association was not found between THICKNESS and teat position.

MTVAC, COMPR, AVGFLOW and PEAKFLOW were significantly associated with THICKNESS in multivariable models ($P < 0.01$). The model with AVGFLOW as explanatory variable had lowest BIC (281.38) also for this

Table 2 Results from univariable analysis for teat-end callosity roughness and thickness

Variable	ROUGHNESS				THICKNESS			
	Odds ratio	P	95% CI		Odds ratio	P	95% CI	
			Lowe	Upper			Lower	Upper
MTVAC, kPa	2.126	0.001	1.357	3.331	1.890	0.008	1.185	3.016
COMPR, kPa s	1.380	0.014	1.069	1.781	1.443	0.016	1.072	1.942
AVGFLOW, kg/min	0.040	0.001	0.006	0.265	0.049	0.005	0.006	0.406
PEAKFLOW, kg/min	0.082	0.001	0.018	0.382	0.146	0.022	0.028	0.761
MPCVAC, kPa	1.008	0.708	0.967	1.050	0.965	0.132	0.921	1.011
MPCOM, kPa	0.979	0.480	0.922	1.039	0.928	0.040	0.865	0.997
Length, cm	1.137	0.665	0.635	2.034	1.054	0.874	0.549	2.024
Apex, cm	0.241	0.099	0.045	1.305	0.843	0.853	0.138	5.136
Base, cm	0.907	0.865	0.295	2.793	1.252	0.714	0.376	4.170
Overmilking time, min	0.916	0.858	0.352	2.384	0.558	0.402	0.143	2.182

MTVAC, average vacuum level in the short milk tube during the main milking period; COMPR, teat compression intensity; AVGFLOW, quarter average milk flow rate; PEAKFLOW, quarter peak milk flow rate; MPCVAC, average vacuum level in the mouthpiece chamber during the main milking period; MPCOM, average vacuum level in the mouthpiece chamber in the overmilking period. Random intercepts at cow level were included in all analyses to account for within cow dependency of the outcome variables

ROUGHNESS, dichotomized outcome variable where smooth teat-end callosity rings form the comparison group and teat-ends with a roughened callosity ring is considered abnormal

THICKNESS, dichotomized outcome variable where teat-ends having a thin or not visible teat-end callosity ring form the comparison group, and medium, thick or extreme are considered abnormal

Table 3 Final multivariable logistic regression models describing the likelihood of a teat having a roughened or thickened teat-end callosity ring, respectively [22]

Outcome and BIC	Variable	Odds ratio	P	95% CI	
				Lower	Upper
ROUGHNESS	DIM	1.016	0.032	1.001	1.030
BIC = 290.59	Parity 1 (reference)	–	–	–	–
	Parity 2	0.291	0.201	0.044	1.928
	Parity ≥ 3	2.943	0.073	0.903	9.593
	AVGFLOW, kg/min	0.020	0.001	0.003	0.160
THICKNESS	DIM	1.024	0.006	1.007	1.041
BIC = 281.38	Parity 1 (reference)	–	–	–	–
	Parity 2	0.386	0.381	0.046	3.249
	Parity ≥ 3	3.969	0.056	0.963	16.362
	AVGFLOW, kg/min	0.019	0.001	0.002	0.181

The models were selected based on having the lowest Bayesian information criterion (BIC) among other models for the same outcome

DIM, days in milk; AVGFLOW, quarter average milk flow rate

ROUGHNESS, dichotomized outcome variable where smooth teat-end callosity rings form the comparison group and teat-ends with a roughened callosity ring is considered abnormal

THICKNESS, dichotomized outcome variable where teat-ends having a thin or not visible teat-end callosity ring form the comparison group, and medium, thick or extreme are considered abnormal

outcome (Table 3). BIC for the models using MTVAC, COMPR and PEAKFLOW were 283.22, 282.86 and 285.33, respectively.

The random intercept term signifying the correlation between teats within the same cow was highly significant ($P < 0.001$) in all models.

Discussion

Relationships among milk flow-variables, MTT-variables and teat dimensions

The clustering of variables identified by the PCA shows that recording and evaluating a smaller number of variables might be sufficient for MTT in AMS herds. Cluster 1 was based on vacuum recordings from the MPC, cluster 2 represented the measured teat dimensions, cluster 3 displayed milk flow recordings, and cluster 4 was based on vacuum recordings from the short milk tube.

We observed a strong negative relationship between MTVAC and AVGFLOW. This is in agreement with previous studies [16, 28]. The relationship between system vacuum, claw vacuum and milk flow has been described in previous experimental studies [10, 15, 16]. The system vacuum was the same for all observations, and the system has no claw. Based on the strong relationship between MTVAC and AVGFLOW it seems possible to use average milk flow as a proxy for the vacuum level in the short

milk tube during milking in an AMS. Increasing the system vacuum will increase the physical forces acting on the teat. Our findings indicates that cows with a low milk flow responded with poor teat-end condition even at a standard system vacuum level. Increasing system vacuum level is likely to increase the number of cows with this problem.

The PCA showed that MTVAC and COMPR were closely related. This indicates that COMPR and MTVAC contain similar information. Because COMPR accounts for both duration of the pulsation cycle, vacuum level in the pulsation tube and liner type, this variable might be better suited for comparisons between herds [12]. Since every milking was performed using the same liner and the same pulsation settings, and there was little variation in vacuum conditions in the pulsation tube between cows, COMPR was mainly influenced by the vacuum level in the short milk tube. The PCA also showed a negative relationship between the milk flow variables (AVGFLOW and PEAKFLOW) and COMPR, which is likely due to the strong association between the milk flow variables and vacuum level in the short milk tube.

In agreement with previous research on teat anatomy and milk flow rate in CMS [17], the PCA showed that there were no apparent association between teat dimensions and the milk-flow variables AVGFLOW and PEAKFLOW. Accordingly, no evident association was found between teat dimensions and the MTT-variables MTVAC and COMPR. The quarter milk flow rate has been shown to be a consequence of the canal anatomy, such as length and diameter, but sophisticated tools such as ultrasonography is required to acquire this kind of information [17].

The PCA also showed that teat dimensions were related to MPC vacuum; larger teat dimensions were associated with lower MPC vacuum. This finding is in agreement with results from a previous study performed in a CMS [19]; a high vacuum level in the MPC can be observed when the teat is too small relative to the diameter of the liner barrel, allowing the vacuum in the short milk tube to propagate to the MPC. A low vacuum level in the MPC is observed when the liner fits the teat, making a tight seal in the liner barrel. Low MPC vacuum levels may also be a result of air leakage due to the mouthpiece opening being too large relative to the base of the teat [19].

Relationships between teat-end callosity and milk flow- and MTT-variables

The multivariable logistic regression models showed that MTVAC, COMPR, AVGFLOW, and PEAKFLOW were all associated with the outcome variables THICKNESS and ROUGHNESS. The negative relationship between the milk flow variables AVGFLOW and PEAKFLOW and the variables based on vacuum recordings; MTVAC and COMPR, as shown in the PCA (Fig. 1), is indicating that these four variables contain similar information.

Difference in BIC of two models < 2 is considered to be a weak evidence for superiority of the model with the lowest BIC, whereas values from 2 to 6 are considered positive evidence [26]. For both outcome variables, the models using AVGFLOW had the lowest BIC. However, the differences only provided weak evidence that the models using AVGFLOW was superior to the other. Nevertheless, this is a relevant finding because the milk flow is readily available in the herd management system and might be used instead of or in addition to vacuum measurements to indicate whether the milking procedure is involved as a cause for teat-end condition problems in a herd. If cows with poor teat-end condition also show low milk flow rates, it should be suspected that the milking settings (e.g. system vacuum) is not suited for this group of cows. In contrast, if poor teat-ends occur frequently across the whole range of milk flow rates, one might suspect that environmental and genetic factors are the dominating causes for the condition, or that the milking system has major defects affecting all cows. Further research is needed to test this hypothesis before it is implemented in herd health management protocols.

Because we used a cross sectional study design, it is relevant to ask whether the associations between teat-end callosity and milk flow rate could be interpreted in two directions; (1) milk flow affecting (vacuum levels and thereby) teat-end callosity, or (2) teat-end callosity affecting milk flow (and thereby vacuum levels). The length of the teat canal, measured by ultrasonography, has been shown to correlate with average and peak milk flow rate [17]. Our study showed that quarter average and peak milk flow rates were associated with vacuum levels in the short milk tube, which is in agreement with previous research from CMS [14]. Previous research have also shown that vacuum conditions at the teat-end during milking is involved in the development of teat-end callosity [10]. We therefore think it is plausible that the difference in development of teat-end callosity between cows primarily is a result of teat canal anatomy, manifested as differences in milk flow rate, rather than the opposite. However, we cannot rule out that a high degree of teat-end callosity may also act in concert with narrow teat canals, leading to further reduced milk flow rate in affected cows. Few authors, if any, have discussed the possible effects of teat-end callosity on milk flow rate.

Neijenhuis et al. [22] found less teat-end callosity in first parity cows than cows in third and later lactations. Although not statistically significant in our models, the OR estimates indicated a higher likelihood of having a thickened or roughened teat-end callosity ring in cows

in third or later lactations compared to first lactation cows (Table 3). The cows in third or later lactations had been milked by CMS in two or more previous lactations. Although less likely, we cannot rule out that this have interfered with the degree of teat end callosity in the present investigation. Sterret et al. [29] investigated teat-end callosity in a group of Holstein cows before and after converting to quarter-based milking, and found a decrease in teat-end callosity approximately 1 month after installing the new system. This shows that teat-end callosity is a dynamic condition, and that previous milking machine settings might be of minor importance. Because our main focus was associations between teat-end callosity and milk flow, not prevalence of teat end callosity, we consider possible effects of earlier exposure to CMS to be of minor importance for our conclusions.

We used vacuum recordings to split the milking into two main phases; main milking and overmilking. Because this is an indirect method, it is not possible to determine exactly when the milk flow starts to decline and when it drops below the take-off limit, which might lead to some misclassification if compared to methods using data from milk meters. The duration of the main milking phase was used as the denominator in the calculation of MTVAC and MPCVAC. Furthermore, duration of the overmilking period was used both as an explanatory variable and as the denominator in the calculation of MPCOM. Thus, it is obvious that erroneous calculations of the transition between the main milking and overmilking periods would hamper the results of the present investigation. However, we have used accepted and standardized methods commonly implemented in herd health advisory services around the world [30]. It is also worth noting that both MTVAC and AVGFLOW were significantly related with teat-end callosity thickness and roughness, and that AVGFLOW was calculated by the AMS software independent of how we defined the transition between milking phases. This indicates that the definition of the main milking and overmilking phases cannot have had a major impact on our models. We also acknowledge that the varying time span between teat-end scoring and vacuum measurements and the non-standardized time from milking to teat measurement may have added variability to the results. It is reasonable to expect that less variability due to a more uniform sampling regime would strengthen rather than weaken the reported associations, which already by the present approach have shown to be quite significant.

No associations were found between THICKNESS and ROUGHNESS and overmilking time. This is likely due to short duration and little variation in the overmilking periods in AMS, as described in previous studies [4, 5]. In our data, 90% of the observations had an overmilking time between 16 and 48 s. Because the AMS settings included a low-vacuum period of 6 s and a delay from initiation of detachment to actual detachment of 4 s, there might be a slight overestimation of the duration of the overmilking time. However, this overestimation is similar for all milkings, and we do not expect this to bias our assessments of overmilking time and teat end condition.

A recent study revealed a relationship between MPC vacuum and congestion at the teat-ends [20]. Our outcome variables can be considered long-term changes in the teat tissue, whereas the congestion shown by Penry et al. [20] was observed by ultrasonography immediately after milking. Despite short overmilking periods, the variable MPCOM showed a significant association with THICKNESS in the univariable analysis ($P < 0.05$). However, none of the MPC variables showed significant associations with THICKNESS or ROUGHNESS in the multivariable models when DIM and parity were accounted for. Conclusively, further research is warranted to increase the knowledge on how overmilking in AMS can be evaluated by vacuum recordings.

The data were obtained from a single AMS herd in which all cows were milked with the same milking machine settings and the same liner, and we acknowledge that this may lower the external validity. However, because relationships between teat-end condition and milking machine performance has traditionally been studied in CMS [14–16, 31], our study in an AMS herd under field condition represents a step forward for the knowledge on associations between teat-end condition and milk flow in AMS herds. Due to the differences between CMS and AMS, e.g. milking cluster vs. individual attachment and detachment, our findings should be used with precaution in CMS.

A previous study have indicated that breeds differs concerning the development of teat-end hyperkeratosis [31]. Although we expect associations between milk flow variables in AMS and teat end condition to be similar also for other breeds than Norwegian Red, it is feasible to investigate these associations separately in other breeds.

Conclusion

Quarter milk flow rate obtained from the AMS software may be used as a proxy for vacuum level in the short milk tube. Furthermore, quarter milk flow rate obtained from the AMS provided useful information for evaluating associations between the milking procedure and risk factors for impaired teat-end condition.

Authors' contributions

The study was initiated by ACW, who also participated in planning the study together with GD, HN and ORe. GD and HN performed teat-end scoring, teat measurements and recorded vacuum levels for MTT. HN analysed the vacuum recordings in close cooperation with ORø. ORe, GD, AR and HN did the data

analysis and drafted the manuscript. All authors have read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

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The dataset used and analysed during the current study is available from the corresponding author on reasonable request.

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Not applicable.

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References

1. Neijenhuis F, Barkema HW, Hogeveen H, Noordhuizen JPTM. Relationship between teat-end callosity and occurrence of clinical mastitis. J Dairy Sci. 2001;84:2664–72.
2. Breen JE, Green MJ, Bradley AJ. Quarter and cow risk factors associated with the occurrence of clinical mastitis in dairy cows in the United Kingdom. J Dairy Sci. 2009;92:2551–61.
3. Hamann J, Burvenick C. Physiological status of the bovine teat. Bull Int Dairy Fed. 1994;297:3–12.
4. Svennersten-Sjaunja KM, Pettersson G. Pros and cons of automatic milking in Europe. J Anim Sci. 2008;86:37–46.
5. Hogeveen H, Ouweltjes W, de Koning CJAM, Stelwagen K. Milking interval, milk production and milk flow-rate in an automatic milking system. Livest Prod Sci. 2001;72:157–67.
6. TINE. Annual report. Oslo: Tine Dairy Company; 2013.
7. Stræte EP, Vik J, Hansen BG. The social robot: a study of the social and political aspects of automatic milking systems. Proc Food Syst Dyn. 2017. https://doi.org/10.18461/pfsd.2017.1722.
8. Rutten CJ, Velthuis AGJ, Steeneveld W, Hogeveen H. Sensors to support health management on dairy farms. J Dairy Sci. 2013;96:1928–52.
9. International Organization for Standardization (ISO). Milking machine installations–vocabulary. ISO 3918:2007.
10. Besier J, Lind O, Bruckmaier RM. Dynamics of teat-end vacuum during machine milking: types, causes and impacts on teat condition and udder health—a literature review. J Appl Anim Res. 2016;44:263–72.
11. Mein GA, Williams DM, Thiel CC. Compressive load applied by the teatcup liner to the bovine teat. J Dairy Res. 2009;54:327–37.
12. Spohr M, Uhlenbruck F. Melktechnische einflüsse auf die asprägung von hyperkeratosen In: DVG-Tagung. Herausforderungen in der Zukunft der Mastitisbekämpung. Grub: Deutsche Veterinärmesizinische Gesellschaft e.V.; 2012. p. 120–5.
13. Leonardi S, Penry JF, Tangorra FM, Thompson PD, Reinemann DJ. Methods of estimating liner compression. J Dairy Sci. 2015;98:6905–12.
14. Besier J, Bruckmaier RM. Vacuum levels and milk-flow-dependent vacuum drops affect machine milking performance and teat condition in dairy cows. J Dairy Sci. 2016;99:3096–102.
15. Rasmussen MD, Madsen NP. Effects of milkline vacuum, pulsator airline vacuum, and cluster weight on milk yield, teat condition, and udder health. J Dairy Sci. 2000;83:77–84.
16. Bade RD, Reinemann DJ, Zucali M, Ruegg PL, Thompson PD. Interactions of vacuum, b-phase duration, and liner compression on milk flow rates in dairy cows. J Dairy Sci. 2009;92:913–21.
17. Weiss D, Weinfurtner M, Bruckmaier RM. Teat anatomy and its relationship with quarter and udder milk flow characteristics in dairy cows. J Dairy Sci. 2004;87:3280–9.
18. Williams DM, Mein GA, Brown MR. Biological responses of the bovine teat to milking: information from measurements of milk flow-rate within single pulsation cycles. J Dairy Res. 1981;48:7–21.
19. Borkhus M, Rønningen O. Factors affecting mouthpiece chamber vacuum in machine milking. J Dairy Res. 2003;70:283–8.
20. Penry JF, Upton J, Mein GA, Rasmussen MD, Ohnstad I, Thompson PD, Reinemann DJ. Estimating teat canal cross-sectional area to determine the effects of teat-end and mouthpiece chamber vacuum on teat congestion. J Dairy Sci. 2017;100:821–7.
21. Østerås O, Solbu H, Refsdal AO, Roalkvam T, Filseth O, Minsaas A. Results and evaluation of thirty years of health recordings in the Norwegian dairy cattle population. J Dairy Sci. 2007;90:4483–97.
22. Neijenhuis F, Barkema HW, Hogeveen H, Noordhuizen JPTM. Classification and longitudinal examination of callused teat ends in dairy cows. J Dairy Sci. 2000;83:2795–804.
23. Mein G, Williams DM, Reinemann DJ. Effects of milking on teat-end hyperkeratosis: 1. Mechanical forces applied by the teatcup liner and responses of the teat. In: National Mastitis Council, 42nd annual meeting proceedings. Madison: National Mastitis Council Inc; 2003. p. 114–23.
24. Postma E. New technology for milking vacuum diagnostics helps veterinarians better understand and manage udder health problems. In: Proceedings of the British Mastitis Conference. Taunton: The Dairy Group; 2012. p. 87–88.
25. Mein GA, Reinemann DJ. In: Machine milking: Volume 1. Mein and Reinemann; 2014. p. 38.
26. Rønningen O. Measurement of liner movement. In: Proceedings of the international symposium on advances in milking. Cork: Teagasc and Int. Dairy Fed.; 2007. p 32–41.
27. Dohoo IR, Martin W, Stryhn H. Veterinary epidemiologic research. 2nd ed. Charlottetown: VER Inc; 2009.
28. Rose-Meierhöfer S, Hoffmann G, Öz H, Ströbel U, Ammon C. Milking-time tests in conventional and quarter-individual milking systems. Landbauforschung Völkenrode. 2010;60:11–5.
29. Sterrett AE, Wood CL, McQuerry KJ, Bewley JM. Changes in teat-end hyperkeratosis after installation of an individual quarter pulsation milking system. J Dairy Sci. 2013;96:4041–6.
30. Rønningen, O. Milking time tests—a tool for milking advisory services. In: 6. Tänikoner Melktechniktagung, Aspekte zur Optimierung der maschinellen Milchgewinnung. Ettenhausen: Forschungsanstalt Agroscope; 2017. p. 5–15.
31. Gleeson DE, O'Callaghan EJ, Rath M. The effects of genotype, milking time and teat-end vacuum pattern on the severity of teat-end hyperkeratosis. Ir J Agric Food Res. 2003;42:195–203.

III

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Factors associated with milking-to-milking variability in somatic cell counts from healthy cows in an automatic milking system

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ABSTRACT

Fully automated on-line analysis equipment is available for analysis of somatic cell count (SCC) at every milking in automatic milking systems. In addition to results from on-line cell counters (OCC), an array of additional cow-level and quarter-level factors considered important for udder health are recorded in these systems. However, the amount of variability in SCC that can be explained by available data is unknown, and so is the proportion of the variability that may be due to physiological or normal variability. Our aim was to increase our knowledge on OCC as an indicator for disturbances in udder health by assessing the variability in OCC in cows free from clinical mastitis. The first objective was to evaluate how much of the variability in OCC could be explained by different potential sources of variability, including intramammary infection (IMI) status (assessed by bacterial culture of quarter milk samples). The second objective was to evaluate the repeatability of the OCC sensor used in our study and the agreement between OCC values and SCC measured in a dairy herd improvement (DHI) laboratory. A longitudinal study was performed in the research herd of the Norwegian University of Life Sciences from January 5th 2016 to May 22nd 2017. Data from 62,471 milkings from 173 lactations in 129 cows were analyzed. We used ln-transformed OCC values (in 1000 cells/ml) as the outcome (lnOCC) in linear mixed models, with random intercepts at cow-level and lactation-level within cow. We were able to explain 15.0% of the variability in lnOCC with the following fixed effects: lactation stage, parity, milk yield, OCC in residual milk from the previous milking, inter-quarter difference between the highest and lowest conductivity, season, IMI status, and genetic lineage. When including the random intercepts, the degree of explanation was 55.2%. The individual variables explained only a small part of the total variability in lnOCC. We concluded that physiological or normal variability is probably responsible for a large part of the overall variability in OCC in cows without clinical mastitis. This is important to consider when using OCC data for research purposes or in decision-support tools. Sensor repeatability was evaluated by analyzing milk from the same sample multiple times. The coefficient of variation was 0.11 at an OCC level relevant for detection of subclinical mastitis. The agreement study showed a concordance correlation coefficient of 0.82 when comparing results from the OCC with results from a DHI laboratory.

1. Introduction

Management of udder health is essential for maintaining an efficient and sustainable dairy production. Somatic cell count (SCC) is a widely used indicator of udder health status in dairy cows, and is used both at quarter level, cow level, and bulk-tank level (Schukken et al., 2003). Dairy herd improvement (**DHI**) programs commonly include monthly or bimonthly measurements of cow-level SCC for assessing udder health and implementing selective dry cow therapy strategies (Østerås et al., 1999; Torres et al., 2008). In automatic milking systems (**AMS**), fully automated on-line analysis equipment is available for on-farm analysis of SCC at every milking (Sørensen et al., 2016). This represents a substantial increase in the amount of data containing information, e.g., for udder health management, which may also serve as phenotypes for breeding programs. In addition to frequent measurements of SCC, a whole array of additional cow-level and quarter-level factors considered of importance for udder health are recorded in the AMS at every milking (Hogeveen et al., 2010). This raises the question regarding the

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extent to which the variability in SCC can be explained by different explanatory factors, and the proportion of the variability that may be due to physiological variation within and between cows. Therefore, it is important that the relevance of using such frequent measurements is evaluated against known biological states, and that sources of variability are studied within and between animals before a conclusion on a given animal's health status is reached.

A literature review estimated that the geometric mean SCC level in uninfected quarters was 68,000 cells/mL (Djabri et al., 2002). However, SCC in milk can increase by tenfold or more during an intramammary infection (**IMI**) (de Haas et al., 2002). An IMI caused by bacteria is considered to be the most common cause of elevated SCC in dairy cows (Schepers et al., 1997; IDF, 2013). Other reasons for fluctuations in SCC include, among other causes: systemic disease, trauma to the udder, lactation stage, parity, and seasonal variation (IDF, 2013). However, a large proportion of the variability in SCC remains unexplained, even when accounting for these factors (Schepers et al., 1997). The milking-to-milking variability in milk composition, including SCC, has been investigated in previous studies (Quist et al., 2008; Forsbäck et al., 2010). However, in these studies, data on bacteriological udder health status were either not included at all (Quist et al., 2008) or only sparsely (Forsbäck et al., 2010). Both these studies were of short duration, being only five and 21 days, respectively. Hence, milking-to-milking variability in SCC over more prolonged periods in cows with known bacteriological udder health status has, to our knowledge, not previously been described.

Although the detection of clinical mastitis in AMS still receives substantial attention, implementation of preventive measures should be preferable to reduce production losses, to reduce the use of antimicrobial drugs, and to improve animal welfare. Detection of subclinical mastitis by SCC plays an important role in mastitis prevention programs. A recent study investigated the performance of results from an on-line somatic cell counter (**OCC**) as an indicator for subclinical mastitis (Dalen et al., 2019). Although the sensitivity and specificity for detection of subclinical mastitis were reported to be better than those of traditional DHI systems (Reksen et al., 2008; Dalen et al., 2019), the amount of false positive alerts remains challenging. Increasing our knowledge on potential sources of variability in OCC and determining how much of the variability can be attributed to specific measurable factors, might help improve future decision-support tools for udder health management.

Sensor performance can be described by repeatability (the variation in the results when the same sample is measured repeatedly) and the agreement between one method and a reference method (also called reproducibility) (Dohoo et al., 2009). The agreement between OCC measurements and SCC measurements from a DHI system has previously been evaluated in commercial Holstein and Jersey herds (Sørensen et al., 2016). However, the repeatability of the OCC sensor has yet to be reported. As variability caused by suboptimal sensor performance will be incorporated in the total variability in the frequent OCC measurements, this needs to be evaluated separately in order to assess how the precision might influence overall variability.

The aim of this study was to increase our knowledge on OCC as an indicator for disturbances in udder health by assessing the variability in frequently measured OCC in cows free from clinical mastitis. Our first objective was to evaluate how much of the variability between frequently measured OCC could be explained by potential explanatory factors, including subclinical IMI status as determined by bacterial culture in quarter milk samples (QMS), variability between cows, and variability between milkings in the same cow, among other factors. To assess the sensor as a potential source of variability in our data, a second objective was to evaluate the repeatability of the OCC sensor used in this study, and the agreement between OCC results and SCC measured in a DHI laboratory.

2. Material and methods

2.1. Milking-to-milking variability in OCC

2.1.1. Field study and data collection

This study used data collected at the research herd at the Norwegian University of Life Sciences in a study previously described by Dalen et al. (2019). Cows in two lactation pens, each holding approximately 50 cows, were investigated over 17 months from January 5th 2016 to May 22nd 2017. Each lactation pen was equipped with one AMS (DeLaval VMS, DeLaval International AB, Tumba, Sweden) and an On-line Cell Counter (DeLaval International AB, Tumba, Sweden) that recorded cow-level OCC at every milking. Both AMS were adjusted to minimize the amount of residual milk in the system after milking to reduce the effect of carryover of milk from the previous cow. First, when a milking had started, the milk pump was run for a short period to replace the residual milk in the pump with milk from the current milking. Secondly, instead of mixing the milk in the receiver jar before sampling, small pulses of milk were collected during pumping the entire milk volume. The AMS recorded milking interval, electrical conductivity, average milk-flow rate, and milk yield at quarter level in every milking. These data were obtained from the DelPro management system (DeLaval International AB, Tumba, Sweden). Because OCC is reported at cow level, a variable describing the average milk-flow rate per milking (kg/min) at cow level was calculated as the average value for quarters with non-missing values. Conductivity was also reported per quarter, and, to convert this into a cow-level indicator of disturbances in udder health, the difference between the highest and lowest conductivity among the four quarters (inter-quarter difference) was calculated (Sheldrake et al., 1983; Nielen et al., 1992). Milk yield per milking (kg) was reported at cow level. Most cows in the study herd belonged to one of two genetic groups of Norwegian Red cattle, one selected for high milk yield and the other for low incidence of clinical mastitis (Heringstad et al., 2007). Differences in SCC have previously been reported between these two genetic groups (Heringstad et al., 2008).

Aseptic QMS were collected monthly from all lactating cows, and were frozen after collection and during transport to the laboratory for microbiological analyses (Hogan et al., 1999). From the cultured bacteria, species identification was performed by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) microflex LT (Bruker Corporation, Billerica, USA) (Cheuzeville, 2015). Samples with culture results indicating more than 2 morphologically different colony types were treated as contaminated and excluded from further analyses.

2.1.2. IMI status

The culture results from the QMS were used to assign a subclinical IMI status for each cow throughout the study period. Dalen et al. (2019) described the methodology in detail. In short, pathogens were divided into 2 groups; the group of pathogens from which a high cell count would be expected during an IMI episode was named Pat 1, while known mastitis pathogens that were not included in Pat 1, were in the Pat 2 category. Positive culture results were considered to be associated with an episode of subclinical IMI when fulfilling at least one of the following three criteria: (1) $\geq$ 1000 cfu/mL of a single mastitis pathogen were cultured from a single sample in at least 1 quarter, (2) $\geq$ 500 cfu/mL of a mastitis pathogen were cultured from 2 out of 3 consecutive milk samples from the same quarter, or (3) $\geq$ 100 cfu/mL of a mastitis pathogen were cultured from 3 consecutive milk samples from the same quarter. These definitions were adapted from Zadoks et al. (2002). Cows with positive milk cultures that did not meet any of the above criteria were classified as being transiently colonized (Reksen et al., 2012). To assign an IMI status to every milking based on the monthly QMS, we used the mid-point estimation method previously described by Zadoks et al. (2002), assuming that a shift from one udder health status to another happened midway between two sampling

occasions. Furthermore, because the OCC is recorded at the cow level, the udder health status at quarter level were aggregated into cow-level diagnoses. When assigning the IMI statuses, we implemented a hierarchical order in the classification such that a cow could only be assigned to the Pat 2 IMI group when there was no simultaneous diagnosis of a Pat 1 IMI in the same cow during the same period. Based on this set of criteria, cows were assigned one of the following four udder health statuses for every milking: No IMI, Pat 1 IMI, Pat 2 IMI, or transient colonization.

Details on the results from the microbiological analyses performed on the QMS can be found in Dalen et al. (2019). Briefly, mastitis pathogens were cultured from 1222 out of 5330 QMS, and the pathogens detected most frequently were *Staphylococcus epidermidis* (n = 234), *Corynebacterium bovis* (n = 225), *Staphylococcus chromogens* (n = 167), *Staphylococcus aureus* (n = 119), and *Staphylococcus haemolyticus* (n = 116).

2.1.3. Inclusion and exclusion criteria

A total of 96,524 milkings were performed in the two AMS during the study period. This included data from 257 full or partial lactations in 173 cows. Observations fulfilling the following criteria were included in the analysis: days in milk (DIM) from 5 to 305, milking interval of 4–24 h, and milk yield of $\geq$ 3.5 kg per milking. Furthermore, observations with missing or zero OCC values, observations with missing OCC from the previous milking in the same AMS, and lactations with data from fewer than 100 days were omitted. All data from lactations where a case of clinical mastitis had been recorded were excluded from the analysis.

2.1.4. Statistical analysis

The dataset used in the statistical analyses contained 62,471 milkings from 173 lactations in 129 cows; 85 cows contributed with one lactation, and 44 cows with two lactations. At lactation level, the distribution among parities were as follows: 81 first parity, 42 second parity, and 50 third or higher parities.

We used OCC (in 1000 cells/mL) transformed to a logarithmic scale (lnOCC) as the outcome variable in linear mixed models (Schepers et al., 1997; Reksen et al., 2008). The explanatory variables evaluated are described below and summarized in Table 1. We included the lnOCC value from the previous milking in the same AMS to adjust for the carryover effect due to residual milk from the previous cow, as suggested by Løvendahl and Bjerring (2006). Milk yield per milking (kg) was included to account for the dilution effect of milk from healthy quarters in the same cow (Green et al., 2006) and differences in milk production between cows.

To adjust for possible differences between the two sensors used in the study, a categorical variable, distinguishing between the two milking stations, was included in the analysis. The maximum inter-quarter difference in conductivity per milking was included as an indicator of pathological processes in one or more quarters (Sheldrake et al., 1983; Nielen et al., 1992). Previous research has shown that average milk-flow rate is associated with SCC (Berry et al., 2013), and the average milk-flow rate per milking was therefore included. Because both the milk yield and the milk-flow rate per milking are associated with time since last milking, our models adjusted for this by including the milking interval in hours (Hogeveen et al., 2001). To account for changes in SCC related to stage of lactation, DIM and lnDIM were included in the model (Reksen et al., 2008). Cows with different parities differ in SCC level (Laevens et al., 1997), and therefore our model included a categorical variable distinguishing between first, second, and third or later parities. To account for variability in OCC due to genetic differences between cows, a categorical cow-level variable accounting for differences between cows according to genetic group ("low mastitis"; "high yield"; "unknown") was included in our model, with "low mastitis" set as the baseline level (Heringstad et al., 2008). Seasonal variability was accounted for by including a categorical variable distinguishing between winter (Dec., Jan., Feb.), spring (Mar., Apr., May), summer (Jun., Jul., Aug.), and autumn (Sep., Oct., Nov.). Finally, the IMI status (No IMI, Pat1 IMI, Pat2 IMI, or transient colonization) was also included in the analysis as a categorical variable.

Due to the lack of independence between repeated OCC measurements within cows and lactations, we used a multi-level modeling approach. Random intercepts were specified at cow level and lactation level within cows. The significance of the random intercept terms was evaluated against a model with a fixed intercept using the likelihood ratio test. A variance component model was used for calculating the intraclass correlation coefficient to describe how much of the overall variability resided at the cow level and at within-cow lactation level. To model the dependency between the residual error terms within cow and lactation, the following correlation structures were evaluated: exponential, compound symmetry, and no within-lactation correlation between the error terms. The exponential and compound symmetry correlation structures were specified with the same grouping variables as the random intercepts, and data was sorted by milking number within lactations. First-order autocorrelation was also considered, but was not used due to unequal time intervals between observations. The model resulting in the lowest Akaike information criterion (AIC) value was selected. Subsequently, a backwards variable selection procedure was applied, and statistical significance was considered at P-value $<$ 0.05. The regression modeling was performed in the package 'nlme' in the statistical software R, version 3.6.1 (R Core Team, 2019).

Goodness-of-fit was evaluated by calculating the marginal and conditional coefficient of determination (Nakagawa and Schielzeth, 2013), which describes the variance explained by the fixed factors only and the combination of the random and fixed factors, respectively. The estimates were calculated using the package 'MuMin' in the statistical software R, version 3.6.1 (R Core Team, 2019) based on parameter estimates from the final model. To evaluate the approximate

Table 1
Summary of explanatory variables evaluated in the study.

Variable	Brief description[a]
Carryover	ln-transformed OCC (in 1000 cells/mL) from the previous cow milked in the same AMS.
Milking station	Categorical variable distinguishing between the two OCC sensors used in the study.
Milk yield (kg)	Adjustment for dilution effect on SCC from healthy quarters and differences in milk production between cows.
Conductivity	Difference between highest and lowest conductivity among the four quarters.
Milk flow rate (kg/min)	Average milk flow rate from quarters with registered milk flow.
Milking interval (hours)	Time since previous milking for the same cow.
Lactation curve	A lactation curve described by DIM and the natural logarithm of DIM accounting for changes in OCC related to lactation stage.
Parity	Categorical variable for first, second, and third or later lactation.
Lineage	Categorical variable distinguishing between different genetic lineages; low mastitis, high milk yield, and unknown.
Seasonal variability	Categorical variable; winter, spring, summer, autumn.
IMI status	Categorical variable; No IMI, Pat 1 IMI, Pat 2 IMI, transient colonization.

[a] OCC = on-line somatic cell count; AMS = automatic milking system; SCC = somatic cell count; DIM = days in milk; IMI = intramammary infection; Pat 1 IMI = IMI with mastitis pathogens from which a high somatic cell count would be expected; Pat 2 IMI = IMI with other known mastitis pathogens.

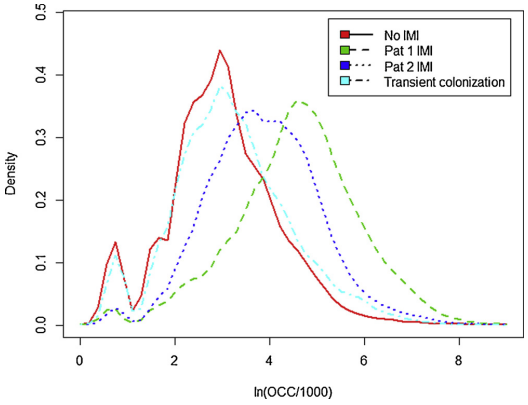

Fig. 1. Smoothed density plot showing the distribution of ln-transformed Online Cell Count (OCC) values (in 1000 cells/mL) in periods of 1) no intramammary infection (No IMI), 2) IMI with known mastitis pathogens from which a high somatic cell count would be expected (Pat 1 IMI), IMI with other known mastitis pathogens (Pat 2 IMI), and 3) Transient colonization.

contribution of the individual variables to the overall fit for the final model, we used the difference in marginal coefficient of determination between the final model and models where one term at a time was omitted. The two parameters of the lactation curve (DIM and lnDIM) were included simultaneously in all models.

Residual diagnostics were performed by graphical assessment of the distribution of the residuals calculated for the individual observations and for the random intercepts, respectively.

To compare the variability in OCC between periods of different IMI status (no IMI, Pat 1 IMI, Pat 2 IMI, or transient colonization), the distribution of lnOCC in periods of different IMI statuses was evaluated graphically using smoothed density curves. In addition, coefficients of variation were calculated for each of the four IMI statuses, assuming a log-normal distribution in OCC.

2.2. Agreement between OCC and SCC

To evaluate the agreement between results from the OCC sensor used in the current study and SCC measurements from a laboratory accredited by the International Committee for Animal Recording (ICAR), additional composite milk samples were collected at 16 occasions over 5 weeks for a subset of milkings in one of the two milking stations. The 64 cows present in one of the two lactation pens were sampled multiple times. The samples were collected with an automated milk sampler (DeLaval, Tumba, Sweden), conserved with bronopol (2-bromo-2-nitropropane-1,3-diol) and shipped refrigerated to the ICAR-accredited laboratory used for routine milk analyses by the Norwegian Dairy Herd Recording System. The samples were analyzed in a Bentley Somacount FCM (Bentley Instruments Inc., Chaska, MN). The dataset included 1661 OCC values with corresponding SCC measurements from 64 cows.

Both SCC and OCC values were transformed to the natural logarithmic scale. Because neither of the methods could be considered a gold standard due to differences in sampling equipment, the concordance correlation coefficient (**CCC**) was chosen for the statistical analysis. A version of CCC modified to account for repeated measurements within cow was used. The analysis was performed in the package 'cccrm' in the statistical software R, version 3.6.1 (R Core Team, 2019). In addition, a scatterplot with a superimposed 45 ° line (representing perfect agreement) was used for graphical assessment of the data.

2.3. Repeatability

To evaluate the repeatability of the OCC sensors, a sample of bulk tank milk (5 L) was collected. The milk was mixed gently, but thoroughly, to ensure an even distribution of the milk constituents, before drawing a number of consecutive 5 mL samples in syringes. These samples were subsequently injected directly in the OCC apparatus, which was operated in manual mode. The process was repeated as many times as possible in the available time slot (n = 62) for both OCC sensors used in the study. The mean OCC value, standard deviation and coefficient of variation (CV) were calculated for both sensors.

3. Results

3.1. Milking-to-milking variability in OCC

3.1.1. Descriptive results

The arithmetic and geometric mean OCC value in the final dataset was 96,629 cells/mL and 35,279 cells/mL, respectively. The lowest OCC value was 1000 cells/mL (detection limit) and the highest was 7,474,000 cells/mL.

The intraclass correlation coefficient calculated from the variance component model was 0.155 at the cow level, and 0.536 at the lactation level. Hence, in our data, 15.5% of the variability in lnOCC could be attributed to differences between cows, and 53.6% to differences between lactations (within cows). Consequently, 46.4% of the variability could be attributed to milking-to-milking differences within lactation.

Smoothed density curves showing the distribution of lnOCC values in periods of no IMI, Pat 1 IMI, Pat 2 IMI and transient colonization are presented in Fig. 1. The no IMI-group has the highest density between lnOCC of 2 and 3 (7400 and 20,000 cells/mL, respectively), whereas the periods of Pat 1 IMI and Pat 2 IMI had their highest densities at an lnOCC value of around 5 and 4, respectively (148,400 cells/mL and 54,600 cells/mL). Periods classified as transient colonization showed a similar distribution as periods of No IMI. There was, however, a large overlap between the lnOCC values between the groups. Supplementing the graphical assessment in Fig. 1, the coefficients of variation for OCC in periods of No IMI, Pat 1 IMI, Pat 2 IMI, and transient colonization were 1.67, 2.13, 1.70, and 1.89, respectively.

3.1.2. Multivariable model

The multivariable linear mixed model, using an exponential correlation structure, was selected based on the lowest AIC. The likelihood ratio test showed that the random intercept terms of "cow" and "lactation" within cow contributed significantly to a better model fit ($P < 0.001$). The estimates from the final model are presented in Table 2. The model showed that, compared with culture-negative periods, the lnOCC increased on average by 0.43 units in periods of subclinical Pat 1 IMI, and by 0.29 units in periods of subclinical Pat 2 IMI. At an SCC of 100,000 cells/mL this corresponds to an increase of 54,000 and 33,000 cells, respectively. The regression coefficients for DIM and lnDIM describe a lactation curve where lnOCC decreases rapidly in early lactation, reaches a minimum around 70 DIM, and slowly increases towards the initial level during the rest of the 305-d lactation. Cows belonging to the genetic group selected for high milk yield had higher lnOCC values than cows in the low mastitis group. The relationship between lnOCC and milk yield was negative; hence higher milk yield was associated with lower lnOCC. The carryover effect showed a positive relationship between the lnOCC in a given milking and the OCC measured in the residual milk from the previous cow milked in the same AMS. No difference was found between lnOCC in the two milking stations, and the variable distinguishing between the two milking stations was omitted from the final model. Only minor changes in the estimates for the other variables were seen after this omission.

Random effect estimates for the final model, reported as standard deviations (95% CI), were 0.41 (0.31 – 0.53) for cow, and 0.72 (0.64 –

Table 2
Parameter estimates from the final multivariable model describing ln-transformed on-line somatic cell count measured by DeLaval on-line cell counter in a Norwegian Red dairy herd. The model included random intercepts at cow- and lactation-level (within cow), and an exponential correlation structure.

Variable[a]	Coefficient	Standard error	P-value	R-squared[b] (%)
Lactation curve:				1.3
Days in milk (DIM)	0.005	0.0003	< 0.001	
lnDIM	−0.358	0.023	< 0.001	
Yield at cow level in the current milking (kg)	−0.038	0.002	< 0.001	1.0
Carryover	0.141	0.002	< 0.001	2.4
Parity:				
First (reference)	–	–	–	–
Second	0.303	0.148	0.047	
Third or later	0.463	0.156	0.005	
Udder health status:				2.9
No IMI (reference)	–	–	–	
Pat 1 IMI	0.434	0.031	< 0.001	
Pat 2 IMI	0.278	0.024	< 0.001	
Transient colonization	0.081	0.024	< 0.001	
Conductivity (inter-quarter difference), mSv	0.381	0.011	< 0.001	1.4
Genetic lineage:				2.7
Low mastitis incidence (reference)	–	–	–	
High milk yield	0.371	0.137	0.008	
Unknown	0.372	0.374	0.322	
Milking interval (hours)	−0.033	0.002	< 0.001	−0.5
Average milk flow rate (kg/min)	0.480	0.052	< 0.001	0.8
Season:				0.1
Winter (reference)	–	–	–	
Spring	−0.051	0.017	0.003	
Summer	−0.117	0.029	< 0.001	
Autumn	−0.048	0.024	0.047	
Intercept	3.787	0.143	< 0.001	

[a] Carryover = lnOCC from the previous cow milked in the same AMS; IMI = intramammary infection; Pat 1 IMI = IMI with mastitis pathogens from which a high somatic cell count would be expected; Pat 2 IMI = IMI with other known mastitis pathogens.

[b] R-squared = the change in marginal coefficient of determination (Nakagawa and Schielzeth, 2013) when a variable was added to a model already containing all other variables in the final model.

0.80) for lactation within cow. Within group standard error (95% CI) was 0.87 (0.86 – 0.88). The correlation structure parameter ρ^2 (95% CI) was 1.81 (1.76–1.85).

The marginal and conditional coefficients of determination showed that the fixed effects in the final model described 15.0% of the variability in lnOCC, while the fixed and random effects together described 55.2% of the milking-to-milking variability of lnOCC in clinically healthy udders. The approximate contributions of the individual variables to the overall marginal coefficient of determination are reported in Table 2.

3.2. Agreement between OCC and SCC

The CCC between the results from the OCC and the DHI laboratory, estimated on ln-transformed data, was 0.82 (95% CI: 0.78 - 0.85). The CCC has a maximum value of 1, representing the situation of perfect agreement between the two methods.

The agreement between OCC and SCC is displayed in Fig. 2. Although most observations was clustered around the superimposed line of perfect agreement, it appears that the agreement increases by increasing lnSCC values.

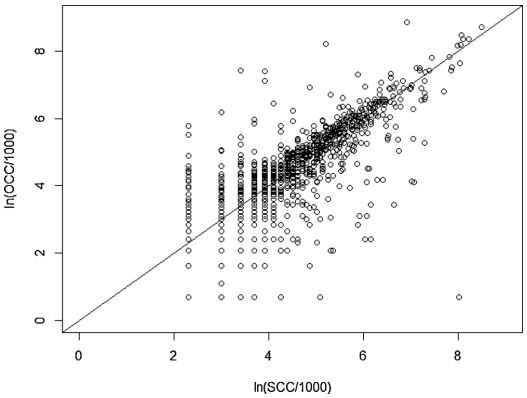

Fig. 2. On-line Cell Count (OCC) results plotted against Somatic Cell Count (SCC) measured in a DHI laboratory. Scatterplot including 1661 observations with corresponding OCC and SCC results with a superimposed 45° line representing the situation of perfect agreement between the two methods.

3.3. Repeatability

Results from the repeatability study showed nearly identical results for the two OCC sensors used in the current study. The 62 analyses performed on OCC 1 resulted in a mean OCC value (in 1000 cells/mL) of 112, a standard deviation of 12.8, and consequently a CV of 0.11. The 62 analyses performed on OCC 2 resulted in a mean OCC value (1000 cells/mL) of 117.9, a standard deviation of 12.7, also resulting in a CV of 0.11.

4. Discussion

To the authors' knowledge, this is the first presentation of the basic characteristics of frequently measured OCC relative to known IMI status. Only lactations with no records of clinical mastitis were included in the analyses. Our findings contribute to a better understanding of the normal variability in OCC; this is important for further improving the use of OCC for research, for udder health management in AMS herds, and for breeding programs.

SCC data are often used in research studies investigating how different aspects of dairy production (e.g., housing, milking routines, treatment protocols, etc.) might affect udder health (Bielfeldt et al., 2004; Erdem et al., 2007; Bhutto et al., 2010). The underlying assumption is that a risk factor affects udder health, which, in turn, results in changes in SCC. A major strength of our study is the close monitoring of IMI status by monthly QMS bacterial cultures together with detailed data recorded by the OCC and AMS at every milking. This enables us to evaluate factors of importance for lnOCC and to assess the variability in lnOCC obtained at every milking in clinically healthy cows. An important finding is that inclusion of subclinical IMI status in our model increased the degree of explanation by only 2.9 percentage points, from 12.1% to 15.0%. This is, however, a conservative estimate because the effect of IMI on OCC is adjusted by other variables included in the model. The IMI status used in this study describes persistent infections with known udder pathogens, which are recognized to be the most important cause of elevated SCC (IDF, 2013). It is therefore relevant to discuss some possible explanations why our study resulted in a relatively low degree of explanation attributed to udder health status: Firstly, this study focused on clinically healthy udders, and lactations with clinical mastitis were excluded from the analysis. By doing so, the range of IMI statuses was restricted to subclinical mastitis, and it is likely that including cases of clinical mastitis would have increased the degree of explanation in our model. Secondly, quarter milk samples

were collected monthly. It is possible that a higher sampling frequency would also have increased the degree of explanation between the subclinical mastitis cases as defined in our study and the OCC values obtained at every milking.

As for all biological variables, some degree of normal or physiological variation should be expected in SCC. Our results obtained from a herd of Norwegian Red cows show that the normal variation is likely to be much higher than can be explained through close monitoring of clinically healthy cows in sensor systems commonly used in AMS. This is underlined by the graphical assessment of the distribution of lnOCC, which shows a large extent of overlap in lnOCC values between periods without IMI and periods with either IMI or transient colonization. Nevertheless, the use of SCC in udder health management has contributed to substantial improvements in dairy production by identifying cows in need of closer attention, e.g., when implementing selective dry cow therapy (Østerås et al., 1999; Zecconi et al., 2018a; Lipkens et al., 2019).

In one of the few reports on variability in SCC, Schepers et al. (1997) estimated variance components for factors affecting SCC at quarter level from data recorded at approximately monthly intervals and reported that their model explained 50.2% of the variation in ln-transformed SCC. The model of Schepers et al. (1997) included herd and cow within herd, in addition to season, bacterial diagnoses, stage of lactation, parity, and clinical mastitis. In their data from seven herds, cow within herd explained 11% of the overall variability, while herd explained only 0.6%. In contrast to Schepers et al. (1997), the present study used OCC data at cow level, and data were recorded at every milking. It is possible that this difference has introduced additional variability to our data. In addition to the monthly QMS, our model used conductivity data measured at every milking as an indicator of changes in udder health status; this is a possible explanation for reaching a similar overall degree of explanation as that of Schepers et al. (1997).

Mastitis has been included in the breeding program for the Norwegian Red breed since 1978, resulting in genetic improvement (Heringstad and Østerås, 2013). More recently, geometric mean SCC over 305-day lactations have been included in the genetic evaluation for Norwegian Red (Interbull, 2012). SCC have also been evaluated as an alternative trait in the absence of reliable data on clinical mastitis, and a genetic correlation of 0.7 between these two traits shows not only that SCC is a relevant indicator for clinical mastitis, but also that SCC and clinical mastitis are genetically different traits (Ødegård et al., 2003). In our study, clinically healthy cows of the genetic group for high milk yield had higher lnOCC values than cows bred for low mastitis risk, also after adjustment for differences in milk yield. Hence, the effect of genetic lineage on lnOCC in our final models is likely to be a true effect of genetic differences in mastitis resistance, rather than a correlated response of differences in production level. This is in agreement with previous research results in the same breed (Heringstad et al., 2008).

The AMS used in our study were adjusted to reduce the amount of residual milk in the system after each milking (carryover effect). Nevertheless, our statistical adjustment for the carryover effect was significant in the multivariable models, and increased the marginal coefficient of determination by 2.4%. Løvendahl and Bjerring (2006) and Løvendal et al. (2010) reported up to 20% carryover in various types of AMS, showing that the impact of carryover, and the need for adjustment, is pronounced in commercial herds for which the sampling equipment has not been optimized. As pointed out by Sørensen et al. (2016), correction of carryover effect is also relevant for DHI samples collected in AMS. In this case, the carryover effect will not only affect the SCC measurement, but also the other milk constituents measured in the same sample. These results show that further improvements in the sampling equipment are necessary. Furthermore, by obtaining data on the sampling order, it might be possible for DHI systems to adjust for the carryover effect and provide more precise estimates for SCC and other milk constituents.

With the high degree of normal variability in OCC and the large extent of overlap in OCC in periods with and without IMI, it seems likely that identification of new biomarkers or combinations of biomarkers that are better at distinguishing pathological from physiological processes in the udder would be of benefit to the dairy industry. The difference in electrical conductivity between the quarter with the highest and lowest value was significantly related to OCC, which is in agreement with previous research (Nielen et al., 1992); a higher difference was associated with increasing OCC. Like SCC, electrical conductivity is used as an indicator of ongoing inflammatory processes in the udder. However, conductivity has been shown to have poor diagnostic test properties for the detection of subclinical mastitis (Norberg et al., 2004). Although the combination of electrical conductivity and SCC has been found to improve detection of clinical mastitis (Kamphuis et al., 2008), it is not known whether this is also the case for subclinical mastitis. A number of alternative biomarkers, such as L-lactate dehydrogenase, N-acetyl-β-D-glucosaminidase activity, and milk amyloid A, have been evaluated for the detection of clinical mastitis (Chagunda et al., 2006; Gerardi et al., 2009). The use of these on commercial farms is limited, and only L-lactate dehydrogenase has been implemented in on-farm systems (DeLaval Herd Navigator; DeLaval, Tumba, Sweden). Furthermore, the concentration of these biomarkers in milk is related to a compromised blood-milk barrier, and they are therefore less suitable for detection of subclinical mastitis. Methods differentiating between cell types in milk have recently been developed for the use in DHI laboratories (Damm et al., 2017), but it is still unclear how much useful information this adds over traditional SCC measurements (Zecconi et al., 2018b). Another aspect is the dilution effect of milk from healthy quarters, which represents an important limitation of using composite milk samples for detection of changes in milk arising in one quarter. Forsbäck et al. (2010) studied the variability in milk constituents at quarter level and argued that repeated measurements at quarter level provides more accurate information on udder health than cow-level data.

Sørensen et al. (2016) evaluated the agreement between OCC results and SCC results from a DHI laboratory in seven commercial herds, and reported generally good agreement between the two methods (mean $R^2 = 0.86$), although their results differed between herds and breeds. In line with Sørensen et al. (2016) the results from the current study indicates that the agreement between the two methods was reasonably good (CCC = 0.82) also in this herd of Norwegian red cows. However, the graphical assessment revealed that the results differed substantially between methods in some cases, and that this trend was more pronounced at low lnSCC values. This needs to be taken into consideration when operating at low thresholds for defining subclinical mastitis.

The repeatability of the OCC sensor, as evaluated by coefficient of variation, was identical for the two devices used in our study (CV = 0.11) at an OCC-level comparable to threshold values for the detection of IMI (e.g. 132.000 cells/mL at Sp = 90% for the detection of Pat 1 IMI; Dalen et al., 2019). For comparison, the manufacturer of the Bentley Somacount, which was used at the DHI lab, reports a CV ≤ 0.06 at 100,000 cells/mL (Bentley Instruments Inc., Chaska, MN). However, the present study was performed by manually injecting the milk sample into the apparatus, hence any additional variability caused by the sampling method could not be quantified. It should also be noted that the current study evaluated the repeatability at one OCC level only, and that data for other OCC levels is needed before concluding on the repeatability for the whole range of possible OCC values.

Milking interval was included in the final model and showed a significant relationship with the outcome variable. Nevertheless, with the chosen method for evaluating the contribution of the individual variables to the overall degree of explanation, milking interval apparently had a negative impact. This can be interpreted as an artifact arising from the combination of milking interval as a fixed effect and the correlation structure included in our model to account for the dependency between residual error terms within cow and lactation.

Because milking interval can be considered a measure of the temporal proximity between two observations, including this variable in the model will affect the correlation structure parameters. This way, when removing milking interval from the model, a larger proportion of the variance was accounted for by the random effects, resulting in the situation where omitting milking interval as a fixed effect apparently led to a higher marginal degree of explanation. In lack of a more sophisticated method, we acknowledge that the results should be interpreted as approximate contributions to the overall model fit.

We recognize that our study has some limitations that should be considered when interpreting the results. The data were obtained from a single Norwegian Red herd, and although the herd, including management practices, housing, milking procedure etc., is comparable to herds on commercial Norwegian farms of the same size, extrapolation to other herds and other breeds should be done with caution.

5. Conclusion

This study identified several factors associated with fluctuations in frequently measured OCC values in clinically healthy cows in an AMS herd. However, these factors only explained a small proportion of the overall variability in the data, and a large degree of the overall variability remain unexplained despite close monitoring of the IMI status by monthly quarter milk samples. The unexplained variability likely represents physiological fluctuations in OCC, which is important to consider when using frequently measured OCC in research or for herd management purposes.

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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.

Bhutto, A.L., Murray, R.D., Woldehiwet, Z., 2010. Udder shape and teat-end lesions as potential risk factors for high somatic cell counts and intra-mammary infections in dairy cows. Vet. J. 183, 63–67. https://doi.org/10.1016/j.tvjl.2008.08.024.

Bielfeldt, J.C., Badertscher, R., Tölle, K.-H., Krieter, J., 2004. Factors influencing somatic cell score in Swiss dairy production systems. Schweiz. Arch. Tierheilk. 146 (12), 555–560. https://doi.org/10.1024/0036-7281.146.12.555.

Chagunda, M.G.G., Larsen, T., Bjerring, M., Ingvartsen, K.L., 2006. L-lactate dehydrogenase and N-acetyl-β-D-glucosaminidase activities in bovine milk as indicators of non-specific mastitis. J. Dairy Res. 73, 431–440. https://doi.org/10.1017/S0022029906001956.

Cheuzeville, L., 2015. Rapid Detection of Microorganisms in the Dairy Value Chain by MALDI-TOF MS. MS Thesis. Faculty of Science and Technology, University of Stavanger, Stavanger, Norway.

Dalen, G., Rachah, A., Nørstebø, H., Schukken, Y.H., Reksen, O., 2019. The detection of intramammary infections using online somatic cell counts. J. Dairy Sci. https://doi.org/10.3168/jds.2018-15295.

Damm, M., Holm, C., Blaabjerg, M., Bro, M.N., Schwarz, D., 2017. Differential somatic cell count — a novel method for routine mastitis screening in the frame of Dairy Herd Improvement testing programs. J. Dairy Sci. 100, 4926–4940. https://doi.org/10.3168/jds.2016-12409.

de Haas, Y., Barkema, H.W., Veerkamp, R.F., 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.

Djabri, B., Bareille, N., Beaudeau, F., Seegers, H., 2002. Quarter milk somatic cell count in infected dairy cows: a meta-analysis. Vet. Res. 33, 335–357. https://doi.org/10.1051/vetres:2002021.

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

Erdem, H., Atasever, S., Kul, E., 2007. Some environmental factors affecting somatic cell count of Holstein cows. J. Appl. Anim. Res. 32, 173–176. https://doi.org/10.1080/09712119.2007.9706871.

Forsbäck, L., Lindmark-Månsson, H., Andrén, A., Åkerstedt, M., Andrée, L., Svennersten-Sjaunja, K., 2010. Day-to-day variation in milk yield and milk composition at the udder-quarter level. J. Dairy Sci. 93, 3569–3577. https://doi.org/10.3168/jds.2009-3015.

Gerardi, G., Bernardini, D., Azzurra Elia, C., Ferrari, V., Iob, L., Segato, S., 2009. Use of serum amyloid A and milk amyloid A in the diagnosis of subclinical mastitis in dairy cows. J. Dairy Res. 76, 411–417. https://doi.org/10.1017/S0022029909990057.

Green, L.E., Schukken, Y.H., Green, M.J., 2006. On distinguishing cause and consequence: do high somatic cell counts lead to lower milk yield or does high milk yield lead to lower somatic cell count? Prev. Vet. Med. 76, 74–89. https://doi.org/10.1016/j.prevetmed.2006.04.012.

Heringstad, B., Klemetsdal, G., Steine, T., 2007. Selection responses for disease resistance in two selection experiments with Norwegian Red cows. J. Dairy Sci. 90, 2419–2426. https://doi.org/10.3168/jds.2006-805.

Heringstad, B., Sehested, E., Steine, T., 2008. Correlated selection responses in somatic cell count from selection against clinical mastitis. J. Dairy Sci. 91, 4437–4439. https://doi.org/10.3168/jds.2008-1330.

Heringstad, B., Østerås, O., 2013. More than 30 years of health recording in Norway. In: ICAR 2013 Health Data Conference: Challenges and Benefits of Health Data Recording in the Context of Food Chain Quality, Management and Breeding. May 30-31, 2013, Århus, Denmark. pp. 39–45. http://www.icar.org/Documents/technical_series/tec_series_17_Aarhus.pdf.

J. S. Hogan, R. N. Gonzalez, R. J. Harmon, S. C. Nickerson, S. P. Oliver, J. W. Pankey, K. L. Smith. Laboratory Handbook on Bovine Mastitis. Revised Edition 1999 National Mastitis Council Madison, WI.

Hogeveen, H., Kamphuis, C., Steeneveld, W., Mollenhorst, H., 2010. Sensors and clinical mastitis — the quest for the perfect alert. Sensors 10, 7991. https://doi.org/10.3390/s100907991.

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.

IDF, 2013. Guidelines for the use and interpretation of bovine somatic cell counts (SCC) in the dairy industry. Bull. Int. Dairy Fed. Int. Dairy Fed Brussels, Belgium.

Interbull, 2012. International Bull Evaluation Service Official Website. Accessed Mar. 14, 2019. http://www.interbull.org.

Løvendahl, P., Bjerring, M., Larsen, T., 2010. In: Determination of carry-over in automated milking, recording and sampling systems using fluorescent tracers. In: Proc. ICAR 37th Annual Meeting, Riga, Latvia from 31 May to 4 June. pp. 147–152.

Kamphuis, C., Sherlock, R., Jago, J., Mein, G., Hogeveen, H., 2008. Automatic detection of clinical mastitis is improved by in-line monitoring of somatic cell count. J. Dairy Sci. 91, 4560–4570. https://doi.org/10.3168/jds.2008-1160.

Laevens, H., Deluyker, H., Schukken, Y.H., De Meulemeester, L., Vandermeersch, R., De Muêlenaere, E., De Kruif, A., 1997. Influence of parity and stage of lactation on the somatic cell count in bacteriologically negative dairy cows. J. Dairy Sci. 80, 3219–3226. https://doi.org/10.3168/jds.S0022-0302(97)76295-7.

Lipkens, Z., Piepers, S., Verbeke, J., De Vliegher, S., 2019. Infection dynamics across the dry period using Dairy Herd Improvement somatic cell count data and its effect on cow performance in the subsequent lactation. J. Dairy Sci. 102, 640–651. https://doi.org/10.3168/jds.2018-15130.

Løvendahl, P., Bjerring, M.A., 2006. Detection of carryover in automated milk sampling equipment. J. Dairy Sci. 89, 3645–3652. https://doi.org/10.3168/jds.S0022-0302(06)72404-3.

Nakagawa, S., Schielzeth, H., 2013. A general and simple method for obtaining R^2 from generalized linear mixed-effects models. Methods Ecol. Evol. 4, 133–142. https://doi.org/10.1111/j.2041-210x.2012.00261.x.

Nielen, M., Deluyker, H., Schukken, Y.H., Brand, A., 1992. Electrical conductivity of milk: measurement, modifiers, and meta analysis of mastitis detection performance. J. Dairy Sci. 75, 606–614. https://doi.org/10.3168/jds.S0022-0302(92)77798-4.

Norberg, E., Hogeveen, H., Korsgaard, I.R., Friggens, N.C., Sloth, K.H.M.N., Løvendahl, P., 2004. Electrical conductivity of milk: ability to predict mastitis status. J. Dairy Sci. 87, 1099–1107. https://doi.org/10.3168/jds.S0022-0302(04)73256-7.

Ødegård, J., Klemetsdal, G., Heringstad, B., 2003. Genetic improvement of mastitis resistance: validation of somatic cell score and clinical mastitis as selection criteria. J. Dairy Sci. 86, 4129–4136. https://doi.org/10.3168/jds.S0022-0302(03)74027-2.

Østerås, O., Edge, V.L., Martin, S.W., 1999. Determinants of success or failure in the elimination of major mastitis pathogens in selective dry cow therapy. J. Dairy Sci. 82, 1221–1231. https://doi.org/10.3168/jds.S0022-0302(99)75345-2.

Quist, M.A., LeBlanc, S.J., Hand, K.J., Lazenby, D., Miglior, F., Kelton, D.F., 2008. Milking-to-milking variability for milk yield, fat and protein percentage, and somatic cell count. J. Dairy Sci. 91, 3412–3423. https://doi.org/10.3168/jds.2007-0184.

Reksen, O., Gröhn, Y.T., Barlow, J.W., Schukken, Y.H., 2012. Transmission dynamics of intramammary infections with coagulase-negative staphylococci. J. Dairy Sci. 95, 4899–4910. https://doi.org/10.3168/jds.2011-4675.

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.

Core Team, R., 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. https://www.R-project.org/.

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.
Sheldrake, R.F., Hoare, R.J.T., McGregor, G.D., 1983. Lactation stage, parity, and infection affecting somatic cells, electrical conductivity, and serum albumin in milk. J. Dairy Sci. 66, 542–547. https://doi.org/10.3168/jds.S0022-0302(83)81823-2.
Sørensen, L.P., Bjerring, M., Løvendahl, P., 2016. Monitoring individual cow udder health in automated milking systems using online somatic cell counts. J. Dairy Sci. 99, 608–620. https://doi.org/10.3168/jds.2014-8823.
Torres, A.H., Rajala-Schultz, P.J., DeGraves, F.J., Hoblet, K.H., 2008. Using dairy herd improvement records and clinical mastitis history to identify subclinical mastitis infections at dry-off. J. Dairy Res. 75, 240–247. https://doi.org/10.1017/S0022029908003257.
Zadoks, R.N., Allore, H.G., Hagenaars, T.J., Barkema, H.W., Schukken, Y.H., 2002. A mathematical model of Staphylococcus aureus control in dairy herds. Epidemiol. Infect. 129, 397–416. https://doi.org/10.1017/S0950268802007483.
Zecconi, A., Sesana, G., Vairani, D., Cipolla, M., Rizzi, N., Zanini, L., 2018a. Somatic cell count as a decision tool for selective dry cow therapy in Italy. Ital. J. Anim. Sci. https://doi.org/10.1080/1828051X.2018.1532328.
Zecconi, A., Sesana, G., Vairani, D., Cipolla, M., Rizzi, N., Zanini, L., 2018b. Assessment of subclinical mastitis diagnostic accuracy by differential cell count in individual cow milk. Ital. J. Anim. Sci. https://doi.org/10.1080/1828051X.2018.1533391.

IV

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The detection of intramammary infections using online somatic cell counts

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ABSTRACT

Timely and accurate identification of cows with intramammary infections is essential for optimal udder health management. Various sensor systems have been developed to provide udder health information that can be used as a decision support tool for the farmer. Among these sensors, the DeLaval Online Cell Counter (DeLaval, Tumba, Sweden) provides somatic cell counts from every milking at cow level. Our aim was to describe and evaluate diagnostic sensor properties of these online cell counts (OCC) for detecting an intramammary infection, defined as an episode of subclinical mastitis or a new case of clinical mastitis. The predictive abilities of a single OCC value, rolling averages of OCC values, and an elevated mastitis risk (EMR) variable were compared for their accuracy in identifying cows with episodes of subclinical mastitis or new cases of clinical mastitis. Detection of subclinical mastitis episodes by OCC was performed in 2 separate groups of different mastitis pathogens, Pat 1 and Pat 2, categorized by their known ability to increase somatic cell count. The data for this study were obtained in a field trial conducted in the dairy herd of the Norwegian University of Life Sciences. Altogether, 173 cows were sampled at least once during a 17-mo study period. The total number of quarter milk cultures was 5,330. The most common Pat 1 pathogens were *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Streptococcus dysgalactiae*. The most common Pat 2 pathogens were *Corynebacterium bovis*, *Staphylococcus chromogenes*, and *Staphylococcus haemolyticus*. The OCC were successfully recorded from 82,182 of 96,542 milkings during the study period. For episodes of subclinical mastitis the rolling 7-d average OCC and the EMR approach performed better than a single OCC value for detection of Pat 1 subclinical mastitis episodes. The EMR approach outperformed the OCC approaches for detection of Pat 2 subclinical mastitis episodes. For the 2 pathogen groups, the sensitivity of detection of subclinical mastitis episodes was 69% (Pat 1) and 31% (Pat 2), respectively, at a predefined specificity of 80% (EMR). All 3 approaches were equally good at detecting new cases of clinical mastitis, with an optimum sensitivity of 80% and specificity of 90% (single OCC value).
Key words: intramammary infection, sensor, somatic cell count, online cell count

INTRODUCTION

From an economic perspective, mastitis is one of the most important diseases in dairy production (Halasa et al., 2007; Hogeveen et al., 2011). Much of the economic losses are due to reduced milk production following subclinical mastitis (Hogan et al., 2016). Therefore, detection and management of both subclinical and clinical mastitis are of importance for milk quality, animal welfare, and economic return.

The SCC can to some extent be used for the surveillance of IMI (Schukken et al., 2003), and the industry has advanced toward developing new sensors that are specifically designed for udder health surveillance. One of these is the DeLaval Online Cell Counter (DeLaval, Tumba, Sweden). With this, we can obtain repeated measurements of online cell counts (**OCC**) at cow level. These may be implemented in automated detection systems for the management of udder health in automatic milking systems (**AMS**).

Several studies of associations between SCC and IMI have used treatment of clinical mastitis as the gold standard for their evaluation (Kamphuis et al., 2008, 2013; Sørensen et al., 2016). However, these studies used different sensors to estimate SCC. Sørensen et

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al. (2016) used direct optical counting of somatic cells, whereas Kamphuis et al. (2008, 2013) used an indirect measurement of SCC based on viscosity measurements. Also, the ability of OCC to discriminate between IMI and physiological fluctuations in SCC not related to IMI has not been reported. This may be because longitudinal studies of IMI are both time consuming and costly, and because the detection of IMI is not straightforward.

An elevated SCC is usually a response to an IMI (IDF, 2013), and we are therefore measuring response to an infection, rather than the infection itself. Automated detection of the response to an IMI by using OCC must therefore take into account that the immune systems of different cows may respond differently to the same IMI pathogens (Rivas et al., 2013). Thus, the OCC from different cows with IMI due to the same pathogen may vary. However, with frequent sampling of OCC we may be able to distinguish between measurement noise and true changes resulting from biological processes. That is, arbitrary changes in OCC can be viewed as within-animal deviations and corrected for by calculating rolling averages or by using smoothing functions (Sørensen et al., 2016). To detect cases of clinical cases of mastitis, Sørensen et al. (2016) created an elevated mastitis risk (**EMR**) indicator, based on smoothed OCC. The EMR is a continuous variable (from 0–1), where values close to 0 indicate low risk of mastitis and higher values, approaching 1, indicate an increased risk of mastitis (Sørensen et al., 2016).

The OCC can also be elevated due to reasons other than IMI, including other systemic diseases, stage of lactation, stress, trauma, previous IMI, milking interval, day-to-day variation, and diurnal variation (IDF, 2013). Thus, the ability to distinguish between elevated OCC due to IMI or for other reasons is crucial for udder health management. Detection systems with a high specificity are often preferred by farmers using AMS (Claycomb et al., 2009) because a large number of false-positive alerts is a concern (Hogeveen et al., 2010). The diagnostic test properties of sensor systems should therefore be investigated and reported, so that farmers have an evidence-based foundation for choosing systems that match their requirements.

The primary aim of this study was to detect episodes of subclinical mastitis caused by mastitis pathogens. A secondary aim was to detect new cases of clinical mastitis. Specifically, we first wanted to test the predictive abilities of single values and rolling averages of OCC and an EMR indicator for detection of periods of subclinical mastitis or new cases of clinical mastitis. Second, we wanted to compare the diagnostic properties of these different approaches.

MATERIALS AND METHODS

Field Study

Data were obtained during a 17-mo longitudinal observational study in the research herd at the Norwegian University of Life Sciences. Two groups, each of approximately 50 Norwegian Red cows, housed in immediate proximity to each other, were milked on average 2.6 times per day by 2 identical AMS (DeLaval VMS) during the study period. The monthly mean number of lactating cows was 96, the mean milk production per cow per day was 27.9 kg, and the average cow composite OCC was 115,103 cells/mL. The farm had reliable identification of animals and used standardized mastitis control practices, such as monthly milk quality testing in a DHIA program, postmilking teat disinfection, and selective dry-cow therapy.

The OCC were recorded from every milking from January 5, 2016, to May 22, 2017.

Trained veterinary personnel collected quarter milk samples (**QMS**) from all lactating cows on a monthly basis according to recommended guidelines (Hogan et al., 1999). Samples were frozen after collection and during transport to the laboratory for microbiological analyses. Samples were thawed in the laboratory and bacteriological culture was performed according to standard procedures (Hogan et al., 1999). Briefly, 0.1 mL of milk from each quarter was spread on cattle blood agar plates with esculin and incubated at 37°C. Plates were read at 24 and 48 h. Species identification of cultured bacteria was performed with MALDI-TOF MS microflex LT (Bruker Corporation, Billerica, MA; Cheuzeville, 2015). Samples with culture results indicating more than 2 morphologically different colony types were treated as contaminated and excluded from further analyses.

Mastitis Status

Based on the culture results, the cows were given an udder health status for subclinical mastitis throughout the study period. In this way, every milking was either associated with an episode of subclinical mastitis or not. A cow was considered to have an episode of subclinical mastitis when meeting at least 1 of the following criteria: (1) $\geq$1,000 cfu/mL of a single mastitis pathogen was cultured from a single sample in at least 1 quarter, (2) $\geq$500 cfu/mL of a mastitis pathogen was cultured from 2 out of 3 consecutive milk samples from the same quarter, or (3) $\geq$100 cfu/mL of a mastitis pathogen was cultured from 3 consecutive milk samples from the same quarter. These definitions were adapted

from those of Zadoks et al. (2002). Cows with positive milk cultures that did not meet any of the above criteria were classified as being transiently colonized (Reksen et al., 2012).

Because the OCC is recorded at the cow level, the bacteriological diagnoses at the quarter level were aggregated into cow-level diagnoses. The same cow could experience an episode of subclinical mastitis in more than 1 quarter simultaneously, and in some cases, these 2 episodes could be caused by different mastitis pathogens. Hence, pathogens were divided into 2 groups (**Pat 1** and **Pat 2**), according to characteristics of the bacteria. The group of pathogens from which a high cell count would be expected during a subclinical mastitis episode was named Pat 1. The Pat 1 group consisted of the following species: *Staphylococcus aureus*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Enterococcus faecalis*, *Enterococcus faecium*, *Lactococcus lactis*, *Staphylococcus epidermidis*, and *Staphylococcus simulans* (Djabri et al., 2002; Reksen et al., 2008; Simojoki et al., 2009, 2011; Fry et al., 2014). Known mastitis pathogens not included in Pat 1 were assigned to the Pat 2 category. These Pat 2 pathogens included *Corynebacterium bovis*, *Staphylococcus chromogenes*, *Staphylococcus haemolyticus*, *Aerococcus viridans*, *Staphylococcus hominis*, *Staphylococcus xylosus*, and other bacteria cultured.

Cows were given the status of subclinical mastitis when 1 or more quarters were positive for either a subclinical mastitis with a Pat 1 mastitis pathogen or a Pat 2 mastitis pathogen. For milkings where a cow was found positive for subclinical mastitis for mastitis pathogens from both categories (Pat 1 and Pat 2) simultaneously, we regarded the OCC response to be primarily due to the mastitis pathogen in the Pat 1 category. That is, we implemented a hierarchical order in the classification such that a cow could only be assigned to the Pat 2 subclinical mastitis category when there was no simultaneous diagnosis of a Pat 1 subclinical mastitis in the same cow during the same infectious period.

As sampling was performed monthly, we did not know exactly when each episode of subclinical mastitis started, and duration of infection was therefore calculated using the mid-point estimation method previously described by Zadoks et al. (2002). Thus, the start of the subclinical mastitis episode was defined as the middle of the time interval between a negative culture and the first positive culture event, and the end of the subclinical mastitis episode was defined as the middle of the time interval between the last positive culture event and the first negative culture for a cow defined as cured (Zadoks et al., 2002).

A veterinary treatment for clinical mastitis was defined as a new case of clinical mastitis.

Farm personnel identified cows with suspected clinical mastitis based on generalized clinical symptoms, including anorexia, lethargy, or elevated rectal temperature. These cows underwent clinical examination by the herd veterinarian. Cows that were treated for clinical mastitis were transferred to a treatment pen without AMS, and we do not have OCC records throughout the period of treatment for the clinical mastitis cases. Therefore, the last milking in the AMS before veterinary treatment was associated with each new case of clinical mastitis.

OCC

Statistical analyses were conducted using Stata (Stata SE/14, Stata Corp., College Station, TX).

The raw OCC values were smoothed using 1 of 3 different methods: (1) rolling 7-d average of available OCC, (2) rolling 48-h average of available OCC, and (3) calculation of the EMR for all milkings. The rolling 7-d and 48-h average of OCC were not transformed before calculating rolling averages and correspond to the values given in the AMS software DelPro (DeLaval). The EMR was computed as described by Sørensen et al. (2016) for all milkings. Briefly, we checked the validity of all recorded OCC measurements before ln-transformation. Only milkings from 5 to 305 DIM with a milking interval of 4 to 24 h and a milking yield of $\geq$3.5 kg were included. Online cell count values of 0 were omitted from further analyses. We used the Wood lactation curve to calculate lactation-specific OCC curves for first, second, and third and above lactations (Wood, 1967). Periods with missing OCC data were corrected for by slowly approaching the lactation-specific OCC curves by 5% for each milking with missing observations (Sørensen et al., 2016). The ln-transformed OCC data were adjusted for aberrations and drift at the sensor level by single exponential smoothing (Hyndman et al., 2008) before double exponential smoothing of the adjusted OCC values was employed according to the description by Sørensen et al. (2016).

The lactation-specific OCC curves were used for rapid initialization of the double exponential smoothing (Sørensen et al., 2016). The output from the double exponential smoothing (level and trend) were used to calculate EMR values on a continuous scale from 0 to 1 (Sørensen et al., 2016).

Diagnostic Test Properties

The raw OCC values, rolling 7-d OCC values, and the EMR were evaluated against the subclinical mastitis status of the cow at every milking. For subclinical mastitis, the diagnostic properties were explored

separately for the 2 pathogen groups, Pat 1 and Pat 2. Furthermore, the raw OCC values, rolling 48-h OCC values and the EMR were evaluated against the clinical mastitis status of the cow at every milking. For each of the approaches, alert thresholds were calculated for 4 different levels of specificity for detection of Pat 1 subclinical mastitis episodes, Pat 2 subclinical mastitis episodes, and new cases of clinical mastitis using the "roctab" and "diagt" functions in Stata (Stata SE/14, Stata Corp.). The "roctab" function was used to identify the cut-point for each predefined level of specificity. It uses all registered outcomes of the diagnostic test variable as a classification cut-point and computes the corresponding sensitivity and specificity. The sensitivity of the identified diagnostic test variable cut-point for specificities of 80, 85, 90, and 99% were further evaluated with "diagt." This displays summary statistics for a diagnostic test as compared with the true status, in our case an episode of subclinical mastitis or a new case of clinical mastitis. Alert thresholds for each level of specificity for Pat 2 subclinical mastitis were calculated after removing observations with a Pat 1 subclinical mastitis alert at the same level of specificity. The 4 different detection approaches were compared using the "roccomp" function in Stata, which compares the area under multiple receiver operating characteristics curves (Stata SE/14, Stata Corp.). The receiver operating characteristics area under the curve for detection of Pat 2 subclinical mastitis were calculated after removing observations with Pat 1 subclinical mastitis alerts at the level of 80% specificity for Pat 1 subclinical mastitis.

RESULTS

Field Study

Altogether, we collected 5,330 QMS from a total of 257 lactations in 173 cows. Each cow was on average sampled 8 times, ranging from 1 to 16. Cows entered the study with an average of 38 DIM, ranging from 4 to 269. The average DIM of cows at sampling was 119, ranging from 5 to 303. Bacteria were cultured from 1,222 samples, with 1 and 2 pathogens cultured in 1,152 and 67 samples, respectively. We excluded 3 samples from the analysis due to contamination. Pathogens were found in 222 lactations in 155 cows. The distribution of bacterial culture results are shown in Table 1.

Mastitis Status

According to our definition of subclinical mastitis status, there were 106 cow-level episodes of Pat 1 subclinical mastitis during the course of the study. These episodes were based on 324 positive cow-level culture results. A total of 23,409 AMS milkings from 97 lactations in 80 cows were associated with these episodes of Pat 1 subclinical mastitis. An additional 65 Pat 1 positive cow-level samples from 55 lactations in 53 cows did not meet any of our defined criteria for subclinical

Table 1. Distribution of 1,286 microbiological diagnoses in 1,219 samples with positive bacteriological culture results out of 5,327 quarter milk samples

		Culture result (cfu/mL)		
Bacterial species detected	N	≥100 and <500	≥500 and <1,000	≥1,000
Staphylococcus epidermidis	234	54	36	144
Corynebacterium bovis	225	31	41	153
Staphylococcus chromogenes	167	14	12	141
Staphylococcus aureus	119	45	11	63
Staphylococcus haemolyticus	116	43	17	56
Aerococcus viridans	91	57	14	20
Enterococcus faecalis, *Enterococcus faecium*, and *Lactococcus lactis*	81	16	3	62
Streptococcus dysgalactiae	66	9	3	54
Staphylococcus simulans	32	6	5	21
Staphylococcus hominis	31	19	6	6
Streptococcus uberis	25	1	2	22
Staphylococcus xylosus	8	1	1	6
Streptococcus agalactiae	—	—	—	—
Other[1]	91	48	17	26
Not detected	4,108			

[1]Other bacteria cultured: *Acinetobacter lwoffii*, *Bacillus pumilus*, *Corynebacterium amycolatum*, *Corynebacterium* spp., *Corynebacterium stationis*, *Macrococcus caseolyticus*, *Macrococcus luteus*, *Staphylococcus auricularis*, *Staphylococcus capitis*, *Staphylococcus equorum*, *Staphylococcus hyicus*, *Staphylococcus* spp., *Streptococcus canis*, *Streptococcus lutetiensis*, *Streptococcus* spp., and *Trueperella pyogenes*.

Table 2. Pathogen-specific 7-d average cow-level online cell count (OCC) at the time of microbiological sampling in cows with subclinical mastitis

Pathogens in episodes of subclinical mastitis	N	OCC (1,000 cells/mL)	95% CI
Staphylococcus epidermidis	141	191	150–232
Corynebacterium bovis	104	114	77–150
Staphylococcus chromogenes	136	154	111–197
Staphylococcus aureus	77	355	281–430
Staphylococcus haemolyticus	67	112	82–142
Aerococcus viridans	20	119	14–224
Enterococcus faecalis, *Enterococcus faecium*, and *Lactococcus lactis*	56	292	183–400
Streptococcus dysgalactiae	56	308	204–412
Staphylococcus simulans	27	310	217–404
Staphylococcus hominis	6	119	0–309
Streptococcus uberis	22	298	162–434
Staphylococcus xylosus	7	180	122–238
Other[1]	25	131	76–185

[1]Other bacteria cultured: *Acinetobacter lwoffii*, *Bacillus pumilus*, *Corynebacterium amycolatum*, *Corynebacterium* spp., *Corynebacterium stationis*, *Macrococcus caseolyticus*, *Macrococcus luteus*, *Staphylococcus auricularis*, *Staphylococcus capitis*, *Staphylococcus equorum*, *Staphylococcus hyicus*, *Staphylococcus* spp., *Streptococcus canis*, *Streptococcus lutetiensis*, *Streptococcus* spp., and *Trueperella pyogenes*.

mastitis and were classified as being transiently colonized.

Similarly, 117 episodes of Pat 2 subclinical mastitis occurred during the study period. These were associated with 288 positive cow-level culture results. A total of 22,182 milkings from 107 lactations in 84 cows were associated with these episodes of Pat 2 subclinical mastitis. An additional 106 Pat 2 positive cow-level culture results from 55 lactations in 51 cows were isolated in connection with an episode of Pat 1 subclinical mastitis. Following our hierarchical definition of subclinical mastitis, these culture results were not included in the Pat 2 subclinical mastitis episodes. Furthermore, 101 Pat 2 positive cow-level culture results from 78 lactations in 70 cows did not meet any of our defined criteria for subclinical mastitis and were classified as being transiently colonized.

During the study period, we recorded 16 veterinary treatments for clinical mastitis in 15 cows.

OCC

The OCC was successfully recorded from 82,182 of 96,542 milkings; the 14,360 missing values were because of equipment failure or failure to service and refill the OCC unit with reagent.

The Pat 1 subclinical mastitis pathogens were generally associated with a higher OCC than the Pat 2 subclinical mastitis pathogens. An overview of the pathogen-specific subclinical mastitis diagnoses and corresponding 7-d average OCC at the time of sampling are given in Table 2. The 7-d average OCC of cows with different subclinical mastitis status, as well as the 48-h average OCC of cows with a new case of clinical mastitis, are shown in Table 3. The 7-d average OCC of cows with Pat 1 subclinical mastitis and the 48-h average OCC of cows with a new case of clinical mastitis are clearly higher than the corresponding values from other groups, but this is not the case for Pat 2 subclinical

Table 3. Pathogen group-specific 7-d average of cow-level online cell count (OCC) values at the time of microbiological sampling in cows with subclinical mastitis (SCM), and average of cow-level OCC from milkings during the 48 h before a new case of clinical mastitis

Group	N	OCC (1,000 cells/mL)	95% CI
Pat[1] 1 SCM	311	260	224–298
Pat 2 SCM	269	83	70–96
Any SCM	580	178	157–200
Transient colonization	134	124	56–192
No SCM and no bacteria cultured from any teat in cow	519	53	44–61
New case of clinical mastitis	16	1,280	721–1,838

[1]Pat = pathogen group.

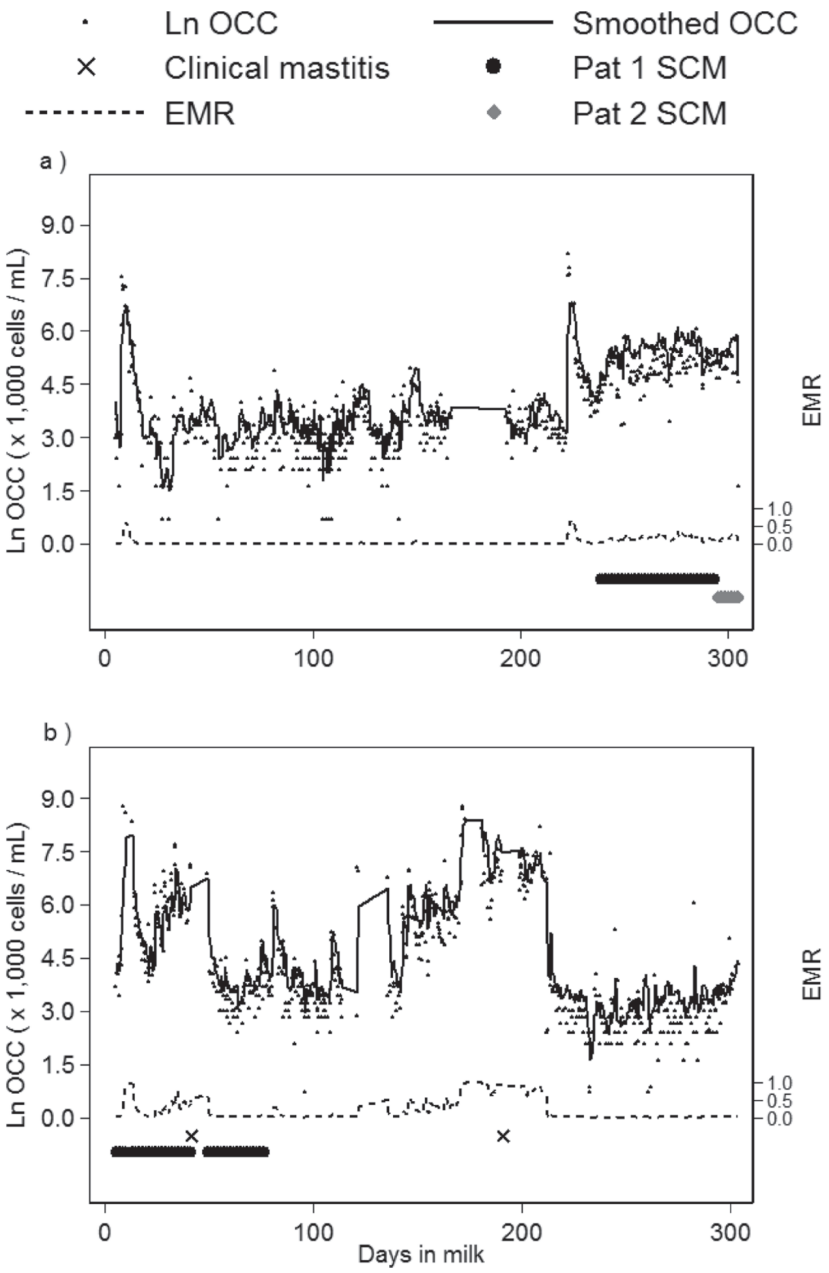

Figure 1. Examples of online cell count (OCC) values and mastitis status. The cow in (a) is a typical example of a cow with low OCC values until the onset of a case of Pat 1 subclinical mastitis (SCM). The Pat 1 SCM is followed by a case of Pat 2 SCM. This cow was not treated for clinical mastitis. The cow in (b) is an example of a cow with Pat 1 SCM and 2 treatments for clinical mastitis. EMR = elevated mastitis risk. Pat 1 and Pat 2 = different groups of mastitis pathogens.

Table 4. Sensitivities at set specificities for detection of cases of Pat 1 subclinical mastitis (SCM), cases of Pat 2 SCM, and new cases of clinical mastitis (CM) using online cell count (OCC) or elevated mastitis risk (EMR) values[1]

Item	Sp = 80 Se (95% CI) [cut-off]	Sp = 85 Se (95% CI) [cut-off]	Sp = 90 Se (95% CI) [cut-off]	Sp = 99 Se (95% CI) [cut-off]
Pat 1 SCM				
Single OCC	63 (63–64) [≥74]	54 (54–55) [≥97]	43 (42–44) [≥132]	7 (6–7) [≥814]
7-d average OCC	69 (69–70) [≥77]	62 (61–62) [≥101]	51 (50–51) [≥137]	7 (6–7) [≥726]
EMR	69 (68–69) [≥0.03]	59 (59–60) [≥0.05]	48 (48–49) [≥0.08]	8 (7–8) [≥0.62]
Pat 2 SCM				
Single OCC	29 (28–30) [≥40]	20 (19–20) [≥57]	12 (12–13) [≥88]	0.5 (0.4–0.7) [≥642]
7-d average OCC	29 (29–30) [≥42]	21 (20–21) [≥58]	14 (14–15) [≥90]	0.3 (0.3–0.4) [≥626]
EMR	31 (30–31) [≥0.01]	19 (18–20) [≥0.02]	12 (11–12) [≥0.04]	0.5 (0.4–0.6) [≥0.54]
New CM				
Single OCC	87 (60–98) [≥121]	87 (60–98) [≥159]	80 (52–96) [≥232]	60 (32–84) [≥1,397]
48-h average OCC	81 (54–96) [≥126]	69 (41–89) [≥167]	69 (41–89) [≥243]	44 (20–70) [≥1,336]
EMR	81 (54–96) [≥0.08]	81 (54–96) [≥0.12]	75 (48–93) [≥0.20]	38 (15–65) [≥0.83]

[1]Cut-off values for the levels of sensitivity (Se) and specificity (Sp) are in 1,000 cells/mL for OCC and between 0 and 1 for the EMR. Calculated sensitivities for Pat 2 subclinical mastitis were conducted for each level of specificity after the removal of milkings with a sensor alert for subclinical Pat 1 subclinical mastitis at that level of specificity. Pat 1 and Pat 2 = different groups of mastitis pathogens.

mastitis and transient colonizations. Examples of OCC curves and mastitis status are shown in Figure 1.

Diagnostic Test Properties

The diagnostic test properties for the predefined specificities of a single OCC value, the rolling 7-d or 48-h average of OCC and the EMR, are shown distributed by Pat 1 subclinical mastitis episodes, Pat 2 subclinical mastitis episodes, and new cases of clinical mastitis in Table 4. The sensitivity of the different approaches decreases when higher specificity is demanded.

The receiver operating characteristics area under the curve of Pat 1 subclinical mastitis episodes, Pat 2 subclinical mastitis episodes, and new cases of clinical mastitis are shown in Table 5. In our study, we found that the EMR and the rolling 7-d average of OCC performed better than the single OCC for detection of Pat 1 subclinical mastitis episodes. The ROC curves of the different approaches for detecting Pat 1 subclinical mastitis episodes are shown in Figure 2. For the detection of Pat 2 subclinical mastitis episodes, the EMR approach performed better than both OCC approaches. The 3 different detection approaches performed equally well for detection of new cases of clinical mastitis. The ROC curves of the different approaches for detecting new cases of clinical mastitis are shown in Figure 2.

DISCUSSION

This is a study exploring the practical application of OCC to detect episodes of subclinical mastitis and new cases of clinical mastitis. The study is limited to one farm, which hampers the generalizability of the results. However, the aim of the study was to test the practical applicability of the approach rather than to generalize the results on a larger proportion of the population.

In this study we demonstrate that OCC may be used to identify cows with an episode of subclinical mastitis and new cases of clinical mastitis. The diagnostic test properties of the system can be adapted according to the required practical application, with settings selected on the basis of the tradeoff between sensitivity and specificity. A farmer with a high tolerance of false positives may choose to increase the sensitivity at the cost of lower specificity.

No systems, including our approach in this study, currently operate at the desired level for sensor systems

Table 5. Receiver operating characteristics area under the curve (ROC area) of the 3 detection approaches for detection of cases of Pat 1 subclinical mastitis (SCM), Pat 2 SCM, and new cases of clinical mastitis (CM)[1]

Detection approach	ROC area	95% CI
Pat 1 SCM		
Single OCC[2]	0.783	0.779–0.787
Rolling 7-d average OCC	0.809	0.806–0.813
EMR[3]	0.804	0.800–0.808
Pat 2 SCM		
Single OCC	0.587	0.581–0.593
Rolling 7-d average OCC	0.597	0.591–0.603
EMR	0.641	0.635–0.647
New CM		
Single OCC	0.931	0.859–1.000
Rolling average 48 h OCC	0.925	0.869–0.980
EMR	0.904	0.802–1.000

[1]The ROC area under the curve for detection of Pat 2 subclinical mastitis is calculated after excluding milkings with Pat 1 subclinical mastitis alerts at the level of 80% specificity for Pat 1 subclinical mastitis. Pat 1 and Pat 2 = different groups of mastitis pathogens.

[2]OCC = online cell count.

[3]EMR = elevated mastitis risk.

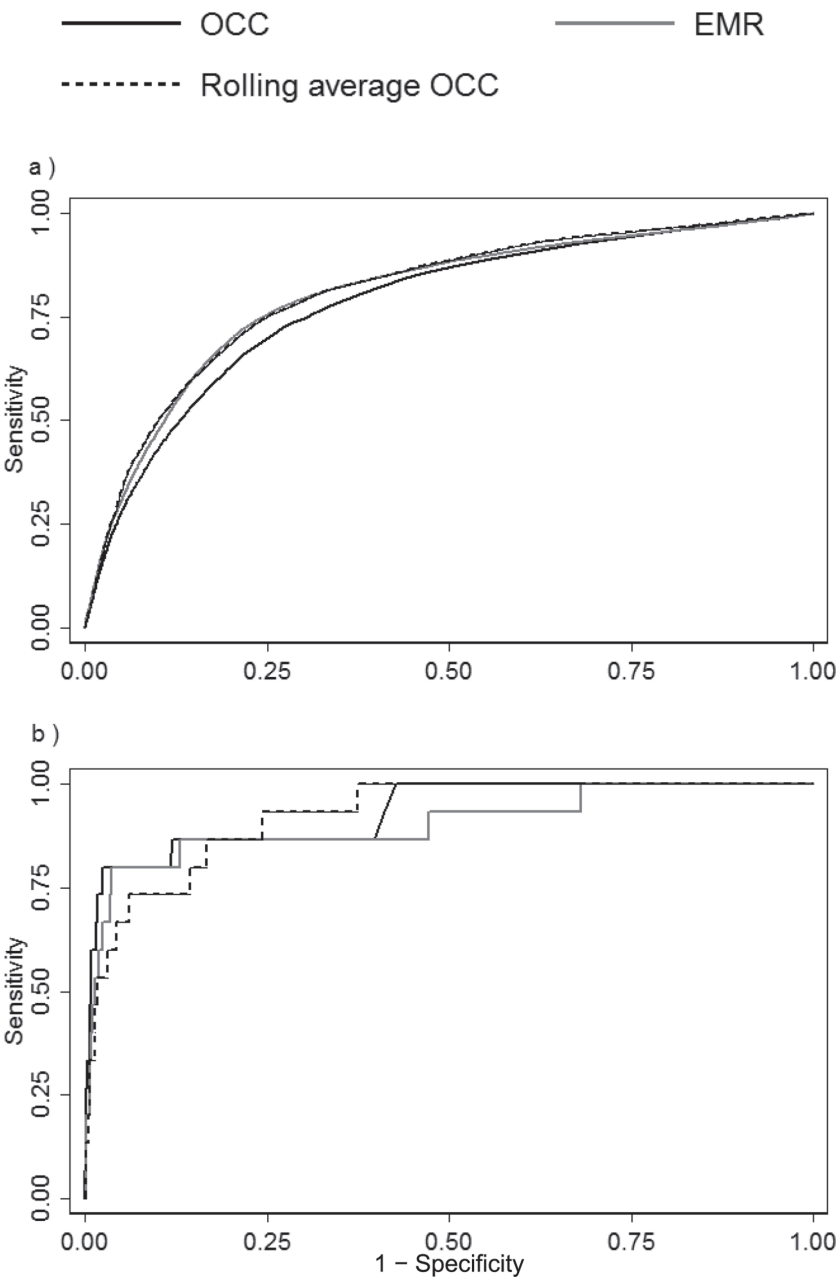

Figure 2. Receiver operating characteristic curves for detection of (a) Pat 1 subclinical mastitis and (b) new cases of clinical mastitis. The time windows for rolling averages are 7 d and 48 h for (a) and (b), respectively. EMR = elevated mastitis risk; OCC = online cell count. Pat 1 and Pat 2 = different groups of mastitis pathogens.

in dairy production (ISO, 2007). We suggest that using sensor systems could still be advantageous for the herd manager and that the usefulness of the system depends on the desired application. Thus, we suggest that sensitivity should be high for detection of cows for dry-cow treatment, but specificity should be high for detection of IMI during lactation. This suggestion is related to the number of detection events. For selective dry-cow therapy, for which there is a single detection event, high sensitivity at the cost of lower specificity will result in a moderate number of false-positive IMI alerts. However, a test with similar characteristics will lead to numerous false alerts during lactation, when every sensor measurement would represent a possible detection event. Both single OCC values and smoothed values, like the EMR of Sørensen et al. (2016), can be used to detect episodes of subclinical mastitis and new cases of clinical mastitis. Based on this, we propose that systems should be adjusted according to the lactation stage of the individual cow and the tolerance of the farmer for false positives, such that farm-specific everyday practical udder health management is accommodated. This is in line with current work undertaken by the International Dairy Federation (Hogeveen et al., 2018).

Sørensen et al. (2016) used treatment of mastitis with antibiotics as their gold standard for mastitis cases, but they also suggested that the EMR could identify cows with chronic infections. From our data, the single EMR and the rolling 7-d average OCC performed better than a single OCC value for the detection of Pat 1 subclinical mastitis episodes. Furthermore, the EMR approach performed better than both the OCC approaches for the detection of Pat 2 subclinical mastitis episodes. We suggest that this reflects the variation in immune response between individuals (Rivas et al., 2013), and that the cow-specific smoothing inherent in the EMR provides more information that improves the diagnostic properties of the system.

Our results shows that the confidence interval (**CI**) of the 7-d average OCC for episodes of Pat 1 subclinical mastitis or new cases of clinical mastitis differed from those of the other groups. The corresponding CI for Pat 2 subclinical mastitis episodes and transient colonization overlapped, and the CI of transient colonization overlapped with those of cows with both no subclinical mastitis and no transient colonization. This overlap in CI makes it difficult to separate the latter groups from each other. However, as the aim is to manage udder health, as measured by bulk tank SCC, the most important goal is to identify cows with a true IMI accompanied by a high SCC.

We consider the results are applicable to dairy cows with frequent OCC measurements in AMS. However, Sørensen et al. (2016) reported large differences in test algorithm performance between herds. Assuming that this is the case for our study also, a limitation of the external validity of our results is that we have only studied a single herd that may have a quite specific OCC pattern. Furthermore, because the results are based on OCC only, they cannot be extrapolated to other udder health sensors or SCC from DHI samples without further evaluation.

Our aim was to use OCC measurements to detect cows with subclinical mastitis associated with infection over time or high colony-forming unit counts of mastitis pathogens. We chose bacteriological culture of QMS combined with our criteria of infection over time or high colony-forming unit counts to define episodes of subclinical mastitis. This is an imperfect gold standard and the results may be biased by misclassification of subclinical mastitis status (Dohoo et al., 2011). Because of our conservative definition of subclinical mastitis status, there were likely few false positive cases. Therefore, the misclassification mainly includes cows with subclinical mastitis that were falsely defined as healthy. This results in a negative bias in specificity. Furthermore, as cows were sampled for bacteriological culture once monthly, some cows may have had an episode of subclinical mastitis between our visits. When cured, these would not be detected by bacteriological culture, but they might have been identified by the sensor system. Also, the sensor system may have detected the true start and end of subclinical mastitis episodes, whereas our defined start and end were set by the midpoint estimation method described. Thus, we may have treated the sensor alerts as false, although they could actually have been correct. This is a challenge for all detection approaches that are based on SCC (IDF, 2013).

We grouped the bacteriological diagnoses in our study in the Pat 1 and Pat 2 groups, and not the traditional major and minor pathogen groups. This was because the non-aureus staphylococci is a heterogeneous group of bacterial species (Vanderhaeghen et al., 2015). In our study, we included *Staphylococcus epidermidis* and *Staphylococcus simulans* of the non-aureus staphylococci in the group expected to cause elevated OCC (Pat 1). This was based on reports of these pathogens' ability to cause IMI over time and elevated SCC (Simojoki et al., 2011; Fry et al., 2014). Furthermore, we chose a hierarchical approach to grouping bacteriological diagnoses in Pat 1 subclinical mastitis episodes and Pat 2 subclinical mastitis episodes, such that a cow could

not be positive for both Pat 1 subclinical mastitis and Pat 2 subclinical mastitis simultaneously. The reason for choosing this approach was that Pat 1 subclinical mastitis episodes were likely to have a greater effect on the OCC than Pat 2 subclinical mastitis episodes. The benefit of this approach is that we could evaluate the ability of the system to identify episodes of Pat 2 subclinical mastitis after removing cows with Pat 1 subclinical mastitis alerts. Thus, the system divided the herd into 4 mastitis status groups: cows with Pat 1 subclinical mastitis episodes, cows with Pat 2 subclinical mastitis episodes, cows with no subclinical mastitis and cows with a new case of clinical mastitis.

Rapid detection of clinical mastitis is important for both animal welfare, milk quality, and economic return. At the same time, more data may improve the diagnostic test properties of sensor equipment (Hogeveen et al., 2010). To balance this issue, the rolling average OCC for detection of clinical mastitis in our study was set to use OCC data from 48 h. This did, however, not improve the detection of new cases of clinical mastitis in our study. Although, in our study, all 3 approaches performed equally for the detection of new cases of clinical mastitis, we have relatively few cases. There could be differences in the operating characteristics of the 3 approaches for the detection of new cases of clinical mastitis that we are unable to estimate with our material. Also, because we do not have OCC readings throughout the clinical mastitis treatment period, we cannot evaluate which approach is best for continued alerts for clinical mastitis. However, the sensitivities and specificities are both likely to improve when the time window for matching the gold standard of clinical mastitis is increased (Hogeveen et al., 2010).

Furthermore, to improve the detection of subclinical mastitis in our study, the rolling average OCC for detection of subclinical mastitis was set to use data from 7 d. This significantly improved the diagnostic test properties for detection of Pat 1 subclinical mastitis episodes. We propose that this longer detection window is acceptable because subclinical mastitis does not necessarily require immediate action for animal welfare reasons.

Frequent sensor alerts can be a concern for herd managers. Therefore, information from the sensor system has to be actionable, and the level of alert should be adapted to the urgency of the situation. That is, whenever there is an alert, the system should be able to evaluate whether there is a need for immediate notification of the herd manager. Furthermore, optimal actions should be suggested, with predictions of outcome for each suggestion. For the convenience of the user and to limit overtreatment of cows, the number of false positives should be minimized.

CONCLUSIONS

We investigated detection of subclinical mastitis episodes and new cases of clinical mastitis based on OCC from every milking. For diagnosis of Pat 1 subclinical mastitis episodes, the EMR and a rolling 7-d average of OCC outperformed a single OCC value. For diagnosis of Pat 2 subclinical mastitis episodes, the EMR outperformed the OCC approaches. For detection of new cases of clinical mastitis, all approaches performed equally well. By combining different alerts, the systems can be adapted to the needs of individual farmers regarding udder health management in their herds.

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REFERENCES

Cheuzeville, L. 2015. Rapid detection of microorganisms in the dairy value chain by MALDI-TOF MS. MS Thesis. Faculty of Science and Technology, University of Stavanger, Stavanger, Norway.

Claycomb, R. W., P. T. Johnstone, G. A. Mein, and R. A. Sherlock. 2009. An automated in-line clinical mastitis detection system using measurement of conductivity from foremilk of individual udder quarters. N. Z. Vet. J. 57:208–214.

Djabri, B., N. Bareille, F. Beaudeau, and H. Seegers. 2002. Quarter milk somatic cell count in infected dairy cows: A meta-analysis. Vet. Res. 33:335–357.

Dohoo, I., S. Andersen, R. Dingwell, K. Hand, D. Kelton, K. Leslie, Y. Schukken, and S. Godden. 2011. Diagnosing intramammary infections: Comparison of multiple versus single quarter milk samples for the identification of intramammary infections in lactating dairy cows. J. Dairy Sci. 94:5515–5522.

Fry, P. R., J. R. Middleton, S. Dufour, J. Perry, D. Scholl, and I. Dohoo. 2014. Association of coagulase-negative staphylococcal species, mammary quarter milk somatic cell count, and persistence of intramammary infection in dairy cattle. J. Dairy Sci. 97:4876–4885.

Halasa, T., K. Huijps, O. Osteras, and H. Hogeveen. 2007. Economic effects of bovine mastitis and mastitis management: A review. Vet. Q. 29:18–31.

Hogan, J. S., E. A. Berry, J. E. Hillerton, H. Hogeveen, S. C. Nickerson, S. P. Oliver, G. M. Pighetti, P. Rapnicki, Y. H. Schukken, and K. L. Smith. 2016. Current concepts of bovine mastitis. 5th ed. Current concepts of bovine mastitis. National Mastitis Council, Madison, WI.

Hogan, J. S., R. N. González, S. C. Harmon, S. C. Nickerson, J. W. Pankey, and K. L. Smith. 1999. Laboratory handbook on bovine mastitis. 1st ed. National Mastitis Council, Madison, WI.

Hogeveen, H., G. Dalen, and I. C. Klaas. 2018. Rethinking definitions for mastitis detection: Leaving the paradigm of only clinical mastitis. IDF Animal Health Report 12:23–24.

Hogeveen, H., K. Huijps, and T. J. Lam. 2011. Economic aspects of mastitis: New developments. N. Z. Vet. J. 59:16–23.

Hogeveen, H., C. Kamphuis, W. Steeneveld, and H. Mollenhorst. 2010. Sensors and clinical mastitis–The quest for the perfect alert. Sensors (Basel) 10:7991–8009.

Hyndman, R., A. Koehler, K. Ord, and R. Snyder. 2008. Forecasting with Exponential Smoothing: The State Space Approach. The State Space Approach. Springer Berlin Heidelberg, Berlin, Germany.

IDF. 2013. Guidelines for the use and interpretation of bovine somatic cell counts (SCC) in the dairy industry. Bull. Int. Dairy Fed. Int. Dairy Fed., Brussels, Belgium.

ISO. 2007. ISO 20966:2007. Automatic milking systems - Requirements and testing. Annex C: Example of methods of evaluating detection systems for milk demand as abnormal due to blood or changes in homogeneity. International Organization for Standardization, Geneva, Switzerland.

Kamphuis, C., B. Dela Rue, G. Mein, and J. Jago. 2013. Development of protocols to evaluate in-line mastitis-detection systems. J. Dairy Sci. 96:4047–4058.

Kamphuis, C., R. Sherlock, J. Jago, G. Mein, and H. Hogeveen. 2008. Automatic detection of clinical mastitis is improved by in-line monitoring of somatic cell count. J. Dairy Sci. 91:4560–4570.

Reksen, O., Y. T. Gröhn, J. W. Barlow, and Y. H. Schukken. 2012. Transmission dynamics of intramammary infections with coagulase-negative staphylococci. J. Dairy Sci. 95:4899–4910.

Reksen, O., L. Solverod, and O. Osteras. 2008. Relationships between milk culture results and composite milk somatic cell counts in Norwegian dairy cattle. J. Dairy Sci. 91:3102–3113.

Rivas, A. L., M. D. Jankowski, R. Piccinini, G. Leitner, D. Schwarz, K. L. Anderson, J. M. Fair, A. L. Hoogesteijn, W. Wolter, M. Chaffer, S. Blum, T. Were, S. N. Konah, P. Kempaiah, J. M. Ong'echa, U. S. Diesterbeck, R. Pilla, C. P. Czerny, J. B. Hittner, J. M. Hyman, and D. J. Perkins. 2013. Feedback-based, system-level properties of vertebrate-microbial interactions. PLoS One 8:e53984.

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

Simojoki, H., T. Orro, S. Taponen, and S. Pyorala. 2009. Host response in bovine mastitis experimentally induced with *Staphylococcus chromogenes*. Vet. Microbiol. 134:95–99.

Simojoki, H., T. Salomaki, S. Taponen, A. Iivanainen, and S. Pyorala. 2011. Innate immune response in experimentally induced bovine intramammary infection with *Staphylococcus simulans* and *S. epidermidis*. Vet. Res. (Faisalabad) 42:49.

Sørensen, L. P., M. Bjerring, and P. Lovendahl. 2016. Monitoring individual cow udder health in automated milking systems using online somatic cell counts. J. Dairy Sci. 99:608–620.

Vanderhaeghen, W., S. Piepers, F. Leroy, E. Van Coillie, F. Haesebrouck, and S. De Vliegher. 2015. Identification, typing, ecology and epidemiology of coagulase negative staphylococci associated with ruminants. Vet. J. 203:44–51.

Wood, P. D. P. 1967. Algebraic model of lactation curve in cattle. Nature 216:164–165.

Zadoks, R. N., H. G. Allore, T. J. Hagenaars, H. W. Barkema, and Y. H. Schukken. 2002. A mathematical model of *Staphylococcus aureus* control in dairy herds. Epidemiol. Infect. 129:397–416.

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