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

Philosophiae Doctor (PhD)
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Using online cell counts for detection and prediction of subclinical intramammary infections in dairy cows

Bruk av celletalsmålar til oppdaging av og prediksjon av subkliniske intramammære infeksjonar hjå mjølkekyr

Gunnar Dalen

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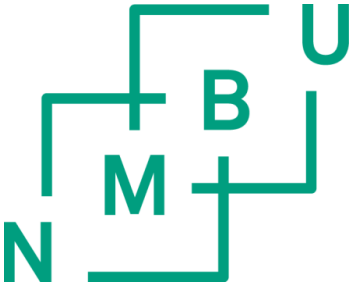

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LIST OF ABBREVIATIONS

AMR	Antimicrobial resistance
AMS	Automatic milking system
CFU	Colony-forming units
DHI	Dairy herd improvement
EMR	Elevated mastitis risk
IMI	Intramammary infection
MALDI-TOF MS	Matrix-assisted laser desorption ionization-time of flight mass spectrometry
NOK	Norwegian kroner (crowns)
OCC	Online cell count
ODE	Ordinary differential equations
Pat 1	Mastitis pathogens expected to result in a marked elevation of OCC values
Pat 2	Mastitis pathogens not included in the Pat-1 group
PLF	Precision livestock farming
QMS	Quarter milk samples
R_0	Reproductive number
SCC	Somatic cell count
SIS	Susceptible-Infectious-Susceptible
SOP	Standard operating procedure

LIST OF PAPERS

I. Dalen G., Rachah A., Nørstebø H., Schukken Y.H. Reksen O.

The detection of intramammary infections using online somatic cell counts

J. Dairy Sci. 2019. 102:1–11

II. Nørstebø H., Dalen G., Rachah A., Heringstad B., Whist A.C., Nødtvedt A., Reksen O.

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

Prev. Vet. Med. 2019. 172:104786.

III. Dalen G., Rachah A., Nørstebø H., Schukken Y.H., Gröhn Y.T., Barlow J.W., Reksen O.

Transmission dynamics of intramammary infections caused by Corynebacterium species.

J. Dairy Sci. 2018. 101(1):472-479

IV. Dalen G., Rachah A., Nørstebø H., Schukken Y.H. Reksen O.

Dynamics of somatic cell count patterns as a proxy for transmission of mastitis pathogens

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SUMMARY

Mastitis is an inflammation of the mammary gland that can result in an elevated somatic cell count (SCC). It is mainly caused by intramammary infections (IMI). Cows with mastitis can have clinical signs (clinical mastitis) or no clinical signs (subclinical mastitis). From an economic perspective, mastitis is one of the most important diseases in dairy production, and most of the economic losses are due to reduced milk production following subclinical mastitis. Because subclinical IMI are the commonest cause of subclinical mastitis, detection and management of subclinical IMI are of considerable importance for dairy production.

The detection of subclinical IMI using laboratory analysis of milk samples is, however, both time consuming and costly. Therefore, subclinical IMI are normally detected using SCC as part of a dairy-herd improvement program (DHI). The challenge with this approach is, first, the moderate association between SCC and subclinical IMI, and, second, that the time lag between readings of SCC based on DHI samples is often too long for the prediction of future episodes of subclinical IMI. More recently, various on-farm sensor systems have been developed to detect IMI. These provide data registrations that, to varying extents, are linked to the status of the animal. Therefore, algorithms using such sensor data can be seen as diagnostic tests, where the ability to classify disease status correctly based on sensor data represents the diagnostic test properties of the sensor system. A major challenge with these systems is that the diagnostic test properties for detection of subclinical IMI are either only moderately accurate or not known. This, in turn, hampers implementation of such systems for decision support.

Therefore, the main objective of this thesis was to evaluate the use of SCC data from online cell count (OCC) values obtained from each milking of cows in an automatic milking system (AMS). Specifically, we wanted both to evaluate the detection of cows with subclinical IMI using OCC values, and to use the OCC values to predict the future prevalence of subclinical IMI at the herd level.

We expected considerable variation in OCC values from milking to milking. Therefore, we used the elevated mastitis risk (EMR) index as a diagnostic test to evaluate the association of the OCC values with subclinical IMI (Paper I). The EMR index is the

output of an algorithm that preprocesses and parameterizes the raw OCC values into an EMR indicator, ranging from 0 - 1, where higher EMR values indicate an elevated risk of mastitis. Our findings showed that the diagnostic test properties of the EMR were too low to be used as the sole method of detection of subclinical IMI in individual cows during lactation. It may, however, be useful for detection of cows with subclinical IMI at drying off (Paper I).

In Paper II, we investigated the variation in OCC values from cows with and without subclinical IMI and found that only 15% of the variation in OCC values could be described by subclinical IMI and by other fixed effects like lactation stage, parity, milk yield, OCC in residual milk from the previous milking, inter-quarter difference between the highest and lowest conductivity, genetic constitution, milking interval and season. However, the fixed and random effects (cow and lactation within cow) together described 55% of the milking-to-milking variability of OCC. This means that 45 % of the variation in OCC values is not explained. Therefore, moderate diagnostic test properties should be expected when using EMR as a diagnostic test for detection of subclinical IMI in individual cows during lactation.

In order to predict the future prevalence of subclinical IMI at the herd level, we developed a Susceptible-Infectious-Susceptible transmission model for IMI based on bacteriological culture results of quarter milk samples (Paper III). Simulations, based on parameters for transmission and cure rate, can be used to generate predictions for any given time. We used *Corynebacterium* spp., which are bacteria known to cause persistent subclinical IMI, as the infectious pathogen to establish this model. In Paper IV, this transmission model was applied to the EMR, and we demonstrated that the transmission model can also be used to predict future prevalence of subclinical IMI in a herd, using the EMR as a proxy for infection. Although the detection of subclinical IMI using the EMR is not optimal for individual cows, predictions of herd-level prevalence will be relatively accurate and consistent. Changes in the parameters of the EMR in such dynamic models, will alter the predicted subclinical IMI prevalence. This way, simulations can be used to determine future herd level status of udder health. Such information can be implemented in a decision-support tool, and preventive actions can be taken to avoid an increase in the future prevalence of subclinical IMI.

The research conducted in this PhD has contributed to our understanding of the association between OCC values and subclinical IMI, using the EMR as a diagnostic test. Furthermore, we have shown that the EMR may be used as a proxy for infection in transmission modeling of subclinical IMI at the herd level. Despite suboptimal diagnostic test properties of the EMR, a sensor system based on the EMR can provide useful information in an udder-health management decision-support tool. The transmission model can be further extended to include the effects of different preventive actions to reduce the transmission rate of subclinical IMI in the herd. In order to do this, we need more knowledge of parameterization of preventive actions and the quantification of their effect on transmission dynamics.

SAMANDRAG

Mastitt er ein betennelse i ein eller fleire jurkjertlar som kan gi auka innhald av kjernehaldige celler (SCC) i mjølka. Den vanlegaste grunnen til mastitt er intramammære infeksjonar (IMI). Kyr med mastitt kan visa kliniske teikn (klinisk mastitt) eller ingen kliniske teikn (subklinisk mastitt). Mastitt er ein av dei viktigaste sjukdommane i mjølkeproduksjonen over heile verda. Den gir dårlegare dyrevelferd, redusert produksjon og økonomiske tap. Storparten av det økonomiske tapet kjem som følgje av redusert mjølkeproduksjon frå kyr med subklinisk mastitt. Sidan subklinisk IMI er den vanlegaste grunnen til subklinisk masttitt, er det viktig for mjølkekvalitet, dyrevelferd og bondens økonomi at subklinisk IMI vert oppdaga og handtert så raskt og så godt det lar seg gjera.

Det er ei utfordring at laboratorieundersøking av mjølkeprøver for å oppdaga subklinisk IMI er både tidkrevjande og kostbart. Difor vert subklinisk IMI i dag vanlegvis oppdaga ved analyse av SCC frå prøver tekne i samband med mjølkeveging. Utfordringa med denne tilnærminga, er at det er moderat samanheng mellom SCC og subklinisk IMI, og at tida mellom analyse av SCC frå prøver tekne i samband med mjølkeveging er for lang til å predikera framtidige episodar av IMI. Dei siste åra har det vorte utvikla fleire sensorar til bruk på garden for å oppdaga IMI. Slike sensorsystem leverer data som, i varierande grad, er knytta til dyrets status. Difor kan ein sjå på algoritmer, som bruker slike data, som diagnostiske testar, der evna til å klassifisera eit dyr sin sjukdomsstatus gir dei diagnostiske testeigenskapane til sensorsystemet. Diverre har desse systema anten moderate eller ukjente diagnostiske testeigenskaper for å oppdaga subklinisk IMI, noko som gjer at systema har moderat verdi som beslutningsstøtte for bonden.

Målet med denne avhandlinga var difor å få meir kunnskap om korleis me kan bruka data frå celletalsmålaren i automatiske mjølkingssystem (AMS). Denne gir eit celletal (OCC) frå kvar mjølking og me ville sjå om dette kan brukast til å oppdaga kyr med subklinisk IMI og til å predikera framtidig prevalens av IMI på buskapsnivå.

Først undersøkte me korleis endringar i OCC over tid heng saman med subklinisk IMI (Artikkel I). Til dette brukte me ein algoritme som omarbeider og parameteriserer data til ein «Elevated Mastitis Risk» (EMR) indeks. Dette er ein indikator for auka

mastittrisiko som går frå 0 til 1, der stigande verdiar indikerer auka risiko for mastitt. Våre funn viste at EMR har for dårlege diagnostiske testeigenskapar til å kunne brukast som einaste rutine for å oppdaga individuelle kyr med subklinisk IMI i laktasjon. Den kan likevel brukast til å oppdaga kyr med subklinisk IMI før avsining.

I Artikkel II såg me på variasjonen i OCC hjå kyr med og utan subklinisk IMI. Då fann me at berre 15% av variasjonen i OCC kunne forklarast med subklinisk IMI og andre faste variablar som laktasjonsstadium, paritet, yting, OCC i restmjølk frå førre mjølking, skilnad mellom høgaste og lågaste leiingsevne mellom kjertlar, genetisk linje, mjølkingsintervall og sesong. Til saman forklarte dei faste og tilfeldige variablane (ku og laktasjon innan ku) 55 % av variasjonen i OCC frå mjølking til mjølking. Dette tyder at 45 % av variasjonen i OCC ikkje er forklart. Difor må ein forventa moderate diagnostiske testeigenskapar ved bruk av EMR til å oppdaga subklinisk IMI hjå individuelle kyr i laktasjon.

Me laga ein «Suscpetible-Infectious-Susceptible»-modell av transmisjonsdynamikken for subklinisk IMI for å predikera framtidig prevalens av subklinisk mastitt på buskapsnivå. Denne vart utvikla på dyrkingsresultat frå kjertelmjølkeprøver (Artikkel III), og me brukte *Corynebacterium* spp. som infeksiøst agens i denne modellen. Dette er bakteriar som er kjent for å gi persistent subklinisk IMI. I Artikkel IV vart denne transmisjonmodellen brukt på EMR, og me viste at EMR kan brukast i modellen til å predikera framtidig prevalens av subklinisk IMI i ein buskap. Tanken bak dette er at sjølv om statusen for kvar enkelt ku er upresis, så vil dette jamna seg ut i buskapen og antatt framtidig prevalens av IMI vil difor vera nokolunde rett. Ei endring i prediksjonen av framtidig IMI-prevalens kan difor tyda på ei kommande endring i jurhelsa i buskapen. Denne informasjonen kan brukast i verktøy for beslutningsstøtte til bonden, slik at førebyggande tiltak kan settast inn tidleg for å unngå ei framtidig auke i prevalensen av subklinisk IMI.

Samla sett har forskinga i denne avhandlinga bidrege til vår forståing av samanhengen mellom OCC og subklinisk IMI, med EMR som ein diagnostisk test. Vidare har me vist at EMR kan brukast i transmisjonsmodellering av subklinisk IMI på buskapsnivå. Eit sensorsystem basert på OCC kan gi verdifull informasjon til bruk i styring av jurhelsa, sjølv om dei diagnostiske testeigenskapane er suboptimale. Transmisjonsmodellen kan

utvidast til å ta inn effekt av ulike førebyggande tiltak for å redusera overføringa av subklinisk IMI i buskapen. Men for å gjera dette er det nødvendig å studera effekten av aktuelle tiltak og bruka desse parameterane når transmisjonsdynamikken skal modellerast.

INTRODUCTION

Background

Dairy farming provides nutritional, social, and economic benefits to a large proportion of the world's population and, as such, is a vital component of the global food system (IDF, 2018). In Norway, the dairy industry is important for both food production and the gross domestic product, contributing to over 25,000 jobs throughout the country and a wealth creation of 19.5 billion Norwegian kroner (**NOK**) (Samfunnsøkonomisk analyse AS, 2017). In 2018, the average dairy-herd size in Norway was 28 cows (TINE Rådgiving, 2019), and, although the majority of cows are still milked in either tie-stalls or milking parlors, the number of farms with automatic milking systems (**AMS**) is increasing (Figure 1). However, the number of AMS per farm is low in Norway (1.1) compared with Denmark (2.9) and the overall average of 1.6 AMS per farm in the Nordic countries (Sigurdsson et al., 2019). In 2018, 45% of cows in Norway were milked in an AMS and they produced 48% of milk delivered to the dairies (TINE Rådgiving, 2019). Therefore, Norway may be viewed as a "laboratory" for research on AMS, including testing auxiliary technology and new approaches to dairy production.

An AMS performs the entire process of milking the cows, and thus the manual labor associated with the milking process is largely reduced to maintenance of the system and follow-up of cows that either do not show up for milking or are registered with failed milking attempts. Although this increases labor efficiency, one disadvantage might be that contact between the farmer and the animals during the milking process is considerably reduced. This is especially challenging regarding the detection of sick animals and abnormal milk. Therefore, AMS should include technologies for rapid and accurate detection of sick cows.

From the AMS, we are able to obtain even more data than ever before. Instead of periodically sending milk samples to the laboratory for different analyses, sensors can inform us about the various milk components at every milking. This provides new possibilities for monitoring an individual animal, and also has the potential to result in improvements in herd-health management. However, in order to achieve this, there are some obstacles that must be overcome. While the amount of data increases substantially in AMS, it remains a challenge to extract the relevant information and to use it to provide decision

support for the farmer. Therefore, information from Dairy Herd Improvement (**DHI**) programs are still commonly used for herd-health management even when AMS is used. These DHI programs are based on analyzing composite milk samples from lactating cows, usually on a monthly or bimonthly basis. The DHI results are then used to determine the current status and historical development at both the cow level and the herd level. If applicable, the information can be used to change standard operating procedures (**SOP**) in order to achieve a desired improvement or to reach a future goal. While this is a proven and effective way of managing herd health, progress may be slow. Furthermore, the changes in SOP may not always address the cause of the herd-health challenges. An example of this could be that a revised SOP emphasizes checking intramammary infection (**IMI**) status and rapid treatment of infected cows to reduce the pressure of infection in the herd. Although this might be a successful approach for limiting the prevalence of IMI, it does not solve the problem should the underlying cause for new infections be predominantly associated with factors such as poor hygiene in the lactation pen or with animal traffic to and from the lactation pen. Therefore, prediction of future developments and establishing decision-support systems based on new sensors and new algorithms have the potential to improve herd-health management substantially.

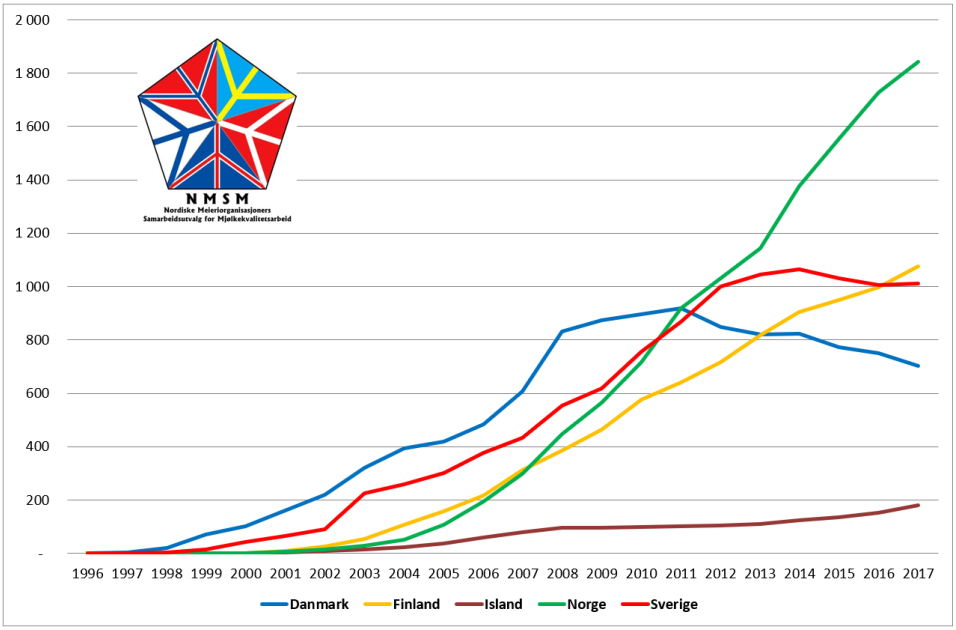

Figure 1. Number of herds with AMS in the Nordic countries by year. Figure from The Nordic Dairy Associations' Committee for Milk Quality Issues.

Mastitis and intramammary infection

Mastitis is, economically, one of the most important diseases in dairy production (Halasa et al., 2007; Hogeveen et al., 2011). It is an inflammation of the mammary gland, and can be clinical or subclinical. Whereas clinical mastitis is an udder inflammation that is characterized by visible abnormalities in the milk and or udder (IDF, 2011), subclinical mastitis is an inflammation of the mammary gland that requires a diagnostic test for detection. Milk somatic cell count (**SCC**) is routinely used for detection of subclinical mastitis, with a diagnostic cut-off of 200,000 cells/mL at the cow level (IDF, 2011).

Mastitis is the most common disease in Norwegian dairy production. In 2018, there were 18 veterinary treatments for mastitis per 100 cow-years in Norway (TINE Rådgiving, 2019). Treatment of mastitis frequently involves antimicrobial therapy, and therefore mastitis contributes to a large proportion of antimicrobial use in Norwegian dairy production. This is a challenge, as antimicrobial resistance (**AMR**) can occur following antimicrobial treatment. However, prudent use can reduce the development

of AMR (Abdi et al., 2018). Therefore, in addition to improving animal welfare and reducing economic losses, the prevention of mastitis can also contribute to less use of antimicrobials and reduced development of AMR.

Mastitis is almost always caused by a bacterial IMI (Hogan et al., 2016), which is an infection occurring in the secretory tissue or the ducts and tubules of the mammary gland, or all of the above (IDF, 2011). Different bacteria can cause IMI (Dohoo et al., 2011), and IMI have received considerable focus in research on udder-health management (Ruegg, 2017). Following an infection, the immune system of the cow mounts a response to this infection. The purpose of the response is to clear the infection, but it often also changes the milk composition and reduces milk production. The severity and duration of these changes will be dependent on several factors, including the causative pathogen, and both the genetic composition and/or physiological status of the cow (Nash et al., 2002; Rivas et al., 2013). Figure 2 shows the development of IMI and subsequent mastitis.

An IMI is commonly diagnosed by microbiological culture of aseptically obtained milk samples (IDF, 2011). The definition of IMI is not straightforward. Zadoks et al. (2002) and Reksen et al. (2012) used a combination of number of colony forming units (**cfu**)/mL and duration of persistency for the definition of cases of IMI in their studies on transmission of *Staphylococcus aureus* and non-aureus staphylococci, respectively. In these studies, a cow was considered to be harboring an IMI when ≥ 1000 cfu/mL of the pathogen were cultured from a single milk sample, or when ≥ 500 cfu/mL of the pathogen were cultured from two out of three consecutive milk samples, or when ≥ 100 cfu/mL were cultured from three consecutive milk samples, or when ≥ 100 cfu/mL were cultured from a clinical sample. The advantage of this approach is that cows that are defined as infected (harboring an infection) are likely to be truly infected. However, with sampling intervals of 3 (Zadoks et al., 2002) and 4 (Reksen et al., 2012) weeks, some infections are likely to be missed with this approach. In contrast, Dohoo et al. (2011) argued that a single sample is sufficient for diagnosing an IMI, while still providing the opportunity for an adaptation of the cfu/ml thresholds for diagnosing an IMI depending on the intended use of the information. Other studies have used the SCC, alone or in combination with clinical signs, as determinants in the definition of IMI status. In a review article, Schukken et al. (2003) argued that SCC in

composite milk can be used as a proxy for IMI status, and used 200,000 cells/mL as the threshold. Using 200,000 cells/mL as the threshold, Dufour and Dohoo (2013) found that quarter level SCC is necessary for computing and monitoring the incidence of IMI during lactation. The advantage of using bacteriological culture results to define IMI, is that a specific pathogen can be isolated and considered in association with changes in milk composition. Knowledge of the specific causative pathogen may be relevant for selecting the most appropriate management actions that are known to be effective at reducing IMI caused by this pathogen (Whist et al., 2007). This knowledge can also be used to improve the herd SOP for udder-health management (Østerås and Sølverød, 2009). However, due to the costs associated with sampling and bacteriological analysis of the milk samples, often a considerable period of time elapses between milk sampling events. Therefore, there is some uncertainty concerned with the IMI status of cows in the period between the two samples. This is particularly challenging for defining the IMI status of cows that have either acquired a new IMI or have recovered from an existing IMI. Zadoks et al. (2002) addressed this challenge by using a mid-point estimation approach, in which they argued that a new IMI or recovery from a previous IMI will, on average, occur mid-way between the two sampling events.

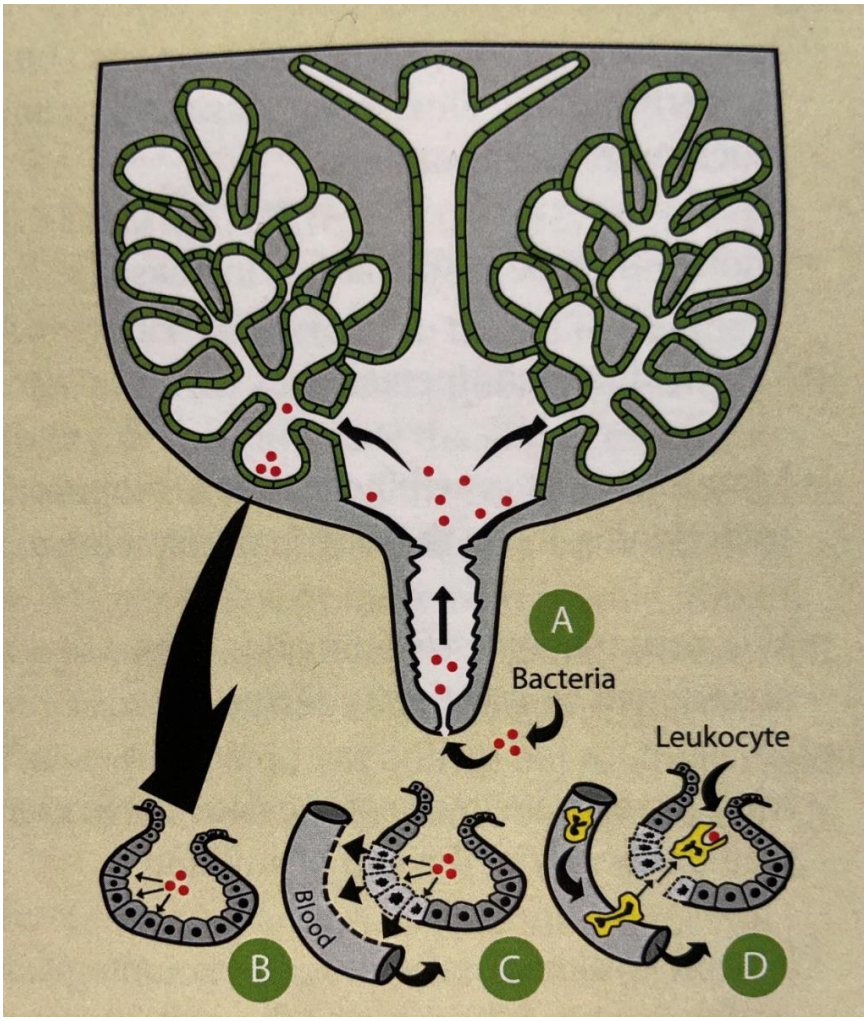

Figure 2. Development of mastitis following intramammary infection. Figure from Hogan et al. (2016). (A) A mastitis pathogen enters the udder via the teat canal and teat cistern. (B) When the mastitis pathogen gains access to the small ducts and glandular tissue, it can potentially affect the alveolar cells. (C) Toxins produced by the mastitis pathogen (small arrows) have the potential to harm or kill the alveolar cells, which, in turn, release inflammatory substances that increase blood-vessel permeability (larger arrows). (D) The increased blood-vessel permeability allows influx of leukocytes from the blood and into the alveolus, where they attempt to remove the mastitis pathogen from the udder. This recruitment of leukocytes into infected alveoli is the main cause of increased SCC in milk from cows with IMI.

An important aspect of IMI is the potential for transmission of an infection from infected cows to susceptible cows. This, combined with the fact that much of the economic loss from mastitis is due to reduced milk production following subclinical mastitis (Hogan et al., 2016), makes detection and management of subclinical IMI an important task in dairy production.

Milk somatic cell counts and udder-health management

Somatic cells are normally present in low concentrations in milk from uninfected mammary glands, and this is usually below 100,000 cells/mL in dairy cows (Leitner et al., 2012; Nyman et al., 2014). Following a challenge to the udder, there is recruitment of inflammatory cells to the mammary gland and this rapidly increases the SCC in milk (Figure 2) (Persson and Sandgren, 1992). The most common cause of elevated SCC in milk is IMI. However, in a previous study, Nyman et al. (2014) found that the IMI status explained only 24% of the SCC. This is mainly because the SCC can be affected 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). When an infected cow recovers from the infection, the SCC usually return to normal levels within 21 days (Pyörälä, 1988). However, the duration of elevated SCC following an IMI is influenced by, among other things, genetic constitution and the causative mastitis pathogen (Nash et al., 2002). In cases where the cow does not recover from a subclinical IMI, the SCC can remain elevated for a prolonged period. The elevation of inflammatory somatic cells during subclinical IMI is thus the basis for using SCC as an indicator of infection status (Rivas et al., 2013).

The SCC obtained through DHI programs are widely used to diagnose subclinical IMI (IDF, 2011), and they have been evaluated and found to be a valuable component in udder-health monitoring programs (Schukken et al., 2003). Udder-health management based on SCC can be seen as part of a health-management cycle, where farm-specific goals are set, and SCC are used to assess current status relative to these goals. When goals are not met, the farmer can take actions to improve progress towards achieving them (Kelton, 2006). This process can be illustrated with a Deming circle (Figure 3).

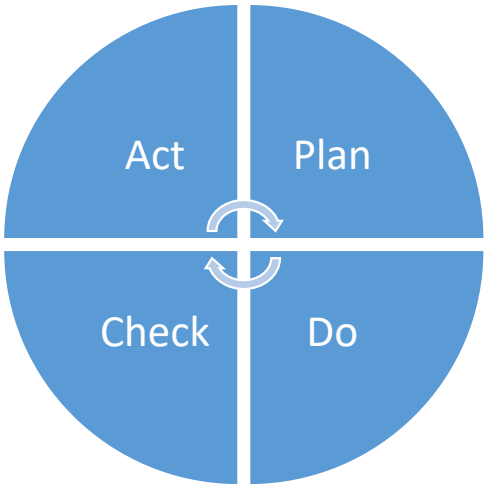

Figure 3. The Deming circle. The farm has a plan to manage udder health ("Plan"). This plan is executed ("Do"), and progress is monitored ("Check"). If deviations occur, further action is taken to improve progress in order to reach the goals ("Act"). These additional actions should be evaluated for possible inclusion into an updated udder-health management plan ("Plan").

Research on mastitis has shown that prevention of new cases of mastitis is particularly effective for management of udder health (Ruegg, 2017). It is therefore a challenge that the current approach of using historical information from DHI programs (Østerås and Sølverød, 2009) mostly allows for only slow improvements that sometimes result in opportunities being missed for preventing new cases of subclinical IMI. With the introduction of on-farm sensors, we may progress to real-time surveillance and predictions of future development. This may allow us to take early preventive actions and thereby achieve improved strategies for avoidance of new cases of subclinical IMI and other undesirable events in the future.

Sensor systems

Sensor systems for udder-health management provide data registrations, which, to a varying extent, are linked to the status of the animal. Therefore, algorithms using such sensor data can be seen as diagnostic tests, where the ability to classify the disease status correctly represents the diagnostic test properties of the sensor system (Dohoo et al., 2009). The basic principles for sensor systems in dairy production are shown in Figure 4. The idea is that algorithms can use sensor data to detect and provide alerts about deviations that are predictive of a specific disease or a defined status. This information is then used, alone or together with a SOP, in a decision-support model intended to assist the farmer in making appropriate management decisions. An optimal sensor system yields only true positive and true negative results. In such a system, the sensitivity and specificity would both be 100%. However, such perfection is never the case, and the information extracted from sensor data is therefore an inherently flawed proxy for an individual cow's biological status. As a result, the sensor system uses both true and false test results as the basis for the sensor-system alerts. An example of a false-negative test result could be that the test classifies a cow as healthy, whereas the cow actually has a subclinical IMI. A false-positive test result could be when the test classifies a cow as infected, whereas in reality the cow has no subclinical IMI. As the current udder-health sensor systems measure a cow's response to the infection, rather than the infection itself, the diagnostic test properties are likely to be imperfect. In addition, there is considerable biological variation within the cow population regarding the level and duration of the response in milk composition following an infection (Nash et al., 2002; Rivas et al., 2013).

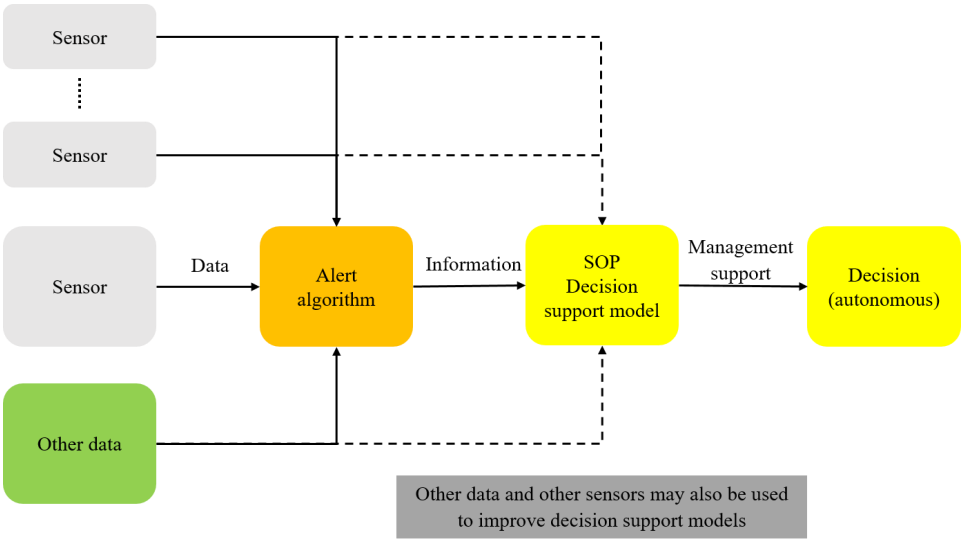

Figure 4. Basic principles of sensor systems. The solid lines signify direct pathways of data into the alert algorithm and through to the decision. The dotted lines signify potential interaction between sensors and possible data input not only to the alert algorithm, but also to the decision support model. Figure from Henk Hogeveen, adapted from Rutten et al. (2013).

In everyday use, farmers with AMS on their farms prefer sensor systems with high specificity rather than high sensitivity (Claycomb et al., 2009). This is mainly because a large number of false sensor-system alerts is of practical concern for farmers (Hogeveen et al., 2010). Also, false sensor-system alerts could result in unnecessary treatment or other actions directed at healthy animals. On the other hand, failure to alert the farmer to a sick animal is a potential concern for animal welfare and herd health. Therefore, the diagnostic test properties of sensor systems should be investigated and reported such that implementation of sensor systems in decision-support tools can be improved.

Online cell counts

One on-farm sensor is the DeLaval Online Cell Counter (DeLaval International AB, Tumba, Sweden). This sensor provides online cell counts (**OCC**) as a proxy for SCC from every milking tested. Previous studies of associations between OCC and IMI have relied on records of clinical mastitis as the gold standard for the evaluation of sensor performance

(Kamphuis et al., 2008; Sørensen et al., 2016). However, the ability of OCC to discriminate between subclinical IMI and physiological alterations in SCC has not yet been determined.

One challenge associated with automated detection of subclinical IMI using OCC, is that because the OCC is a function of a cow's response, this value varies widely (Rivas et al., 2013). Thus, differentiating between physiological normal variation and variation due to pathology remains a major challenge. One advantage of frequent sampling of OCC is that a larger density of records may enable better separation of measurement noise from true changes due to biological processes. In such a system, arbitrary changes in OCC values can be viewed as within-animal deviations and corrected for by calculating rolling averages or by using smoothing functions (Sørensen et al., 2016). Sørensen et al. (2016) showed that by using this approach, OCC may be used to detect cases of clinical mastitis (Figure 5). However, this has not yet been demonstrated for prediction of subclinical IMI status of cows in an AMS.

An elevated mastitis risk (**EMR**) parameter as shown in Figure 5, has, however, only been tested for the ability to detect cases of clinical mastitis (Sørensen et al., 2016), and not for the ability to detect episodes of subclinical IMI. Therefore, EMR threshold values for the detection of subclinical IMI are lacking.

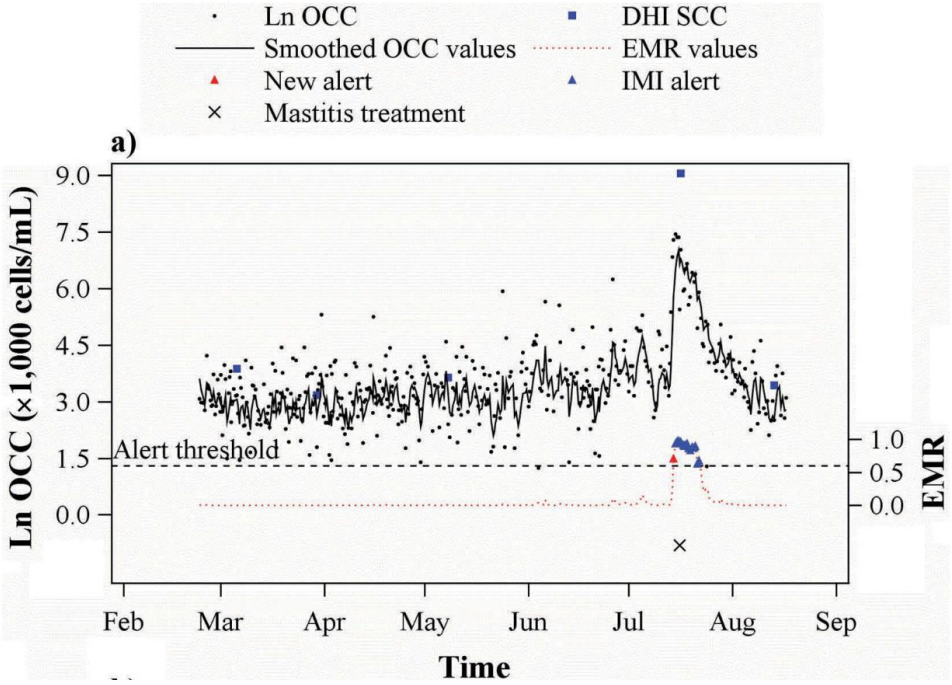

Figure 5. Example of the variations in OCC from milking to milking. Figure from (Sørensen et al., 2016). The figure shows fluctuations in the OCC from milking to milking, before a marked increase in association with a case of clinical mastitis. Smoothed OCC values = OCC level and trend from the double exponential smoothing algorithm developed by (Sørensen et al., 2016). The SCC from DHI samples is shown for comparison. EMR = elevated mastitis risk.

Diagnostic test evaluation

The diagnostic performance of a test is often evaluated by calculating the test's sensitivity and specificity. These parameters quantify the ability of the particular test to determine correctly the biological status of the animal being tested. Table 1 shows the data setup for evaluating the diagnostic sensitivity and specificity of a test.

Table 1. Contingency table for calculation of diagnostic test properties of a test for subclinical IMI

	Subclinical IMI[1] present	**Subclinical IMI absent**	**Totals**
Test positive	a true positive	b false positive	a + b
Test negative	c false negative	d true negative	c + d
Totals	a + c	b + d	a + b + c + d

1 Intramammary infection

Sensitivity (true positive rate) = $\frac{a}{a+c}$

Specificity (true negative rate) = $\frac{d}{b+d}$

For imperfect tests, there is a trade-off between sensitivity and specificity. Hence, if the test threshold is lowered to maximize sensitivity, there will be a greater number of false-positive test results. Conversely, if the test threshold is elevated to maximize specificity, there will be more false-negative test results. Regardless of the approach used for defining IMI, the definition will have an impact on the results, and thus, potentially, on the management actions.

Transmission modeling

Detection of cows with an ongoing subclinical IMI is important. It would, however, be better to avoid episodes of subclinical IMI altogether. Therefore, prediction of the future prevalence of subclinical IMI, and suggested actions to keep this as low as possible, would be preferable. This may be done at the herd level by using a transmission model, based on ordinary differential equations (**ODE**), to predict alterations in the future prevalence of subclinical IMI in the herd. With repeated bacteriological milk culture results of all lactating quarters (**QMS**) in a herd, we can model the transmission dynamics of persistent IMI (Lam et al., 1997; Reksen et al., 2012; Barlow et al., 2013). Such models can be used to predict the future IMI prevalence. However, taking and analyzing monthly QMS of all lactating cows is not cost efficient for management of udder health in commercial dairy herds. With the

introduction of sensors like the OCC, we can obtain frequently repeated cow-level measurements in a relatively cost-efficient way. If the relationship between OCC values and subclinical IMI is sufficiently strong, then repeated measurements of OCC may be implemented in automated detection algorithms for the prediction of the prevalence of subclinical IMI in AMS herds. In this way, the introduction of sensors such as the OCC may facilitate the progression from retrospective herd-health management, to modelling real-time herd-specific udder-health transmission dynamics as an alternative to laboratory analyses of bacteriological milk samples. Transmission parameters can also be used to simulate future udder health status.

Several mastitis pathogens have the potential to spread between cows in a contagious manner (Barkema et al., 2009). The reproductive number (**R_0**) is the number of secondary infections that occur when an infected individual is introduced into a naive population. The R_0 is a function of contacts per unit time, the transmission probability per contact, and the duration of infectiousness (Anderson and May, 1991). When R_0 is greater than 1, there will be an increasing number of infections in the population (outbreak). When R_0 is below 1, then transmission of infection will not be sustained in the population without the influence of other factors, such as the influx of infected cows from outside (e.g., fresh or purchased cows). Therefore, R_0 is often used to describe the epidemic potential of infections (Anderson and May, 1991). Although the transmission of a pathogen is described by R_0 at the population level, it is the rate of both entry and exit of quarters, the transmission parameter, and the cure or recovery rate or duration of infection that determines the value of R_0. This means that the same bacteria may have different potentials for spreading in different herds, depending on factors like udder-health management, culling, and treatment routines within the herd.

Precision livestock farming

Precision livestock farming (**PLF**) is often used as a collective term to describe integrated livestock-management systems based on sensor information. Halachmi and Guarino (2016) defined PLF as "real-time monitoring technologies aimed at managing the smallest manageable production unit's temporal variability". Using PLF, cows may be managed both as a group and as individuals at the same time. Successful application of PLF requires information from monitoring technologies, a mathematical model to predict current status, a defined management goal, and quantification of the effects

from possible management actions (Wathes et al., 2008). Because several of these prerequisites are not available at present, true implementation of PLF is not presently feasible for management of udder health.

KNOWLEDGE GAPS

Detection of subclinical IMI using OCC values

While historical SCC data can be used for management of udder health (Schukken et al., 2003), we do not know how best to use the frequently measured OCC values to manage subclinical IMI. Also, before OCC data can be implemented in algorithms to predict udder health, we need more knowledge regarding the association and correlation between OCC values and subclinical IMI status, including potential threshold values for detection of subclinical IMI using the OCC sensor. Hence, although there is presently an information overload based on a large quantity of data provided from sensor systems, it is not clear how this information can be most usefully used by the farmers. Therefore, there is a need for knowledge on which information is provided by the sensors, and how this information can be applied to improve decision support.

One of the challenges precluding the use of sensor systems for continuous monitoring of udder health and associated decision support, is the suboptimal diagnostic test properties of the current algorithms in sensor systems (Norberg et al., 2004; Rutten et al., 2013). When the sensor-system alerts are not trustworthy, then neither is the management advice obtained from the sensor system.

Causes of variation in OCC values from milking to milking and from day to day

With the introduction of the OCC sensor system, we are gaining access to huge amounts of data. However, extraction of relevant information from sensor data such as OCC and others has proven difficult regarding management of udder health (Rutten et al., 2013). Therefore, investigation of the predictors for variation in OCC values could provide useful information that would be beneficial in understanding how this measure can best be interpreted and used.

Transmission model of subclinical IMI using OCC values as a proxy for infection

Detection of subclinical IMI episodes during lactation is only relevant if there are SOP or predetermined management actions associated with the detection of these cases. During lactation, management actions could be, for example, segregation of infected

animals, improving the hygiene, or reducing the density of stocking. However, prevention of a possible increase in the prevalence of subclinical IMI in the future would be preferable. In order to accomplish this, it is necessary to be able to predict the future situation regarding subclinical IMI. One way to do this is using transmission modeling, with EMR included as a proxy for infection. However, a sensor-based transmission model must first be developed for this approach to be successful.

OBJECTIVES OF THE STUDY

The main objective of this study was to evaluate the use of OCC from every milking in an AMS for the detection of cows with subclinical IMI, and for predicting the future prevalence of subclinical IMI at the herd level.

Secondary objectives:

1. Define criteria for OCC changes associated with subclinical IMI and test the diagnostic test properties of the EMR as a test for detection of subclinical IMI (Paper I).
2. Describe the variation in OCC values related to subclinical IMI and cow-specific factors (e.g. parity, days in milk) (Paper II).
3. Build an SIS (Susceptible-Infectious-Susceptible) transmission model based on bacteriological milk culture results to determine the transmission of subclinical IMI, using *Corynebacterium* spp. as a model pathogen (Paper III).
4. Use dynamic changes in EMR as a proxy for subclinical IMI and build a second SIS transmission model based on daily EMR readings for herd-health surveillance of udder health (Paper IV).

MATERIALS AND METHODS

This section gives an overview of the material and methods used in the thesis. More details are provided in the separate papers. The work included 2 observational longitudinal studies. Papers I, II, and IV used data from a 17-month longitudinal observational study in the dairy research herd at the Norwegian University of Life Sciences. Paper III used data from a 13-month longitudinal observational study in 2 US dairy herds. Figure 6 shows a simplified overview of the material and methods used in the 4 papers.

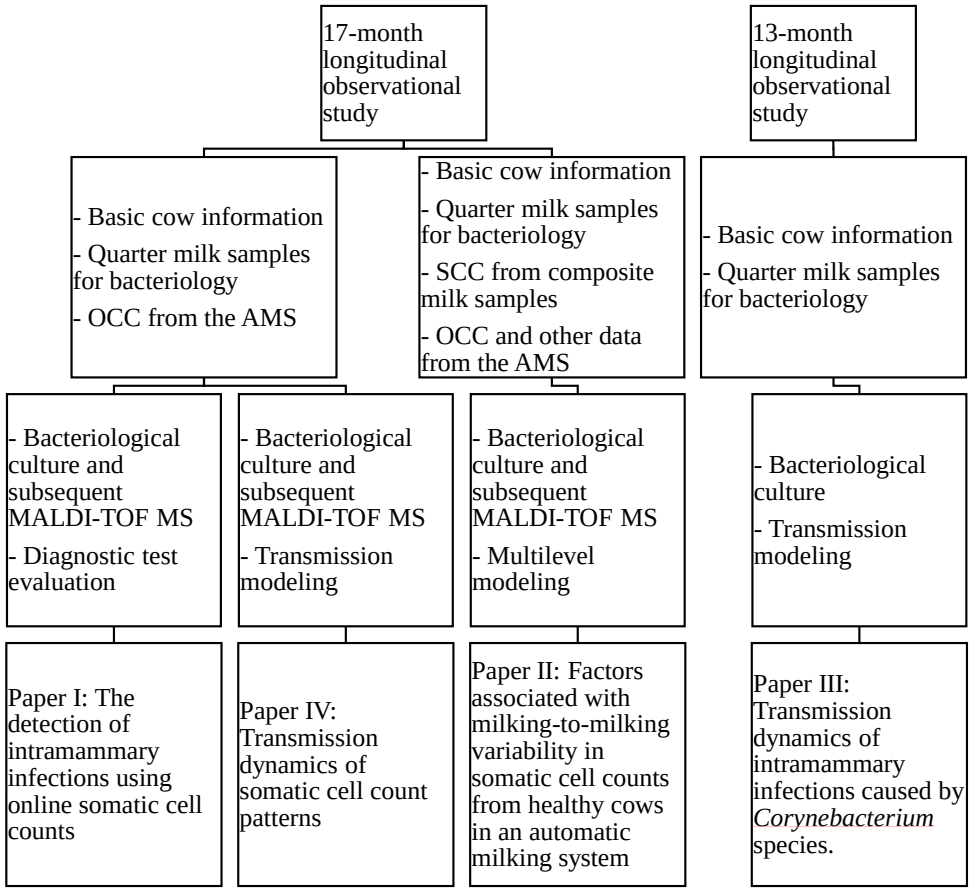

Figure 6. Simplified overview of the material and methods used in this work.

Study samples

17-month longitudinal study (Papers I, II and IV)

Data were obtained during 2016 and 2017 in a 17-month longitudinal study in the dairy research herd at the Norwegian University of Life Sciences. Two groups, each of approximately 50 Norwegian Red cows, housed in the same barn, were milked, on average, 2.6 times per day by two identical AMS (Delaval VMS, DeLaval, Tumba, Sweden) during the study period. The mean monthly 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 used standardized mastitis-control practices, such as monthly milk-quality testing in a DHI program, postmilking teat disinfection, and selective dry-cow therapy.

13-month longitudinal observational study (Paper III)

Data were obtained from a 13-month longitudinal study conducted in two commercial Holstein dairy herds (one in New York and one in Vermont) during 2003 and 2004. In this study, cows were housed in pens of approximately 100 cows and milked 3 times per day in a milking parlor. The mean monthly number of lactating cows was 319 and 346 in the 2 farms, respectively. The corresponding mean milk production was 32.7 kg and 35.0 kg. Similarly, the average cow composite SCC was 404,000 cells/mL and 292,000 cells/mL. The herds participated in a DHI program, with monthly milk-quality testing. Both farms used standardized mastitis-control practices, including pre- and postmilking teat disinfection, and blanket dry-cow therapy.

OCC and EMR

The DeLaval Online Cell Counter (DeLaval International AB, Tumba, Sweden) provides OCC shortly after milking a cow in the AMS. The device samples a fraction of the composite milk and adds a colored reagent that stains the nuclei of somatic cells, before a digital camera takes a picture and counts the number of nuclei in the sample (DeLaval, 2019). Sørensen et al. (2016) evaluated the performance of the OCC relative to DHI analysis (Eurofins, Holstebro, Denmark) using CombiFoss equipment (Foss Electric, Denmark). They found an average R^2 of 0.86, ranging from 0.71 – 0.93, using linear regression to assess the performance. These results indicate that the OCC can be used as a proxy for DHI-based SCC.

A major challenge with using sensor-system data, such as the OCC, is the large physiological variation from milking to milking and from day to day. This makes it difficult to identify moderate deviations from a normal situation. Because subclinical IMI results in a moderate inflammation of the udder, it is difficult to detect it using OCC. Sørensen et al. (2016) used a stepwise process to improve the usability of OCC. This process involves a single exponential smoothing and correction of the raw data from the sensor, before a double exponential smoothing of the individual cow's data. Finally, the smoothed data is parameterized into an EMR indicator, ranging from 0 – 1, where a higher value indicates an elevated mastitis risk.

IMI status

In both study populations, QMS were collected 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).

We decided to focus on detection of subclinical IMI with potential for transmission. We defined this as episodes of subclinical IMI detected in QMS from the same cow in several successive samples or in high amounts in a single sample. This was adapted from Zadoks et al. (2002). Therefore, cows were given an udder-health status for subclinical IMI throughout the study period, based on a combination of persistence and cfu/mL. Using this approach, cows that were assigned the status "subclinical IMI" were likely to be truly infected.

Because the OCC values are recorded at the cow level, the quarter level bacteriological diagnoses were aggregated into cow-level diagnoses. A consequence of this, was that the same cow could experience an episode of subclinical IMI with more than one pathogen at the same time, and we would be unable to determine which pathogen had the most influence on the OCC values. Therefore, we divided the pathogens into 2 groups (**Pat 1** and **Pat 2**). The Pat-1 group consisted of pathogens that are expected to result in a marked elevation of OCC values. These were: *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; Simojoki et al., 2011; Fry et al., 2014). The Pat-2 group consisted of those pathogens that were not included in the Pat-1 group: *Corynebacterium bovis*, *Staphylococcus chromogenes*, *Staphylococcus haemolyticus*, *Aerococcus viridans*, *Staphylococcus hominis*, *Staphylococcus xylosus*, and other bacteria cultured. Whenever a cow was found to have a subclinical IMI with one or more pathogens from both Pat 1 and Pat 2 simultaneously, the change in OCC values was attributed to the Pat-1 subclinical IMI. That is, we implemented a hierarchical classification system, where a cow could only be assigned to the Pat-2 subclinical IMI category when there was no concurrent Pat-1 subclinical IMI.

In the 17-month study in Norway, species of bacteria were identified using matrix-assisted laser desorption ionization-time of flight mass spectrometry (**MALDI-TOF MS**) microflex LT (Bruker Corporation, Billerica, USA) (Cheuzeville, 2015).

The principle of the MALDI-TOF MS is shown and described in Figure 7. Briefly, a sample of a bacterial colony is put on a target plate and covered with a matrix before ionization using a laser. The mass spectrum obtained can be used to identify microorganisms. MALDI-TOF MS provides a fast and reliable way of identifying mastitis pathogens (Cheuzeville, 2015; Nonnemann et al., 2019).

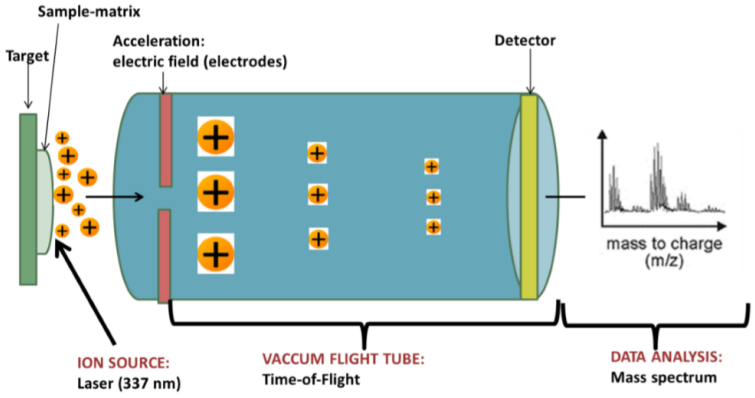

Figure 7: Principle of the MALDI-TOF MS. Figure from Cheuzeville (2015). The matrix-covered sample is ionized by a laser, leading to desorption and transfer of protons from the matrix to the sample that forms ions, with minimal fragmentation. Application of an electric field accelerates the ions, which go through a vacuum flight tube towards a detector. The time of flight through the tube is influenced by the weight of the ions, where lighter ions have greater speed, and thus a shorter time of flight. This difference is used for species identification of the bacterial colonies being tested.

Diagnostic test evaluation

In Paper I, the subclinical IMI status based on QMS results was used as the gold standard for the evaluation of the EMR as a diagnostic test for detection of subclinical IMI. We tested 4 different thresholds of the EMR for their ability to classify subclinical IMI status correctly. The thresholds were set so that the specificities were 80, 85, 90, and 99%, and the corresponding sensitivities were calculated for each threshold, respectively. Thus, each threshold can be considered as a separate diagnostic test for detection of subclinical IMI.

Multilevel modelling

In Paper II, the variation in OCC from milking to milking was evaluated using a linear mixed model (Dohoo et al., 2009). The advantage of using such a model is that it considers the multilevel structure of the data (Dohoo et al., 2009), and we could therefore describe how much of the overall variability resided at the cow-level (between cows) and at the lactation-level (within cow).

Transmission model and transmission parameters

We built a transmission model and used it to evaluate the transmission dynamics of IMI episodes (Paper III and Paper IV). In Paper IV, we used the EMR threshold values from Paper I to assign cows to a subclinical IMI-status category. Cows were categorized with the status of subclinical IMI when the EMR value was greater than a given threshold. We tested 4 different threshold levels with different sensitivities and specificities for the detection of subclinical IMI. The transmission dynamics of the subclinical IMI episodes and the 4 different EMR thresholds were displayed in a Susceptible-Infectious-Susceptible (**SIS**)-transmission model, as shown in Figure 8. The model describes a population divided into two compartments: (1) compartment S denotes susceptible quarters or cows with no subclinical IMI, and (2) compartment I denotes quarters or cows affected with subclinical IMI. The compartments thus represent the proportion of lactating quarters or cows in each state. The dynamics of state transitions are illustrated in Figure 8.

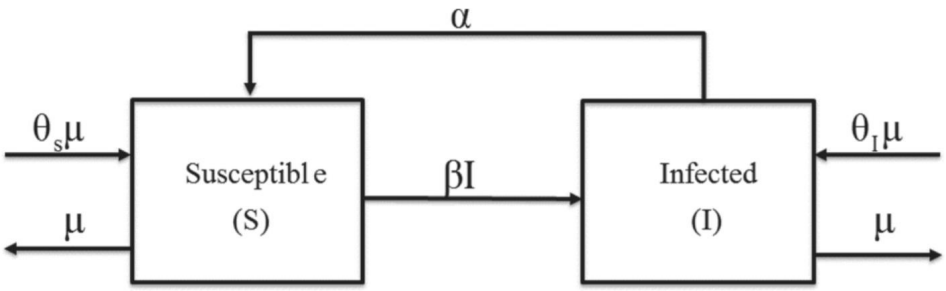

Figure 8. Schematic representation of the mathematical model of transmission of subclinical IMI. The boxes represent the state variables and the arrows represent the flow rates between susceptible (S) and infected (I) states. B = transmission parameter; βI = daily rate of new infections; α = daily rate of cured episodes; μ = daily rate of entry and exit. The proportions of entries into the S and I compartments are determined by θS and θI, respectively.

The model is described mathematically by the following non-linear ODE:

$$\frac{dS}{dt} = -\beta SI + \alpha I + \theta_s N\mu - \mu S \qquad (1)$$

$$\frac{dI}{dt} = \beta SI - \alpha I + \theta_I N\mu - \mu I \qquad (2)$$

The transfer rates in such a model are quantified by the parameters α and β. Parameter β is a function of the contagiousness of the pathogen and the contact rate between animals, and denotes the transmission rate of an infection from a cow with subclinical IMI to a susceptible cow (Keeling and Rohani, 2011). Parameter α describes the daily rate of cow recovery from a subclinical IMI. N represents the sum of susceptible and infected cows in the study at any given time. The daily rate of entry and exit of cows to and from the lactation pen is described by μ. Entries of cows from the fresh pen to the susceptible and infectious compartments within the lactation pen are determined by the proportions θ_s and θ_I, respectively. The transmission parameters in the model are unknown and therefore must be calculated for each herd being considered. This estimate may be made using knowledge of the subclinical IMI status of cows over time from the results of the bacteriological milk samples of QMS. Therefore, we estimated the transmission parameters in the model using OCC-value patterns, assuming that these represent the presence of a subclinical IMI. The estimated transmission parameters were used as input in the dynamic simulation model for prediction of future herd prevalence of subclinical IMI.

MAIN RESULTS

Paper I

In paper I we showed that OCC may be useful for identifying cows with an episode of subclinical IMI. The diagnostic test properties of the OCC were improved when using the EMR compared with using the raw OCC values. The sensitivity of detection of Pat-1 subclinical IMI using EMR was 69% and 8% at the predefined specificities of 80% and 99%, respectively. Examples of the practical implications of the properties of the EMR as a diagnostic test for the number of false sensor-system alerts are shown in Table 2. There is a clear tradeoff between sensitivity and specificity when using EMR to detect Pat-1 subclinical IMI. Although increasing the sensitivity will result in detection of more cases of Pat-1 subclinical IMI, it will also result in more false sensor-system alerts. On the other hand, increasing the specificity in order to reduce the number of false sensor-system alerts will result in the likelihood of detecting cases of subclinical IMI decreasing. A farmer with a high tolerance for false sensor-system alerts may choose to increase sensitivity at the cost of lower specificity, so that more episodes of subclinical IMI are detected and can be managed accordingly.

Table 2. Examples of the practical implication of detection event and specificity level on the number of false sensor-system alerts in an AMS[1] herd with 100 cow-years, milking 2.7 times a day, and using the EMR[2] for detection of subclinical IMI[3].

Test specificity demand	Detection event	False sensor-system alerts	Test sensitivity of the EMR (Paper I)
Specificity ≥ 99	EMR evaluated after every milking	≈ 2 per day	8
	EMR evaluated once before dry off	≈ 1 per year	
Specificity ≥ 80	EMR evaluated after every milking	≈ 35 per day	69
	EMR evaluated once before dry off	≈ 20 per year	

[1] Automatic milking system
[2] Elevated mastitis risk
[3] Intramammary infection

Paper II

In paper II we showed that only 15% of the variation in OCC values could be described by subclinical IMI and by other fixed effects like lactation stage, parity, milk yield, OCC in residual milk from the previous milking, inter-quarter difference between the highest and lowest conductivity, genetic constitution, milking interval and season. However, the fixed and random effects (cow and lactation within cow) together described 55% of the milking-to-milking variability of OCC. Figure 9 shows the distribution of ln-transformed OCC values for cows with no IMI, Pat-1 subclinical IMI, Pat-2 subclinical IMI, and transient colonization. Although the OCC values for cows with Pat-1 subclinical IMI are higher than for the other groups, there is still considerable overlap. Therefore, moderate diagnostic test properties must be expected in studies in which only OCC are used as the indicator of subclinical IMI.

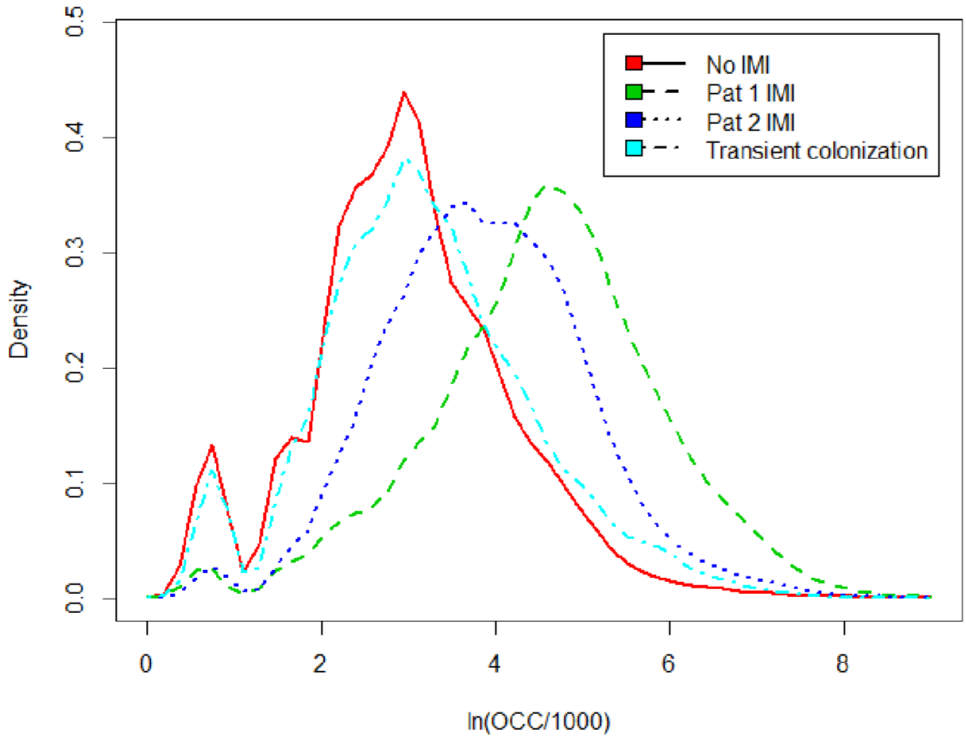

Figure 9. Smoothed density plot showing the distribution of ln-transformed online cell count values (in 1000 cells/mL). Figure from Nørstebø et al. (2019).

Paper III

We studied the transmission dynamics of *Corynebacterium* spp., which are bacteria known to cause subclinical IMI. The statistical analyses in Paper III demonstrated that transmission of IMI due to *Corynebacterium* spp. in the 2 herds studied was influenced by preexisting infections with *Corynbacterium* spp. IMI. In one of the 2 farms studied, there was also an increase in the prevalence of *Corynebacterium* spp. IMI throughout the study, which resulted in an R_0 of 1.18. This was related to a low rate of recovery from *Corynebacterium* spp. IMI in this farm, and this therefore increased the epidemic potential of *Corynebacterium* spp. IMI in this particular situation.

Paper IV

The statistical analyses in Paper IV demonstrated transmission of subclinical IMI, using EMR as a proxy for subclinical IMI. For the EMR thresholds with 80%, 85%, and 90% specificity for detection of subclinical IMI, the R_0 was above 1, indicating an epidemic potential. Furthermore, the transmission of subclinical IMI, using EMR as a proxy for subclinical IMI, was influenced by a preexisting EMR above the EMR thresholds, with 80%, 90%, and 99% specificity for detection of subclinical IMI. That is, cows with an existing EMR above the threshold are significant for the transmission of subclinical IMI. This is proof of concept that a transmission model using EMR can be used for surveillance of subclinical IMI episodes during lactation at different levels of specificity.

DISCUSSION

The work in this thesis has shown that EMR may be used to identify cows with an episode of subclinical IMI, and that the transmission dynamics of subclinical IMI may be modeled using EMR as a proxy for subclinical IMI in a dynamic transmission model.

Detection of subclinical IMI using EMR

None of the sensor systems used in dairy production, including the OCC sensor used in this thesis, currently operate at the desired level of 80% sensitivity and 99% specificity (ISO 20966:2007; Rutten et al., 2013). This is a challenge for animal welfare regarding detection of new cases of clinical mastitis, as this condition should be identified and treated as quickly as possible. Therefore, current sensor systems cannot be used as the sole approach for detection of cases of clinical mastitis in dairy production. However, for episodes of subclinical IMI, we are more concerned with controlling the prevalence of the relevant pathogens at a low level, rather than immediate detection and treatment of all cases. Therefore, use of sensor systems may be rewarding in management of subclinical IMI, despite moderate diagnostic test properties.

A practical consequence of the limited sensitivity and specificity achieved using EMR as a diagnostic test in this work, is that human involvement is essential in the optimal management of udder health in AMS. At the current performance level of the EMR, there is also a large difference between detection and diagnosis. If the diagnostic test properties are suboptimal, the system can only be used for detection of cows that potentially have subclinical IMI. In such settings, secondary investigations and testing must be performed to diagnose a subclinical IMI. This could be, for example, a physical checkup along with bacteriological culture of QMS of cows with sensor-system alerts. However, the use of several tests for the same condition introduces a challenge for the combined interpretation of both tests. If diagnosis of a subclinical IMI is based on the results from both tests being positive, then this is series testing. If, however, a positive result from either one of the tests is sufficient for diagnosing subclinical IMI, then this is parallel testing. In general, series testing decreases sensitivity and increases specificity, whereas parallel testing increases sensitivity and decreases specificity (Dohoo et al., 2009). Therefore, in order to be sure that the additional testing

contributes to successful dairy-health management, guidelines on the interpretation of results from multiple tests should be described in the herd-health management plan.

We decided to evaluate 4 different test thresholds for detection of subclinical IMI using EMR. The reason for using this approach, rather than identifying a single threshold with maximum sensitivity and specificity combined, was to demonstrate the possibility of using different thresholds for different management purposes.

Detection of subclinical IMI using the EMR is relatively straightforward when comparing cows with episodes of, for example, *Staphylococcus aureus*-subclinical IMI with true negative cows (Paper I). However, there will always be a mixture of cows, with some with subclinical episodes or transient colonization with other mastitis pathogens. These cows blur the picture, meaning that strict definitions cannot be easily applied, and thereby the diagnostic test properties of the EMR are diminished. Therefore, we decided to group the bacteria into two groups, Pat 1 and Pat 2. This enabled us to detect episodes of Pat-1 subclinical IMI using EMR (Paper I). However, the detection was rather nonspecific, and secondary testing is necessary to identify those specific bacteria that are presenting the challenge in udder-health management of individual cows and farms. Although the approach in this study can be used to improve detection of subclinical IMI in herds with AMS, more work should be done on pathogen-specific detection of subclinical IMI using sensor systems. With pathogen-specific detection of subclinical IMI, management could be tailored for each individual cow and herd. This would facilitate the progression of udder-health management into PLF.

In this study, the EMR developed by Sørensen et al. (2016) was used for detection of IMI with the OCC sensor. The reason that we chose an already established algorithm was to avoid overfitting of the algorithm to the data from our 17-month field study. Also, it enabled investigation into the diagnostic test properties of the OCC sensor alone, using the EMR to extract information from the OCC values. It has been suggested that little improvement is to be expected from adding more sensors (Hogeveen et al., 2010). However, improvements have recently been made by combining data from several sensors for detection of clinical mastitis cases (Khatun et al., 2018). It is likely that a new algorithm, combining information from several sensors, could improve on

our results. However, we would have needed several farms in our study to validate the results from such an algorithm.

Causes of variation in OCC values from milking to milking and from day to day

The moderate diagnostic test properties of using EMR to detect subclinical IMI are not surprising. As we show in Paper II, only 15% of the variation in OCC values is explained by subclinical IMI, and other fixed effects. However, the fixed and random effects (cow and lactation within cow) together described 55% of the milking-to-milking variability of OCC. Still, 45 % of the variation in OCC values is not explained, and we concluded that this is most likely due to physiological or normal variability. Hence, the sensor information is not a perfect proxy for biological status, and we cannot expect perfect diagnostic test properties from an OCC-based detection system of subclinical IMI.

One practical consequence of the moderate performance level of current sensor systems, is that there are many false sensor-system alerts and the farmer is therefore obliged to undertake several needless checks of a number of cows every day. This is also the case when using EMR to detect subclinical IMI. In order to avoid this unnecessary work, the number of false sensor-system alerts should be minimized (Claycomb et al., 2009). This will, in turn, usually reduce the sensitivity of the sensor system, due to the tradeoff between sensitivity and specificity for imperfect tests. Therefore, the test threshold should be set depending on the desired application of the test results. On this basis, sensor systems probably need to include several algorithms, each adapted to a different management need of the farmer. This will allow tailoring of algorithms to provide decision support for specific management questions, despite the sensor system not being perfect.

Transmission model of subclinical IMI using EMR as a proxy for infection

The challenge of suboptimal diagnostic test properties using EMR as a proxy for infection is exacerbated by the high number of detection events in AMS. Although the EMR aggregates information from several milkings to some extent, the specificity

would have to be very high to minimize the number of false sensor-system alerts if the EMR is evaluated at every milking (Table 2). One way to address the suboptimal diagnostic test properties of the EMR, is to reduce the number of detection events. If the period of interest is reduced to once per lactation (e.g., before dry off), then the sensitivity can be maximized at the expense of lower specificity (Table 2). In such a system, the proportion of false alerts is at the same high level, but the total number of false alerts is tolerable. For detection during lactation, the specificity should be maximized to avoid an overwhelming number of false alerts. The practical implication of this is that such a sensor system will detect fewer subclinical IMI episodes during lactation, but the episodes detected will probably be true episodes of subclinical IMI that warrant secondary testing (serial testing) and, potentially, therapy, culling, or segregation from the herd. For surveillance at the herd level, however, it is not important which individual cow is infected or uninfected, but rather the current prevalence of subclinical IMI, and what it is likely to be in the future. Simulations, based on parameters for transmission and cure rate based on OCC-data (EMR), can be used to generate predictions for the future herd prevalence of subclinical IMI. The premise of this approach is that even though the individual status of the cows is somewhat imprecise, a reliable prediction of the herd-level prevalence will, on average, be obtained. A change in the prediction of future subclinical IMI prevalence may thus be indicative of a future change in the herd-level udder-health status. In such a system, the sensitivity can be increased for the purpose of transmission modelling, as the farmer will not be notified of individual cows that have values above the alert threshold. Instead, the farmer will be alerted when the prediction of the future prevalence shows a negative trend or exceeds the farm-specific udder-health goals. As we showed in Paper IV, both the current and a simulated future cure rate may be described, along with the other transmission parameters, using dynamic simulation modelling based on data from an OCC sensor. The advantage of using OCC data is that herd specific transmission parameters, and thus the dynamic simulation model, can be updated daily. In paper III, we used *Corynebacterium* spp., which are bacteria known to cause persistent subclinical IMI, as the infectious pathogen to establish this model. And we noticed that despite similar management routines in the two farms, the cure rate was significantly different. This underscores the importance of herd specific modelling approaches.

Our application of changes in the EMR at the herd level indicates that the EMR can be used efficiently, despite suboptimal diagnostic test properties at the cow level. Through modeling EMR, these patterns can be predicted as a proxy for the future subclinical IMI prevalence in the herd. These predictions can be made for a prolonged period and be included in a decision-support tool to alert farmers when udder-health management actions against future subclinical IMI episodes are required. This will prove valuable, as management of udder health is particularly focused on preventing new cases (Ruegg, 2017). With herd-specific evaluation of subclinical IMI transmission parameters, management actions can be directed towards preventing a future increase in IMI episodes. In such a system, the concern is not about which particular cows have a subclinical IMI, but the focus is on the future prevalence of subclinical IMI in the herd. Such a sensor system could be integrated as a part of a health-management cycle (Kelton, 2006). This iterative process of continued improvement can be visualized as a part of the previously mentioned Deming circle, where sensor-system alerts indicate the need to "Check" (Figure 10). If the future trend is negative, the farmer should "Act" to prevent this. This could then lead to a change in "Plan" for future udder-health management. If there are no new alerts, the farmer carries out the established plan to manage udder health ("Do"). Moreover, in the "Act" and "Plan" phases, the transmission model can be evaluated to indicate the management area in which actions and new plans should be made. An example of this could be that the sensor system alerts the farmer of a predicted increase in the future subclinical IMI prevalence. The system indicates that the transmission parameters that are having most effect at driving this increase are the number of infected animals and the total number of animals. Suggested actions could then be either to reduce the number of infected animals, to decrease transmission (e.g., teat dipping), or to reduce the stocking density. When applicable, the farmer could also change the herd-health plan to maintain a lower stocking density in the future. The question regarding which cows are infected can then be left for other management-decision moments, (e.g., selection of cows for secondary testing and potential dry-cow therapy).

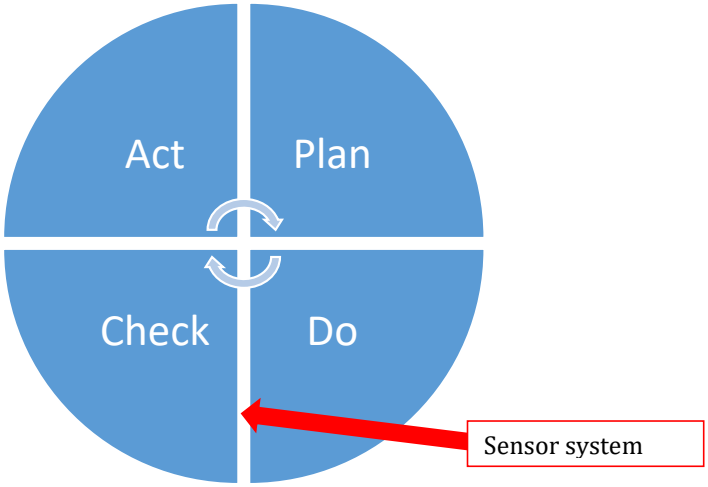

Figure 10. The Deming circle with a sensor system. Each herd has a herd-specific udder-health management plan ("Plan"). The farmer will execute this plan ("Do"). Whenever there is a sensor system alert ("Red arrow"), the farmer should investigate this and (potentially) perform secondary testing ("Check"). Depending on the findings from the investigation, the farmer must either take actions to optimize udder health ("Act") or go back to performing as planned ("Do"). When there is altered management, this can be implemented in a new udder-health management plan ("Plan").

When using models to explain and predict transmission dynamics, it is important to keep causality in mind. The mathematics need to be aligned with the real situation and biological possibilities. This is especially important in the use of algorithms, like the EMR, where there is a lot of pre-processing of OCC values before a parameterized product can be applied as a diagnostic test for detection of subclinical IMI.

Allore and Erb (1999) studied 3 approaches to modelling IMI in dairy cattle: Markov processes, discrete-event simulation, and differential equations. Each approach has its own advantages and limitations, and none of them are perfect. One advantage of the differential equation model is that its simplicity allows for estimation of sensitivity to changes in the different parameters of the model (Allore and Erb, 1999). That is, the model can be extended to evaluate which parameter has most effect on the changing

transmission rates. This could prove useful when evaluating the effects of possible management actions, such as treatment, culling, or segregation of animals, and in coupling this with cost-benefit considerations into the decision-support process. A disadvantage of the differential equation model is that longitudinal information from the population being investigated is needed to estimate the necessary parameters to run the model. However, this information is now available from the AMS, and differential equation models can therefore be implemented in the future management of udder health in AMS.

Limitations

The findings in this work are limited to one farm (Paper I, II, and IV) and two farms (Paper III) only. We believe that the amount and quality of our data means that our results are valid for the herds in our study. That is, the internal validity of our results is good. The limited number of herds is, however, a challenge to the external validity of our results. Therefore, caution is advised regarding extrapolation of our results to other herds. However, the aim of this work was to test the practical applicability of using the OCC sensor, rather than to generalize the results to a larger proportion of the population. We have shown that the EMR may be used for detection of subclinical IMI, but the thresholds identified in Paper I should be tested in other herds before widespread application. Indeed, Sørensen et al. (2016) found that the pattern of change in OCC values differed between herds. Although the EMR reduces some of this variation, the consequence still might be that EMR thresholds for subclinical IMI are farm specific.

In the 17-month longitudinal study (Papers I, II, and IV), we have monthly QMS and OCC data from every milking. Due to the prolonged period between each QMS, some true infections were probably missed. That is, the sensor system may have been correct in some cases, while the gold standard of the biological infectious status (based on monthly QMS) was wrong. Also, the onset of, and recovery from, infection is unlikely to have occurred midway between the QMS for all episodes. Using EMR, the duration of subclinical IMI was found to be 4 days (Paper IV). In order to evaluate whether this was related to a real change in subclinical IMI status or not, we would have needed QMS several times per week. However, despite these imperfect sampling

routines, we were able to demonstrate that the transmission of EMR above the thresholds could be modeled for subclinical IMI between cows ($R_0 > 1$).

When using the EMR to detect subclinical IMI, we are reducing the variation in the OCC values through a series of smoothing operations and parameterization of the smoothed values. The inference is that true changes in subclinical IMI status will still be detected. However, discarding variation may result in loss of information that could have proven useful for identifying the correct subclinical IMI status for individual cows. Potentially, this variation could have been picked up with more advanced big-data techniques (e.g., spatial or temporal analysis). Future studies should therefore attempt to keep more of the sensor data in the analyses, as this may improve the diagnostic test properties.

CONCLUSIONS AND FUTURE PERSPECTIVES

Detection of subclinical IMI using EMR

We have shown that the EMR can be used to detect subclinical IMI in dairy cows (Paper I). Despite suboptimal diagnostic test properties, a sensor system based on OCC values can provide useful information for udder-health management. When every milking is a detection event, specificity should be maximized. In contrast, for a single detection event (e.g., before drying off), a lower specificity is acceptable in order to maximize sensitivity.

Causes of variation in OCC values from milking to milking and from day to day

We demonstrated that a major reason for relatively moderate diagnostic test properties is that the subclinical IMI status explains only a moderate part of the variation in OCC values (Paper II). Thus, only moderate degrees of explanation should be expected from a subclinical IMI sensor system that is based on OCC values alone.

Transmission model of subclinical IMI using EMR as a proxy for infection

We developed an SIS model for transmission of *Corynebacterium* spp. IMI (Paper III). This model was then applied to the EMR, and we demonstrated that, using EMR, the model can be applied to predict the future prevalence of subclinical IMI in a herd based on simulations using a dynamic modelling approach (Paper IV). These predictions can be updated daily when using EMR for estimation of transmission parameters. This transmission model can, theoretically, also be extended to indicate which management area has most effect in driving an undesirable trend. More work on parameterization is necessary in order to use the model to assess the effects of different interventions, such as culling, treatment, and teat dipping. By combining different alerts, the sensor system can be adapted to the needs of individual farmers in the udder-health management on their farm.

Future perspectives

It is likely that future sensor development and research will improve the detection of subclinical IMI using sensor systems. The ability to combine data from different

sources and to retain variation for analysis in the final model will be important. Therefore, the key point of this study is the approach towards an efficient use of suboptimal sensor information, by aggregating sensor information by time. This was accomplished by introducing the EMR for subclinical IMI, and by modelling changes in EMR at herd level using dynamic transmission models.

Sensor-based decision-support tools are likely to improve farm management in the future. Such systems will guide farmers towards animals and management areas in need of attention. This, combined with suggested actions, will enable implementation of PLF to improve both animal welfare and farmer prosperity, as well as environmental and economic viability of future dairy farming.

Further development of transmission models for PLF application requires parameterization of the effects of relevant interventions (e.g., culling, treatment, teat dipping, teat sealant) at the herd level. The current thesis, may, however, be viewed as a first step towards testing the applicability of transmission models, using EMR as a basis for decision making at the herd level.

Potential follow-up projects

The period between QMS being taken for bacteriological culture was a challenge in this study. Therefore, a similar study on using OCC values for detection of subclinical IMI, but with a shorter time period between taking QMS for bacteriological culture, could provide more knowledge on the relationship between change in OCC-value patterns and subclinical IMI.

Parameterization of the effects of different management actions remains a challenge. Therefore, a study that parameterizes the effects of different management actions could contribute greatly to the development of automated decision support in PLF.

Another approach towards closing the knowledge gap could be to couple the data from sensor data in the AMS with DHI information on QMS bacteriological culture results on a larger scale. This way, numerous herds with OCC data from the AMS and bacteriological culture results registered in the DHI system could be included in a study to evaluate the use of OCC-value patterns as a proxy for subclinical IMI. In order to do this, we would need an efficient way to collect the sensor data from farms with

sensor systems and couple this with DHI information on QMS bacteriological culture results.

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Paper I

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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.

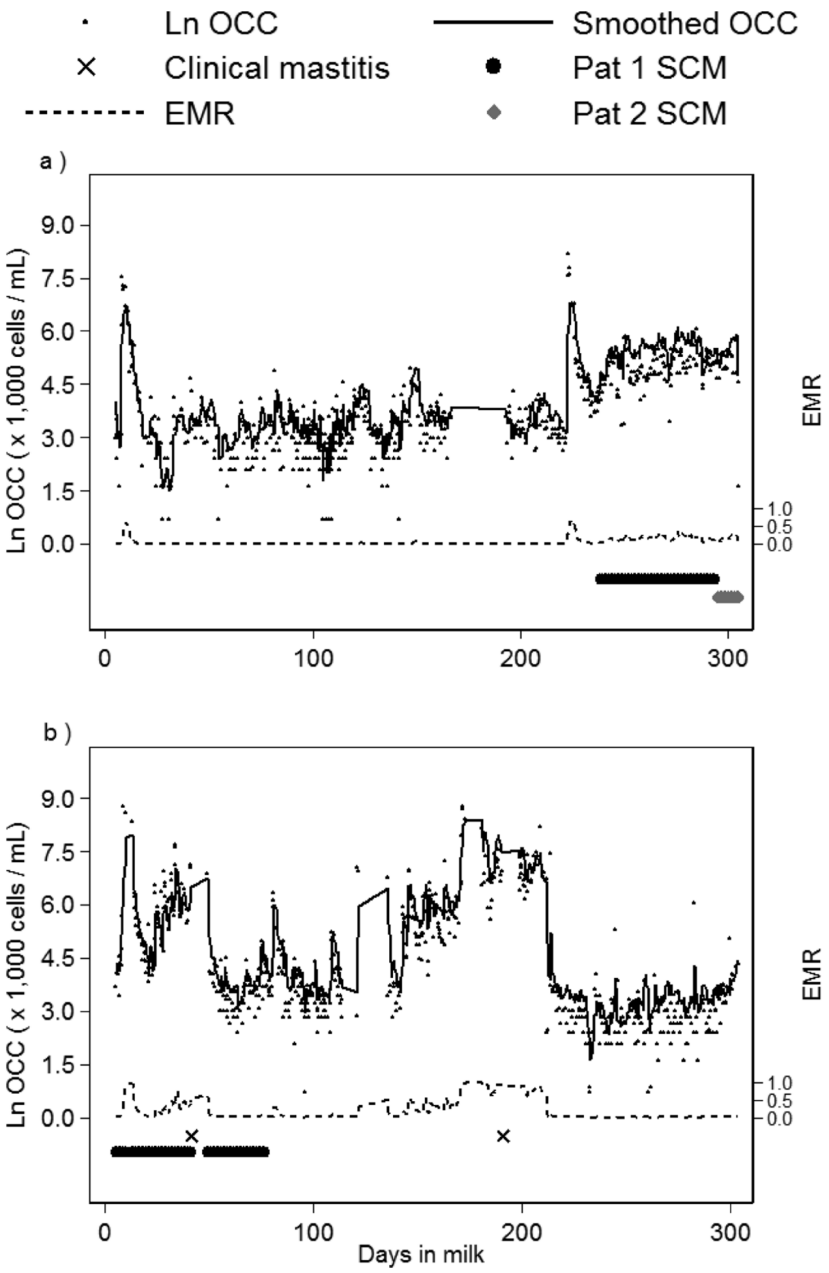

Figure 1. Examples of online cell count (OCC) values and mastitis status. The cow in (a) is a typical example of a cow with low OCC values until the onset of a case of Pat 1 subclinical mastitis (SCM). The Pat 1 SCM is followed by a case of Pat 2 SCM. This cow was not treated for clinical mastitis. The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

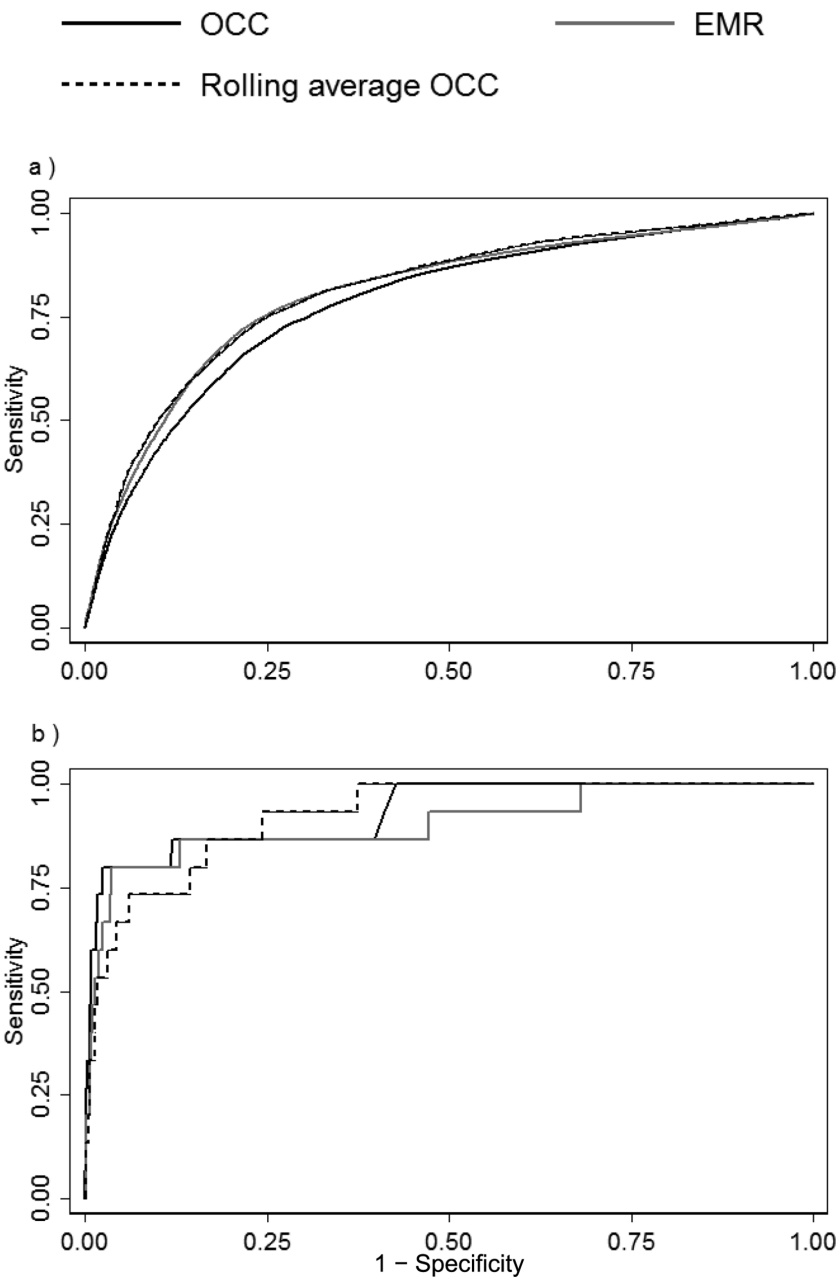

Figure 2. Receiver operating characteristic curves for detection of (a) Pat 1 subclinical mastitis and (b) new cases of clinical mastitis. The time windows for rolling averages are 7 d and 48 h for (a) and (b), respectively. EMR = elevated mastitis risk; OCC = online cell count. Pat 1 and Pat 2 = different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

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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.

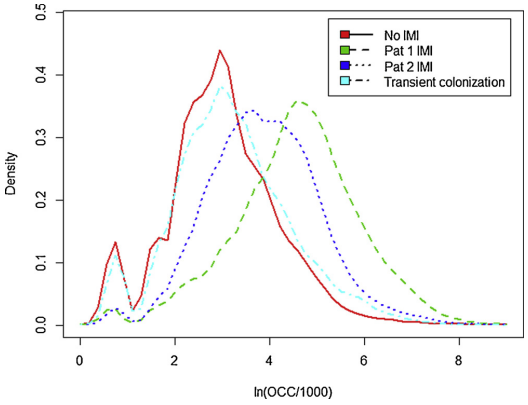

Fig. 1. Smoothed density plot showing the distribution of ln-transformed Online Cell Count (OCC) values (in 1000 cells/mL) in periods of 1) no intramammary infection (No IMI), 2) IMI with known mastitis pathogens from which a high somatic cell count would be expected (Pat 1 IMI), IMI with other known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

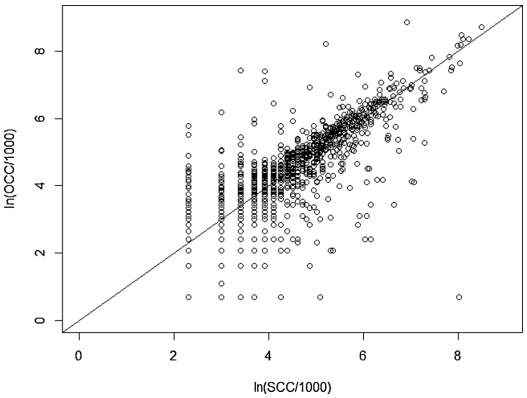

Fig. 2. On-line Cell Count (OCC) results plotted against Somatic Cell Count (SCC) measured in a DHI laboratory. Scatterplot including 1661 observations with corresponding OCC and SCC results with a superimposed 45° line representing the situation of perfect agreement between the two methods.

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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Paper III

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Transmission dynamics of intramammary infections caused by *Corynebacterium* species

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ABSTRACT

The development of reliable models for transmission of intramammary infections (IMI) is the subject of extensive research. Such models are useful to enhance the identification and understanding of factors that affect pathogen-specific IMI dynamics. Longitudinal transmission models are valuable for predicting infection outbreak risks, quantifying the effectiveness of response tactics, and performing response planning. In this work, we focused on modeling *Corynebacterium* spp. by using a compartmental model. Previous investigations have considered modeling the transmission dynamics of several bacterial pathogens, but not *Corynebacterium* spp. We established a *Corynebacterium* spp. Susceptible–Infectious–Susceptible (SIS) model. We simulated the model numerically by using parameters that we estimated by a generalized linear model approach, using month of study as the time variable. The data, from which the parameters of the model were estimated, were obtained in a field trial conducted in 2 US dairy herds. Altogether, 786 cows were sampled at least once during the 13-mo study period. The total number of quarter milk cultures and cases of IMI caused by *Corynebacterium* spp. were 11,744 and 556, respectively, in farm A; the corresponding figures for farm B were 11,804 and 179. Our modeling study included only transmission from persistent IMI caused by *Corynebacterium* spp. within the lactation pens. The rate of new infections was significantly related to preexisting IMI in both farms, underscoring the importance of preexisting *Corynebacterium* spp. IMI for the transmission of *Corynebacterium* spp. within lactation pens. The estimated basic reproduction numbers (R_0) in the 2 farms were 1.18 and 0.98, respectively. The nonsignificant disparity in R_0 was associated with significant differences in cure rates between farms.
Key words: intramammary infection, *Corynebacterium* spp., transmission model

INTRODUCTION

Mastitis is one of the economically most important diseases in dairy production (Halasa et al., 2007; Hogeveen et al., 2011). Much of the economic loss is due to reduced milk production following subclinical mastitis (Hogan et al., 2016). Intramammary infections with *Corynebacterium* spp. are generally mild with limited milk production loss. However, significant elevations in SCC have been observed (Brooks et al., 1983; Brooks and Barnum, 1984a). Although *Corynebacterium* spp. are classified as minor pathogens (Brooks and Barnum, 1984b; Harmon, 1994; Blagitz et al., 2013), the increased prevalence of *Corynebacterium* spp. IMI in some modern dairy farms (Pitkälä et al., 2004) warrants further investigation into the specific properties and roles of the bacteria.

Some authors have reported a protective effect of *Corynebacterium* spp. IMI against IMI caused by other pathogens (Rainard and Poutrel, 1988; Lam et al., 1997a), whereas others report an increased risk of mastitis (Pankey et al., 1985; Berry and Hillerton, 2002; Parker et al., 2007). When investigating the relationship between secondary infections and a preexisting IMI by *Corynebacterium* spp., Parker et al. (2007) suggested that the diverging effects reported for *Corynebacterium* spp. IMI were due to the increased disposition for clinical mastitis of glands with a preexisting IMI. There is also evidence that *Corynebacterium bovis* can colonize the teat canal without affecting the udder past Furstenberg's rosette (Black et al., 1972).

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Mathematical models are powerful tools for understanding infection dynamics by providing predictions about the potential transmission of infections and the effectiveness of control measures (Magal and Ruan, 2008; Otto and Day, 2011). Pathogen-specific transmission patterns have been described for other major and minor mastitis pathogens (Lam, 1996; Zadoks et al., 2002; White et al., 2006; Reksen et al., 2012; Barlow et al., 2013), but not for *Corynebacterium* spp. The basic reproduction number, $\boldsymbol{R_0}$, is used in compartmental transmission models to determine transmission of a disease at the population level. It is defined as the number of secondary cases that one infectious case can produce if introduced into a susceptible population (Grossman, 1980; Diekmann et al., 1990; Hethcote, 2000). Modeling the progression of a disease depends on appropriate parameter values that are often unknown and must be estimated from field data. In this study, we have used a generalized linear model for parameter estimation. The parameters estimated were used in a deterministic state-transition model to describe the transmission dynamics of *Corynebacterium* spp. from preexisting IMI within lactation pens.

The main aim of this study was to develop a novel mathematical description of the transmission dynamics of *Corynebacterium* spp. IMI. Specifically, we first wanted to assess the importance of preexisting IMI by *Corynebacterium* spp. on new IMI caused by this group of bacteria. Second, we wanted to compare transmission parameters and cure rates for *Corynebacterium* spp. IMI between 2 US dairy farms with differing prevalences of *Corynebacterium* spp. IMI.

MATERIALS AND METHODS

Field Study

Data were obtained from a 13-mo longitudinal observational study in 2 commercial Holstein dairy herds (one in New York and one in Vermont). Cows were housed in pens of approximately 100 cows and milked 3 times per day. In farm A, the monthly mean number of lactating cows was 319, the mean milk production per cow per day was 32.7 kg, and the average cow composite SCC was 404,000 cells/mL. In farm B, the monthly mean number of lactating cows was 346, the mean milk production per cow per day was 35.0 kg, and the average cow composite SCC was 292,000 cells/mL. The herds participated in a DHIA program with monthly milk quality testing. Both farms had reliable identification of animals and used standardized mastitis control practices, including pre- and postmilking teat disinfection and blanket dry-cow therapy. Further details on the herds, microbial analyses, and sampling framework have been published previously (Reksen et al., 2012; Barlow et al., 2013).

Quarter milk samples were collected monthly from approximately 200 lactating cows on each farm. Additional samples were collected within 3 d after parturition and when animals were moved to or from the lactation compartment.

Trained field technicians collected the scheduled monthly samples. Selected farm personnel, who had received training for this, obtained the additional samples. All samples were collected according to recommended guidelines (Hogan et al., 1999). Samples collected monthly were kept on ice after collection and during transport to the laboratory, where they were frozen before microbiological analyses. Additional samples collected by farm personnel were frozen immediately after collection. Samples were thawed in the laboratory and bacteriological culture was performed according to standard procedures (Hogan et al., 1999). Samples with culture results presenting more than 3 morphologically different colony types were treated as contaminated and excluded from further analyses.

A quarter was considered to have an IMI with *Corynebacterium* spp. when meeting at least one of the following criteria: (1) $\geq$1,000 cfu/mL of the pathogen were cultured from a single sample, (2) $\geq$500 cfu/mL of the pathogen were cultured from 2 out of 3 consecutive milk samples, (3) $\geq$100 cfu/mL of the pathogen were cultured from 3 consecutive milk samples, or (4) $\geq$100 cfu/mL of the pathogen were cultured from a clinical sample (Zadoks et al., 2002). A case was considered clinical when there was abnormal milk, with or without pain or swelling in the udder, or systemic signs such as anorexia, lethargy, or elevated rectal temperature (Harmon, 1994). Positive bacterial cultures that did not meet any of the above criteria were classified as representing a transient colonization with *Corynebacterium* spp.

Statistical Analysis

Statistical analysis was conducted using SAS software (version 9.1; SAS Institute, Inc., Cary, NC). Transmission parameters (β) and cure rates (α) were calculated using the generalized linear model approach (PROC GENMOD). Evidence of overdispersion was evaluated and models were subsequently adjusted using an overdispersion parameter estimated from the ratio of the Pearson Chi-squared estimate divided by the remaining degrees of freedom (Pscale option).

The transmission parameter (β) was estimated in a linear model with number of new *Corynebacterium* spp. IMI events in each monthly interval (I_M) as the outcome; S = quarter-days in a susceptible udder, I =

quarter-days infected, N = total quarter-days in each interval (study month), β^* is the intercept in the equation $\ln\left(I_M\right) = \beta^* + \ln\frac{SI}{N}$, and the transmission coefficient β is expressed as e^{β}. A log link, assumption of a negative binomial distribution, and offset $\ln\frac{SI}{N}$ (Zadoks et al., 2002) were used. Wald 95% confidence limits were used to compare transmission parameters between farms. To evaluate the effect of an existing *Corynebacterium* spp. IMI on transmission dynamics, we compared the fit of a model with the complete offset term included and a model without the term I/N included in the offset by comparing the 2×log-likelihood ratios.

The cure rate (α) was estimated with number of cured quarters from *Corynebacterium* spp. IMI events in each monthly interval (C_M) as the outcome. A log link, assumption of a negative binomial distribution, and offset ln (I) (Zadoks et al., 2002) were used; I = quarter-days infected in each monthly interval (study month), and α is the intercept in the equation $\ln(C_M) = \alpha + \ln I$, where C_M = cured *Corynebacterium* spp. IMI events in each monthly interval, and the cure rate, α, is expressed as e^{α}. Wald 95% confidence limits were used to compare cure rates between farms.

The population level transmission dynamics were further evaluated by the basic reproduction number, R_0. The expression of R_0 is given by $R_0 = \frac{\beta}{\mu+\alpha}$, where μ is the observed rate of entry and exit of quarters to and from the lactation compartment, and the inverse of the cure rate (α) is the duration of infection. A confidence interval for R_0 was calculated using 1.96 × the standard error obtained from log-transformations of the monthly R_0 expressions.

Model Formulation

The transmission dynamics of *Corynebacterium* spp. were explored by developing a Susceptible–Infectious–Susceptible (**SIS**) model. The model describes a population of lactating udder quarters divided into 2 compartments: (1) S denotes susceptible quarters with no *Corynebacterium* spp. IMI, and (2) I denotes quarters affected with IMI caused by *Corynebacterium* spp., where the compartments represent the proportion of lactating quarters in each state. The dynamics of state transitions are illustrated in Figure 1, and the model is described mathematically by the following nonlinear ordinary differential equations (**ODE**):

$$\frac{\mathrm{d}S}{\mathrm{d}t} = -\beta SI + \alpha I + \theta_S N\mu - \mu S, \qquad [1]$$

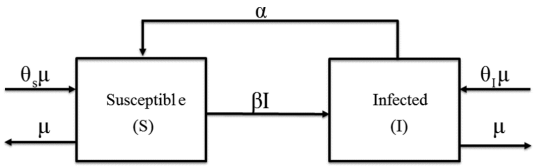

Figure 1. Schematic representation of the mathematical model of transmission of IMI with *Corynebacterium* spp. The boxes represent the state variables and the arrows represent the flow rates between susceptible (S) and infected (I) states. β = transmission parameter; βI = daily rate of new infections; α = daily rate of cured quarters; μ = daily rate of entry and exit of lactating quarters. The proportion of quarters into the S and I compartments are determined by θ_S and θ_I, respectively.

$$\frac{\mathrm{d}I}{\mathrm{d}t} = \beta SI - \alpha I + \theta_I N\mu - \mu I, \qquad [2]$$

where the interaction between the classes is quantified by the parameters α and β. The parameter β denotes the transmission of infection from a quarter with an IMI caused by *Corynebacterium* spp. to a susceptible quarter (Keeling and Rohani, 2011). The parameter α describes the daily rate of cured quarters, and N represents the sum of susceptible and infected quarters in the study at any given time. The daily rate of entry and exit of quarters to and from the lactation compartments is described by μ. Entries of quarters from the fresh pen to the different compartments within the lactation pen are determined by the proportions θ_S and θ_I.

The numerical resolution of the nonlinear ordinary equations of the SIS model was solved numerically by using a nonlinear programming solver of Matlab (MathWorks, Natick, MA), "ode45" solver, which is based on the Runge-Kutta method (Dormand and Prince, 1980). The numerical values of the parameters of the ODE of the SIS model, used in the numerical simulations, were obtained from the statistical analysis.

RESULTS

Field Study

In farm A, 11,744 milk samples were collected from a total of 371 cows. Among these, udder pathogens were cultured and identified in 5,021 samples. The distribution of bacterial culture results is given in Table 1. According to our definition of IMI, there were 556 *Corynebacterium* spp. IMI episodes during the study period, from 1,183 positive cultures; the remaining 1,618 positive samples were defined as transient colonizations. Of the 556 IMI episodes, 465 (84%) and 200 (36%) were associated with one or more samples having

Table 1. Distribution of microbiological diagnoses among samples positive for one or more udder pathogens

	Proportion (%)	
Culture result	Farm A	Farm B
Corynebacterium spp.	39.5	23.4
CNS	39.0	48.2
Streptococcus spp.	15.3	21.5
Staphylococcus aureus	2.7	0.9
Coliforms	1.4	4.8
Trueperella pyogenes	0.4	0.3
Streptococcus agalactiae	—	—
Other	1.7	0.9

≥1,000 or ≥5,000 cfu/mL, respectively. The distribution of quarter samples according to this categorization is shown in Table 2. Among IMI episodes, 3 cultures of *Corynebacterium* spp. were isolated in association with clinical cases. These were all co-infections with other minor pathogens and were not treated. Bacteriological milk culture was performed before and after the dry period in 471 quarter samples. Of these, 37 quarters were dried off while harboring a *Corynebacterium* spp. IMI. At the start of the next lactation, 36 of those were cured and 1 IMI persisted. Out of 434 quarters dried off without a *Corynebacterium* spp. IMI, 31 quarters were infected during the dry period.

In farm B, 11,804 milk samples were collected from a total of 415 cows. Among these, udder pathogens were cultured and identified in 3,528 samples. The distribution of bacterial culture results is given in Table 1. According to our definition of IMI, there were 179 *Corynebacterium* spp. IMI episodes during the study period from 255 positive cultures; the remaining 816 positive samples were defined as transient colonizations. Of the 179 IMI episodes, 147 (82%) and 15 (8%) were associated with one or more culture results with more than 1,000 and 5,000 cfu/mL, respectively. The distribution of quarter samples according to this categorization is shown in Table 2. Among IMI episodes, no cultures were isolated in association with clinical cases of *Corynebacterium* spp. IMI. Bacteriological milk culture was performed both before and after the dry period in 506 quarter samples. Of these, 13 quarters were dried off while harboring a *Corynebacterium* spp. IMI. At the start of the next lactation, all 13 quarters were cured. Out of 493 quarters dried off without *Corynebacterium* spp. IMI, 3 quarters were infected during the dry period.

Estimation of Transmission Parameters

From the statistical analyses, we obtained the following values for farm A. The transmission parameter, β, was 0.0188 (95% CI: 0.0159–0.0222), the cure rate, α, was 0.0122 (95% CI: 0.0098–0.0152), the daily rate of udders leaving and entering the lactation pen, μ, was 0.0039 (95% CI: 0.0027–0.0050), and R_0 was 1.1767 (95% CI: 0.9269–1.5760).

The difference in 2×log-likelihood between the model predicting number of new IMI with $\ln\frac{SI}{N}$ used as the offset term and the model with only $\ln S$ as the offset was 138.9. With 1 df, the Chi-squared statistic predicted a highly significant effect of an existing IMI with *Corynebacterium* spp. on the transmission of the bacteria from infected to susceptible quarters ($P < 0.001$).

The proportion of infected by days of study, as obtained from the raw data, is presented in Figure 2. This curve shows the evolution of the infection throughout the study period. The prevalence of infection began to increase after 215 d of study.

From the statistical analyses, we obtained the following values for farm B. The transmission parameter, β, was 0.0239 (95% CI: 0.0197–0.0291). The cure rate, α, was 0.0202 (95% CI: 0.0161–0.0253), the daily rate of udders leaving and entering the lactation pen, μ, was 0.0040 (95% CI: 0.0029–0.0051), and R_0 was 0.9879 (95% CI: 0.6632–1.4846). The 95% CI for α did not overlap between farms, whereas the corresponding CI for β, R_0, and μ were not different between farms.

The difference in 2×log-likelihood between the model predicting number of new infections with $\ln\frac{SI}{N}$ used as the offset term and the model with only $\ln S$ as the offset was 7.27. With 1 df, the Chi-squared statistic predicted a significant effect of an existing IMI with

Table 2. Number of samples with positive *Corynebacterium* spp. culture results

	Corynebacterium spp. positive		*Corynebacterium* spp. IMI	
Count (cfu/mL)	Farm A	Farm B	Farm A	Farm B
≥5,000	218	16	218	16
≥1,000 and <5,000	330	140	330	140
≥500 and <1,000	409	298	158	53
≥100 and <500	1,844	617	477	46

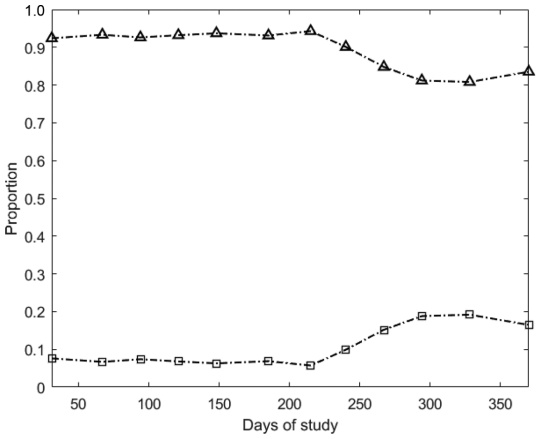

Figure 2. Proportion of quarters in farm A harboring an IMI (I; □) with *Corynebacterium* spp. and susceptible quarters (S; Δ) throughout the study period.

Corynebacterium spp. on the transmission of the bacteria from infected to susceptible quarters ($P < 0.01$).

The proportion of infected quarters by time, as obtained from the raw data, is presented in Figure 3. This curve shows the evolution of the infection throughout the study period. The prevalence of infection on farm B may be characterized as uniformly low throughout the study period.

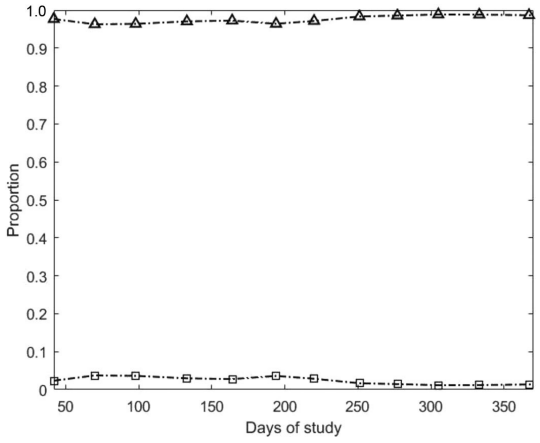

Figure 3. Proportion of quarters in farm B harboring an IMI (I; □) with *Corynebacterium* spp. and susceptible quarters (S; Δ) throughout the study period.

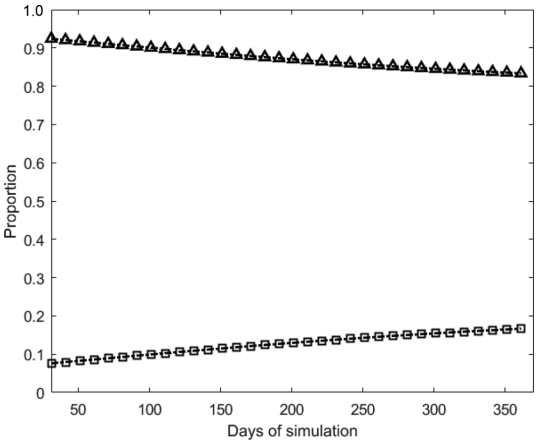

Figure 4. Simulation of the proportion of quarters in farm A harboring an IMI (I; □) with *Corynebacterium* spp. and susceptible quarters (S; Δ) using the Susceptible–Infectious–Susceptible (SIS) model. The values after initialization were $I_0 = 0.07613$ and $S_0 = 0.9239$.

Numerical Simulations

The proportions of I and S quarters from the dynamic simulation for farm A are presented in Figure 4. The proportion of I quarters increased throughout the simulation period, reaching a prevalence of 16.7% at 361 d on farm A. Figure 5 shows the proportion of I and S quarters on farm B. The proportion of I quarters was uniformly low throughout the simulation period on farm B.

DISCUSSION

By plotting the proportion of *Corynebacterium* spp. IMI by study days, we demonstrated an increase in the proportion of infected quarters from 215 d of study (December) and onward in farm A. We did not demonstrate a similar increase in the rate of new infections on farm B. For farm A, we obtained an R_0 of 1.18 that was not significantly different from the corresponding value for R_0 (0.98) on farm B. However, the number of IMI by *Corynebacterium* spp. developed differently between the 2 farms throughout the study period. Although the transmission of a pathogen is described by R_0 at the population level, it is the rate of both entry and exit of quarters, the transmission parameter, and the cure rate or duration of infection that determines the value of R_0. In our investigation, there was no significant difference between farms for the transmission parameters or the rates of entry and exit of quarters. However,

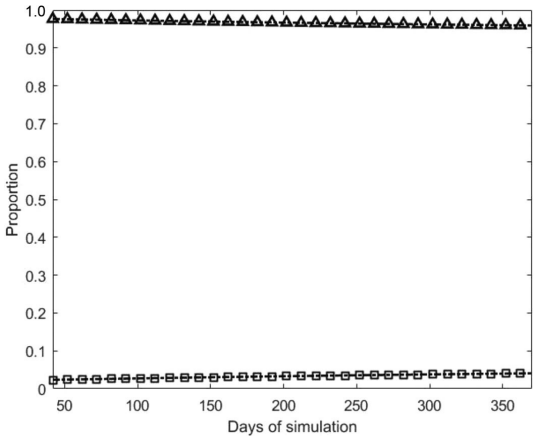

Figure 5. Simulation of the proportion of quarters in farm B harboring an IMI (I; □) with *Corynebacterium* spp. and susceptible quarters (S; Δ) using the Susceptible–Infectious–Susceptible (SIS) model. The values after initialization were $I_0 = 0.02346$ and $S_0 = 0.9765$.

we found that cure rates were significantly different between farm A and farm B. The lower cure rate in farm A increased the R_0 for this herd, which explains the steady increase in new infections caused by preexisting IMI with *Corynebacterium* spp. in this farm. Correspondingly, the significance of a preexisting IMI with *Corynebacterium* spp. was demonstrated for both farms when we compared models with and without an existing IMI included in the offset term. The association between preexisting IMI and new infections was highly significant in farm A.

Biologically, it is plausible to relate the duration of infection to immunological characteristics of the host, or the animal's ability to eliminate an infection. It is worth noting that the cure rate during the dry period was high, with only 1 of 37 *Corynebacterium* spp. IMI persisting from one lactation to the next on farm A, and none out of 13 on farm B. However, we cannot quantify the degree of self-cure because blanket dry-cow therapy was used in the study herds.

Transmission of *Corynebacterium* spp. IMI depending on preexisting IMI has not, to our knowledge, been documented previously. There may be many reasons for the observed increase in new infections in our study, but it is worth noting that the increase started in December, which is the beginning of winter in New York and Vermont. Seasonal factors may thus have contributed to an increase in the infectious transmission of udder pathogens, including more wet and cold udders, damper environments, and so on. Because our diagnostics were limited to classifying at the *Corynebacterium* spp. level, we cannot exclude a shift toward more contagious subtypes, resulting in an alteration of transmission characteristics of the bacterial population in farm A. The deterministic state transmission simulation model shows how the epidemic evolves in a population of cows over time. This model will be suitable for modeling the long-term effect of the transmission parameters on the herd prevalence of *Corynebacterium* spp. IMI, and for modeling the effect of prophylactic interventions.

Very few, if any, studies have attempted to quantify the infection dynamics of *Corynebacterium* spp. in dairy farms. However, observational studies have indicated that the prevalence of this minor pathogen is related to the quality of postmilking teat disinfection in dairy herds (Brooks et al., 1983; Harmon et al., 1986; Hogan et al., 1994; Lam et al., 1997b; Berry and Hillerton, 2002; Williamson and Lacy-Hulbert, 2013). In accordance with this, the present study showed that udder infections contribute significantly in the transmission of *Corynebacterium* spp. IMI. We observed 735 cases of IMI, 3 of which were from clinical cases. From the biological perspective, transient colonization does not necessarily equal IMI. Therefore, we limited our modeling to our definition of IMI.

It should be noted that the results we obtained were from 2 herds with different prevalences of *Corynebacterium* spp. IMI, despite being of similar size and having comparable management routines. We cultured *Corynebacterium* spp. from 23.9% of the quarter samples from farm A, but from only 9.1% of the quarter samples in farm B. The prevalence in farm A was relatively high compared with that reported in other publications (Brooks et al., 1983; Pitkälä et al., 2004; Green et al., 2005). In farm A, a higher proportion of the IMI episodes were associated with culture results having >5,000 cfu/mL than in farm B. This higher shedding level might contribute to an increased transmission potential on farm A. However, the estimated transmission parameter, β, did not differ between the 2 farms. Therefore, the observed difference in duration of infection and proportion of quarters shedding >5,000 cfu/mL might be attributable to host–pathogen factors associated with the ability of the cows to respond to, and clear, the infections. A study on *Salmonella* suggested that the prevalence of different infected states within or between herds could be due to a combined effect of host immunity, herd, and *Salmonella* serotype characteristics (Lanzas et al., 2008).

In the classic infectious disease epidemic SIR models (Anderson and May, 1991), the total population is divided into a susceptible compartment (S), an infected compartment (I), and a recovered compartment (R),

where recovered individuals are often considered to be resistant or removed from the susceptible population. In our modeling study, we adjusted the traditional SIR model with modifications specific to mastitis transmission in dairy herds, where cure and reinfection of individuals are observed, and it is assumed that recovery does not confer absolute resistance to reinfection. This could be described by an SIS model (Lam et al., 1996; White et al., 2001; Reksen et al., 2012), where the total number of quarters is divided into susceptible quarters (S) and infected quarters (I), assuming that susceptibility does not differ between naive individuals and recovered quarters.

CONCLUSIONS

The current study presents an investigation of transmission dynamics of *Corynebacterium* spp. IMI. The statistical analyses demonstrated that transmission of *Corynebacterium* spp. IMI in the 2 herds studied were influenced by preexisting *Corynebacterium* spp. IMI. In 1 of the 2 farms, the prevalence of *Corynebacterium* spp. IMI increased, consistent with an observed $R_0 > 1.0$ related to a low cure rate of *Corynebacterium* spp. IMI in this farm.

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Paper IV

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Dynamics of somatic cell count patterns as a proxy for transmission of mastitis pathogens

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ABSTRACT

Management of udder health is particularly focused on preventing new infections. Data from the DeLaval Online Cell Counter (DeLaval, Tumba, Sweden) may be used in forecasting to improve decision support for improved udder health management. It provides online cell counts (OCC) as a proxy for somatic cell counts from every milking at the cow level. However, these values are typically too insensitive and nonspecific to indicate subclinical intramammary infection (IMI). Our aim was to describe and evaluate use of dynamic transmission models to forecast subclinical IMI episodes using milk cultures or changes in OCC patterns over time. The latter was expressed by an elevated mastitis risk variable. Data were obtained from the dairy herd of the Norwegian University of Life Sciences (Oslo, Norway). In total, 173 cows were sampled monthly for bacteriological milk culture during a 17-mo study period and 5,330 quarter milk samples were cultured. Mastitis pathogens identified were assigned to 1 of 2 groups, Pat 1 or Pat 2. Pathogens from which a high cell count would be expected during a subclinical IMI episode were assigned to the Pat 1 group. Pathogens not in the Pat 1 group were assigned to the Pat 2 group. *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Streptococcus dysgalactiae* were the most common Pat 1 pathogens. *Corynebacterium bovis*, *Staphylococcus chromogenes*, and *Staphylococcus haemolyticus* were the most common Pat 2 pathogens. The OCC were successfully recorded from 82,182 of 96,542 milkings. The current study included 324 subclinical IMI episodes. None of the mastitis pathogens demonstrated a basic reproduction number (R_0) >1. Patterns of OCC change related to an episode of Pat 1 subclinical IMI at specificity levels of 80, 90, and 95% at sensitivity levels of 69, 59, and 48% respectively, demonstrated an R_0 >1. An existing infection was significant for transmission for several Pat 2 pathogens, but only for *Staphylococcus aureus* and *Staphylococcus epidermidis* among Pat 1 pathogens. Dynamic transmission models showed that patterns of OCC change related to an episode of Pat 1 subclinical IMI were significantly related to the same pattern occurring in susceptible cows at specificity levels of 80, 90, and 99% at sensitivity levels of 69, 48, and 8%, respectively. We conclude that changes in herd prevalence of subclinical IMI can be predicted using dynamic transmission models based on patterns of OCC change. Choice of specificity level depends on management goals and tolerance for false-positive alerts.
Key words: intramammary infection, transmission, somatic cell count, online cell count

INTRODUCTION

Management of udder health is particularly focused on preventing new infections (Ruegg, 2017). Common management approaches apply standard operating procedures using historical information (Østerås and Sølverød, 2009; Scherpenzeel et al., 2016), which yields slow-moving improvement. Therefore, real-time detection and management of transmission of subclinical IMI may improve management of udder health.

An IMI is defined as being present when a quarter is infected with a bacterial species (Berry and Meaney, 2006). In many but not all cases, an IMI may be identified based on an increase in SCC (Sargeant et al., 2001). This inflammatory response, often caused by an IMI, is defined as mastitis (Djabri et al., 2002). When clinical symptoms occurs, this is defined as clinical mastitis; when there is an increase in SCC but no clinical signs occur, this is defined as subclinical mastitis (IDF, 2011).

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To some extent, SCC values can be used for surveillance of IMI (Schukken et al., 2003). For reasons of surveillance and precision diagnostics, the industry has advanced toward developing sensors specifically for udder health. One of these sensors is the DeLaval Online Cell Counter (DeLaval International AB, Tumba, Sweden). Using this sensor, we can obtain repeated online cell counts (**OCC**) at the cow level. These data may be implemented in automated detection systems for management of udder health in automatic milking systems (**AMS**). Based on smoothed OCC data, Sørensen et al. (2016) created an elevated mastitis risk (**EMR**) indicator to detect cases of clinical mastitis. This EMR indicator is a continuous variable (from 0 to 1), where values close to 0 indicate a low risk of mastitis and higher values, approaching 1, indicate an increased risk of clinical mastitis (Sørensen et al., 2016).

Dalen et al. (2019) demonstrated that the EMR indicator can be used to detect subclinical mastitis episodes in individual cows. However, when every milking is a potential detection event, the diagnostic test properties are insufficiently sensitive for direct application in a decision-support tool (Dalen et al., 2019). Therefore, the interpretation and use of OCC during lactation should be improved in decision-support tools for dairy farmers.

Compartmental transmission models are powerful tools for understanding infection dynamics by providing predictions about the potential transmission of infections and the efficacy of control measures (Magal and Ruan, 2008; Otto and Day, 2011). Pathogen-specific transmission patterns have been described for major and minor mastitis pathogens (Zadoks et al., 2002; White et al., 2006; Reksen et al., 2012). However, these models have not previously been applied to patterns of OCC change associated with subclinical IMI episodes. By modeling the patterns of OCC change associated with subclinical IMI episodes, as a proxy for transmission of pathogens, the underlying infection pressure in the herd can be continuously monitored. When there is an increase in the prevalence of a particular pattern of OCC change, there are probably more cows in the herd that have the potential to transmit mastitis pathogens to susceptible cows. Forecasting future development of this transmission-associated pattern, by dynamic simulation modeling, could be used to determine whether actions should be taken to reduce transmission risk.

The primary aim of this study was to describe and evaluate the possibility of using dynamic transmission models to forecast the herd prevalence of subclinical IMI episodes by exploiting measured changes in OCC patterns over time. Specifically, we first wanted to estimate the transmission parameters of subclinical IMI episodes based on culture results and associated changes in OCC patterns expressed by the EMR. Second, we wanted to evaluate the effect of preexisting episodes on new subclinical IMI episodes defined by culture results or changes in the OCC patterns.

MATERIALS AND METHODS

Field Study

This study used data obtained during a 17-mo longitudinal observational study in the research herd at the Norwegian University of Life Sciences (Oslo, Norway). On average, 96 cows were milked 2.6 times a day in 2 identical AMS (Delaval VMS, DeLaval, Tumba, Sweden). Mean OCC and milk production per cow per day were 115,103 cells/mL and 27.9 kg, respectively. The farm used standardized mastitis control practices, such as post-milking teat disinfection, selective dry-cow therapy, and monthly milk quality testing in a DHIA program.

The 2 AMS were set to record OCC from every milking during the study period. Quarter milk samples (**QMS**) were collected monthly from all lactating cows, according to recommended sampling guidelines (Hogan et al., 1999). Samples were frozen and transported to the laboratory, where bacteriological culture was performed according to standard procedures (Hogan et al., 1999). Briefly, 0.01 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. We used a MALDI-TOF MS Microflex LT system (Bruker Corp., Billerica, MA; Cheuzeville, 2015) for species identification of cultured bacteria. Further details on the study herd, sampling framework, and microbial analyses were previously published (Dalen et al., 2019).

Subclinical IMI Status

In this study, we investigated subclinical IMI episodes only. Cows treated for clinical mastitis were transferred to a treatment pen without AMS, and we do not have bacteriological samples or OCC records throughout the period of treatment for the clinical mastitis cases. The diagnosis of subclinical IMI was based on bacteriological culture results or using OCC data as a proxy for bacteriological culture results. A cow was considered to have a subclinical IMI episode with an individual mastitis pathogen when meeting at least one of the following criteria: (1) $\geq$1,000 cfu/mL of a single mastitis pathogen cultured from a single sample in at least 1 quarter, (2) $\geq$500 cfu/mL of a mastitis pathogen cultured from 2 out of 3 consecutive milk samples from the

same quarter, or (3) ≥100 cfu/mL of a mastitis pathogen cultured from 3 consecutive milk samples from the same quarter. These definitions are adapted from those of Zadoks et al. (2002).

Because OCC is recorded at the cow level, and our aim was to model transmission of subclinical IMI based on both individual and grouped mastitis pathogens, as well as on patterns of OCC change, we aggregated the bacteriological diagnoses at the quarter level into cow-level diagnoses. Also, because the same cow could experience a subclinical IMI episode with different mastitis pathogens at the same time, 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 IMI episode was designated Pat 1, according to Dalen et al. (2019). The pathogens included in the Pat 1 group were *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). Mastitis pathogens that were not included in Pat 1 were grouped in the Pat 2 category. This included *Corynebacterium bovis*, *Staphylococcus chromogenes*, *Staphylococcus haemolyticus*, *Aerococcus viridans*, *Staphylococcus hominis*, *Staphylococcus xylosus*, and other mastitis pathogens (Dalen et al., 2019). A cow was considered to have a Pat 1 or Pat 2 subclinical IMI when one or more quarters were positive for a Pat 1 or a Pat 2 mastitis pathogen, respectively.

Because sampling was performed monthly, the exact time of infection and cure was not known. Therefore, we used the mid-point estimation method previously described by Zadoks et al. (2002) to calculate the infection period. We defined the start of the subclinical IMI episode as the middle of the time interval between a negative culture and the first positive culture event, and defined the end of the subclinical IMI episode as the middle of the time interval between the last positive culture event and the first negative culture for a quarter defined as cured (Zadoks et al., 2002).

OCC

Online cell counts were successfully recorded from 82,182 of 96,542 milkings (85%); the 14,360 missing values were due either to equipment failure or failure to service and refill the OCC unit with reagent. We computed EMR values (as described by Sørensen et al., 2016; Dalen et al., 2019) for all milkings. Statistical analyses were conducted using Stata (Stata SE/14, Stata Corp., College Station, TX). Briefly, the validity of all recorded OCC measurements were checked before logarithmic transformation. We included only milkings from 5 to 305 DIM with a milking interval of 4 to 24 h and a yield of ≥3.5 kg. Also, OCC values of 0 were omitted from further analyses. Lactation-specific OCC curves were calculated for first, second, and third and later lactations using Wood's lactation curve (Wood, 1967). For milkings with missing OCC data, the missing data were replaced with a value given by 95% of the previous value and 5% of the lactation-specific OCC curve for the cow at the DIM of the milking with missing data. This way, the OCC curve of cows with missing data approached the lactation-specific OCC curves by 5% for each milking where OCC was missing (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 according to 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 for every milking on a continuous scale from 0 to 1 (Sørensen et al., 2016). Because both the level and trend are used in calculation of the EMR, the underlying historic and current OCC values can be different in 2 cows with the same EMR value. Therefore, we use the term "OCC pattern" to describe the OCC changes associated with EMR values. Furthermore, we used the threshold values from Dalen et al. (2019) to assign cows to a subclinical IMI status, based on the OCC pattern given by the EMR value. Cows were classified as having a subclinical IMI when the EMR value was greater than the threshold. For the 4 patterns of OCC change, the EMR value thresholds were 0.03, 0.05, 0.08, and 0.62 at specificity levels of 80, 85, 90, and 99%. The corresponding sensitivities for each pattern of OCC change for detection of Pat 1 subclinical IMI were 69, 59, 48, and 8%, respectively (Dalen et al., 2019).

Transmission Parameters

The transmission parameter (β) was calculated using Poisson regression (Stata SE/14, Stata Corp.) with number of new episodes of subclinical IMI in each monthly interval (I_M) as the outcome, and offset $\ln\frac{SI}{N}$, where S = cow-days of a susceptible cow, I = cow-days infected, N = total cow-days in each interval (study month), and β^* is the intercept in the equation

$\ln(I_M) = \beta * + \ln\frac{SI}{N}$. The transmission coefficient β is expressed as e^{β}.

We estimated the cure rate (α) using Poisson regression (Stata SE/14, Stata Corp.). As outcome, the number of subclinical IMI episodes cured in each monthly interval (C_M) was used, with $\ln(I)$ as offset. Cure rate (α) is the intercept and I = cow-days infected in each monthly interval (study month) in the equation: $\ln(C_M) = \alpha + \ln I$, where the cure rate α is expressed as e^{α} and C_M = cured subclinical IMI episodes in each monthly interval.

Furthermore, we evaluated population-level transmission dynamics using the basic reproduction number, $\mathbf{R_0}$, which is given by the expression $R_0 = \frac{\beta}{\mu + \alpha}$. The observed rate of entry and exit of cows to and from the lactation pen is μ, and the duration of infection is the inverse of the cure rate (α).

Variance of R_0 was obtained using a log-transformation, where $\ln(R_0) = \ln\left(\frac{\beta}{\mu+\alpha}\right)$, and then further simplified to $\ln(\beta) - \ln(\mu+\alpha)$. The variance of $\ln(R_0)$ is then variance $\ln(\beta)$ + variance $\ln(\mu + \alpha)$, assuming no covariance between β and $\mu + \alpha$. The individual variances are obtained from the regression equations as described above. An estimate of the variance of R_0 is then obtained and the standard error (SE) by obtaining the square root of the resulting estimate. Subsequently, we used $\pm 1.96 \times$ SE to calculate a confidence interval for R_0.

Infection dynamics may be studied using the subclinical IMI status of cows, as defined above, or using patterns of OCC change, thereby assuming that patterns of OCC change indicate the presence of a subclinical IMI. Observations from the first 7 d of each cow were omitted for the calculation of transmission parameters for patterns of OCC change. This was done to allow "burn in" of the EMR status, because the EMR for every cow is, by default, initialized with the lactation-specific OCC curves of the herd (Sørensen et al., 2016), and this is likely to be too low for cows with subclinical IMI. Also, as cows were milked several times each day and the transmission models use cow-days as the time variable, only the first observation of the EMR per day was retained in the transmission model of changes in OCC pattern.

Transmission Models

We evaluated the transmission dynamics of the different mastitis pathogens separately and for the groups Pat 1 and Pat 2, as well as the 4 different patterns of OCC change. We modeled subclinical IMI episodes only. The transmission dynamics of the different pathogens, pathogen groups, and patterns of OCC change were displayed in a Susceptible-Infectious-Susceptible (**SIS**) model for each pathogen, pathogen group, and change in OCC pattern. The compartmental model describes a population of lactating cows divided into 2 compartments, where S denotes susceptible cows with no subclinical IMI, and I denotes cows with subclinical IMI, where the compartments represent the proportion of lactating cows in each state. Figure 1 illustrates the state transition dynamics.

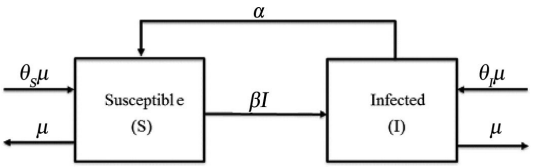

Figure 1. Schematic representation of the mathematical model of transmission of subclinical IMI. The boxes represent the state variables and the arrows represent the flow rates between susceptible (S) and infected (I) states. β = transmission parameter, βI is the daily rate of new infections, α = daily rate of cured cows; μ = daily rate of entry and exit of lactating cows. Proportion of cows entering the S and I compartments are determined by θ_S and θ_I, respectively.

The following nonlinear ordinary differential equations describe the model mathematically:

$$\frac{dS}{dt} = -\beta SI + \alpha I + \theta_S N\mu - \mu S, \quad [1]$$

$$\frac{dI}{dt} = \beta SI - \alpha I + \theta_I N\mu - \mu I. \quad [2]$$

The parameters α and β quantify the transfer rates, where the transmission rate of infection from a cow with subclinical IMI to a susceptible cow is described by β (Keeling and Rohani, 2011). The daily rate of cured cows is described by α. At any given time, the sum of susceptible and infected cows is represented by N. The parameter μ describes the daily rate of entry and exit of cows to and from the lactation pen. The proportions θ_S and θ_I describe cows entering the lactation pen from the fresh pen to the susceptible or the infectious compartment, respectively.

The difference in 2 $\times$ log-likelihood between the model predicting number of new episodes of subclinical IMI with $\ln\frac{SI}{N}$ used as the offset term and the model with only $\ln S$ as the offset was used to evaluate the effect of existing subclinical IMI episodes on the transmission from infected to susceptible cows. The differ-

ence was evaluated with chi-squared statistics with 1 df.

A nonlinear programming solver of Matlab (MathWorks, Natick, MA), "ode45" solver, was used to solve the nonlinear ordinary equations of the SIS model. This approach is based on the Runge-Kutta method (Dormand and Prince, 1980). The parameter values used in the numerical simulations of the SIS model were obtained from the statistical analysis.

RESULTS

Field Study

We collected 5,330 QMS from a total of 257 lactations in 173 cows. Each cow was sampled, on average, 8 times (range 1 to 16). The cows entered the study at an average of 38 DIM (range 4 to 269 DIM). Bacteria were cultured from 1,222 samples, with 1 and 2 pathogens cultured in 1,152 and 67 samples, respectively. According to our criteria for evaluation of contamination, 3 samples were excluded from the analysis. We recorded 16 veterinary treatments for clinical mastitis during the study period. Mastitis pathogens were found in 222 lactations in 155 cows. The most common pathogens found were *Staph. epidermidis*, *C. bovis*, *Staph. chromogenes*, *Staph. aureus*, and *Staph. haemolyticus*. A detailed overview of the distribution of microbial diagnoses can be found in Dalen et al. (2019). According to our definition of subclinical IMI, there were 324 subclinical IMI episodes during the study period.

Estimation of Transmission Parameters

From the statistical analyses, we obtained transmission parameter β, cure rate α, and daily rate of cows leaving and entering the lactation pen μ; based on these parameters, we calculated R_0 for each pathogen and pathogen group, and for the pathogen-proxy pattern of OCC change. The distribution of subclinical IMI episodes and the associated transmission parameters for the different pathogens, pathogen groups, and patterns of OCC change are shown in Table 1. None of the individual mastitis pathogens nor the grouped Pat 1 subclinical IMI or Pat 2 subclinical IMI were found to have an $R_0 > 1$. However, patterns of OCC change with a specificity of 80, 85, and 90% for Pat1 subclinical IMI had an $R_0 > 1$. The average duration of the subclinical IMI episodes, as given by the inverse of the cure rate α, is shown for each pathogen and group in Table 2. The duration of subclinical IMI episodes was significantly lower for the 4 patterns of OCC change than for the Pat 1 subclinical IMI episodes.

Table 1. Transmission parameters[1] for subclinical IMI with individual and grouped (Pat 1 and Pat 2) mastitis pathogens and for 4 online cell count (OCC) patterns with different levels of specificity for detection of Pat 1 subclinical IMI episodes

Subclinical IMI with pathogen, group, or pattern	N[2]	β (95% CI)	α (95% CI)	R_0 (95% CI)
Staphylococcus epidermidis	64	0.0088 (0.0063–0.0123)	0.0080 (0.0058–0.0111)	0.71 (0.45–1.14)
Corynebacterium bovis	70	0.0179 (0.0139–0.0231)	0.0074 (0.0052–0.0107)	1.53 (0.98–2.39)
Staphylococcus chromogenes	36	0.0024 (0.0012–0.0046)	0.0038 (0.0023–0.0062)	0.30 (0.13–0.67)
Staphylococcus aureus	33	0.0093 (0.0060–0.0142)	0.0078 (0.0050–0.0123)	0.76 (0.41–1.42)
Staphylococcus haemolyticus	22	0.0061 (0.0036–0.0106)	0.0062 (0.0037–0.0105)	0.58 (0.27–1.24)
Aerococcus viridans	21	0.0254 (0.0158–0.0408)	0.0203 (0.0121–0.0343)	1.03 (0.51–2.09)
Enterococcus faecalis, *Enterococcus faecium*, and *Lactococcus lactis*	12	0.0023 (0.0009–0.0061)	0.0022 (0.0008–0.0058)	0.35 (0.09–1.41)
Streptococcus dysgalactiae	25	0.0065 (0.0036–0.0117)	0.0077 (0.0045–0.0130)	0.54 (0.25–1.19)
Staphylococcus simulans	6	—	0.0046 (0.0017–0.0123)	—
Staphylococcus hominis	6	0.0294 (0.0132–0.0655)	0.0242 (0.0101–0.0582)	1.03 (0.31–3.38)
Streptococcus uberis	7	0.0030 (0.0007–0.0119)	0.0029 (0.0007–0.0116)	0.41 (0.06–2.93)
Staphylococcus xylosus	2	0.0043 (0.0036–0.0064)	0.0086 (0.0021–0.0342)	0.34 (0.03–3.71)
Other	20	0.0165 (0.0099–0.0273)	0.0139 (0.0080–0.0239)	0.91 (0.43–1.90)
Pat 1	106	0.0069 (0.0053–0.0091)	0.0048 (0.0036–0.0064)	0.76 (0.51–1.13)
Pat 2	147	0.0048 (0.0036–0.0064)	0.0070 (0.0056–0.0087)	0.43 (0.32–0.58)
Elevated mastitis risk (EMR) 80% specificity[3]	1,116	0.1368 (0.1310–0.1428)	0.0889 (0.0851–0.0928)	1.45 (1.36–1.54)
EMR 85% specificity[4]	1,051	0.1465 (0.1377–0.1559)	0.1039 (0.0976–0.1107)	1.34 (1.22–1.46)
EMR 90% specificity[5]	1,045	0.1768 (0.1662–0.1881)	0.1368 (0.1285–0.1456)	1.24 (1.14–1.36)
EMR 99% specificity[6]	261	0.2692 (0.2380–0.3045)	0.2575 (0.2275–0.2914)	1.02 (0.86–1.22)

[1]β = transmission parameter; α = daily rate of cured cows; R_0 = basic reproduction number.

[2]Number of subclinical IMI episodes.

[3]Pattern of OCC change with 80% specificity and 69% sensitivity for detection of Pat 1 subclinical IMI episodes.

[4]Pattern of OCC change with 85% specificity and 59% sensitivity for detection of Pat 1 subclinical IMI episodes.

[5]Pattern of OCC change with 90% specificity and 48% sensitivity for detection of Pat 1 subclinical IMI episodes.

[6]Pattern of OCC change with 99% specificity and 8% sensitivity for detection of Pat 1 subclinical IMI episodes.

The predicted relationships between a preexisting subclinical IMI or EMR threshold and subsequent cases of the same condition in other cows are shown in Table 3 for each pathogen and EMR threshold. For several mastitis pathogens, the number of existing infections had a significant effect on the transmission risk from an infected cow to a susceptible uninfected cow. However, the only Pat 1 mastitis pathogens among these were *Staph. aureus* and *Staph. epidermidis*.

For the patterns of OCC change, the EMR threshold at 80, 90, and 99% specificity for Pat 1 subclinical IMI had a significant effect on whether this pattern would subsequently arise in another cow ($P = 0.009$, 0.011, and 0.009, respectively).

Numerical Simulations

The proportion of cows with alerts for Pat 1 subclinical IMI based on the EMR are shown for the 4 levels of specificity by days of study, as obtained from the raw data in Figure 2. This curve shows the proportion of infected cows throughout the study period. The numerical simulations of the dynamics of I and S cows for each level of specificity are presented in Figure 2. Both the raw data and the dynamic simulations showed a stable transmission dynamic of the 4 patterns of OCC change in this herd throughout the study period. The numerical resolution of the ordinary differential equations describing the model can be used to generate predictions for any given time.

DISCUSSION

In this study, we propose using a transmission model based on frequent OCC measurements to forecast subclinical IMI dynamics at the herd level. By modeling patterns of OCC change, we are able to predict the evolution of OCC patterns, as a proxy for subclinical IMI, in the herd. We used EMR as described by Sørensen et al. (2016) for this purpose. Changes in the proportions of S to I can be simulated and forecast for a prolonged period. This approach could be included in a decision-support tool to alert farmers when udder health management actions against subclinical IMI are required during lactation and at drying off.

Based on research, significant improvements have been made in detection, management, and prevention of mastitis (Ruegg, 2017). We propose further improvement in prevention of new cases of mastitis by using herd-specific evolution of the different transmission pa-

Table 2. Average duration in days of infection with subclinical IMI for individual and grouped (Pat 1 and Pat 2) mastitis pathogens and for 4 online cell count (OCC) patterns with different levels of specificity for detection of Pat 1 subclinical IMI episodes

Subclinical IMI with pathogen	N[1]	Duration (95% CI)
Staphylococcus epidermidis	64	125 (90–172)
Corynebacterium bovis	70	135 (93–192)
Staphylococcus chromogenes	36	263 (161–435)
Staphylococcus aureus	33	128 (81–200)
Staphylococcus haemolyticus	22	161 (95–270)
Aerococcus viridans	21	49 (29–83)
Enterococcus faecalis, *Enterococcus faecium*, and *Lactococcus lactis*	12	455 (172–1,250)
Streptococcus dysgalactiae	25	130 (77–222)
Staphylococcus simulans	6	217 (81–588)
Staphylococcus hominis	6	41 (17–99)
Streptococcus uberis	7	345 (86–1,429)
Staphylococcus xylosus	2	116 (29–476)
Other	20	72 (42–125)
Pat 1	106	208 (156–278)
Pat 2	147	143 (115–179)
Elevated mastitis risk (EMR) 80% specificity[2]	1,116	11 (11–12)
EMR 85% specificity[3]	1,051	10 (9–10)
EMR 90% specificity[4]	1,045	7 (7–8)
EMR 99% specificity[5]	261	4 (3–4)

[1]Number of subclinical IMI episodes.

[2]Pattern of OCC change with 80% specificity and 69% sensitivity for detection of Pat 1 subclinical IMI episodes.

[3]Pattern of OCC change with 85% specificity and 59% sensitivity for detection of Pat 1 subclinical IMI episodes.

[4]Pattern of OCC change with 90% specificity and 48% sensitivity for detection of Pat 1 subclinical IMI episodes.

[5]Pattern of OCC change with 99% specificity and 8% sensitivity for detection of Pat 1 subclinical IMI episodes.

Table 3. Effect of an existing subclinical IMI episode or online cell count (OCC) pattern on the number of subsequent new events (transmissions of the same condition from infected cows to susceptible cows)[1]

Subclinical IMI with pathogen	N[2]	*P*-value of the fit of a model with $S \times I$ versus only S in the offset term
Staphylococcus epidermidis	64	<0.001
Corynebacterium bovis	70	<0.001
Staphylococcus chromogenes	36	0.172
Staphylococcus aureus	33	0.041
Staphylococcus haemolyticus	22	0.040
Aerococcus viridans	21	0.005
Enterococcus faecalis, *Enterococcus faecium*, and *Lactococcus lactis*	12	0.196
Streptococcus dysgalactiae	25	0.069
Staphylococcus simulans	6	—
Staphylococcus hominis	6	0.004
Streptococcus uberis	7	0.524
Staphylococcus xylosus	2	0.284
Other	20	0.016
Pat 1[3]	106	0.065
Pat 2[3]	147	0.063
Elevated mastitis rate (EMR) 80% specificity[4]	1,116	0.009
EMR 85% specificity[5]	1,051	0.156
EMR 90% specificity[6]	1,045	0.011
EMR 99% specificity[7]	261	0.009

[1]The difference in 2 × log-likelihood between the model predicting number of new episodes of subclinical IMI with $\ln\frac{SI}{N}$ used as the offset term and the model with only $\ln S$ as the offset was used to evaluate the effect. S = cow-days of a susceptible cow, I = cow-days infected, N = total cow-days in each interval (study month).

[2]Number of subclinical IMI episodes.

[3]Pathogens from which a high cell count would be expected during a subclinical IMI episode were assigned to the Pat 1 group. Pathogens not in the Pat 1 group were assigned to the Pat 2 group.

[4]Pattern of OCC change with 80% specificity and 69% sensitivity for detection of Pat 1 subclinical IMI episodes.

[5]Pattern of OCC change with 85% specificity and 59% sensitivity for detection of Pat 1 subclinical IMI episodes.

[6]Pattern of OCC change with 90% specificity and 48% sensitivity for detection of Pat 1 subclinical IMI episodes.

[7]Pattern of OCC change with 99% specificity and 8% sensitivity for detection of Pat 1 subclinical IMI episodes.

rameters to indicate which area of management should be improved to prevent an increase in new subclinical IMI episodes. Such transmission models also allow prediction of the effect of culling and treatment decisions on new cases of subclinical IMI, as demonstrated by Reksen et al. (2012).

An interesting finding in our study was the rather low transmission of Pat 1 mastitis pathogens between lactating cows. None of the Pat 1 pathogens demonstrated an $R_0 > 1$. Also, but not unexpectedly, *Staph. aureus* and *Staph. epidermidis* were the only Pat 1 pathogens for which an existing subclinical IMI episode was significantly related to transmission from infected to susceptible cows. That is, existing infections with these pathogens were still transferred from one cow to another but at a lower rate than would be the case if R_0 was >1. With only 2 AMS available for milking the cows in our study herd, we expected a higher degree of transmission of pathogens such as *Staph. aureus* and *Strep. dysgalactiae* between cows. In an AMS, some of the recommended preventive actions to limit transmission of contagious mastitis pathogens during milking (Barkema et al., 2009) are violated, because a large number of cows are milked with the same teat cups and the teat cups are only rinsed with lukewarm water between milkings. However, the observed absence of contagious properties of *Staph. aureus* and *Strep. dysgalactiae* in our study indicate that the AMS is not a major vector of transmission of subclinical IMI in this herd. In line with this, a previous study suggested reduced overmilking and no cross-quarter contamination in AMS as potentially beneficial factors for udder health in AMS (Hogeveen et al., 2001). This may explain the low transmission rates, although this is beyond the scope of the current study. Another potential explanation for this minor rate of transmission is that shedding of bacteria may be too low to enable effective transmission from cows with no clinical symptoms. Furthermore, the duration of subclinical IMI was short for most bacterial species, with the exception of *Staph.*

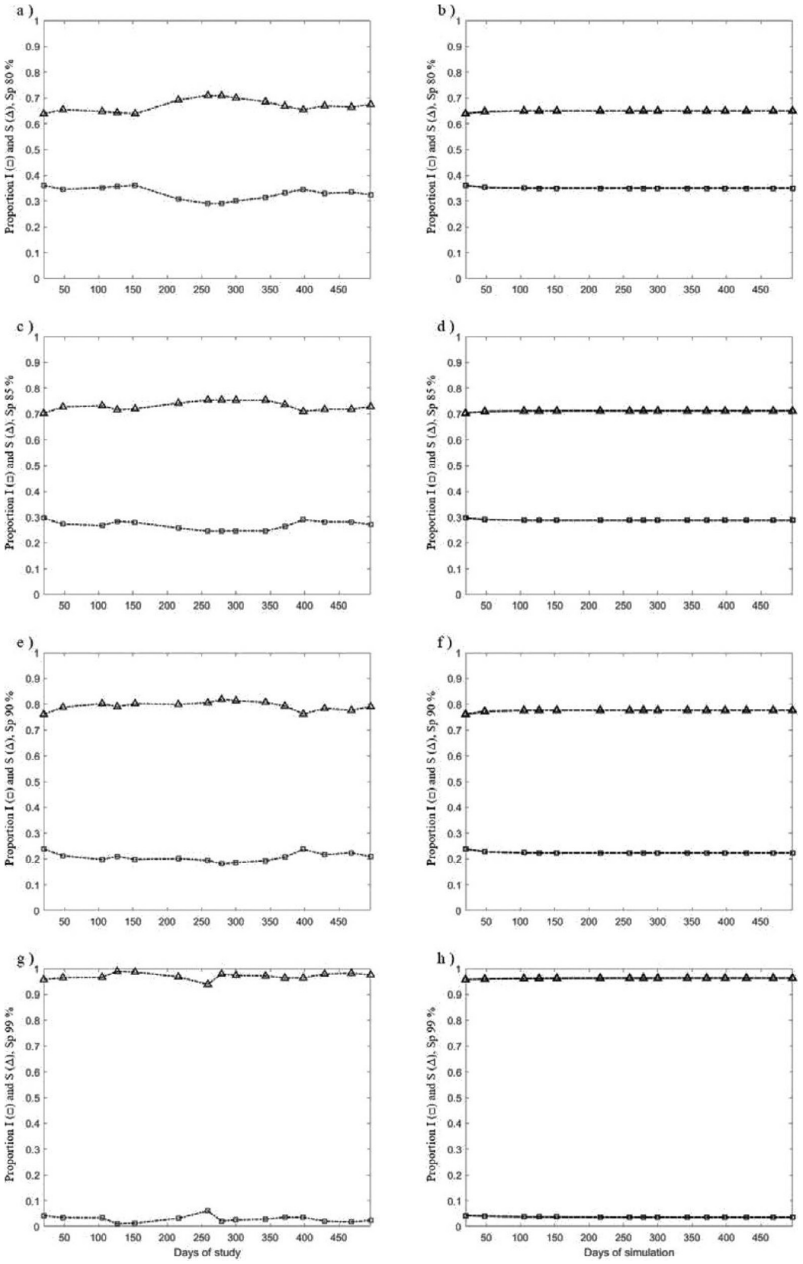

Figure 2. Observed proportion of cows with elevated mastitis risk (EMR) above the threshold (I; □) and susceptible cows (S; Δ) for specificity (Sp) of (a) 80%, (c) 85%, (e) 90% and (g) 99% for Pat 1 (pathogens from which a high cell count would be expected during subclinical IMI) subclinical IMI using EMR. Corresponding dynamic simulation is shown for specificity of (b) 80%, (d) 85%, (f) 90%, and (h) 99%, respectively, for Pat 1 subclinical IMI.

epidermidis and *C. bovis*. The high prevalence of *C. bovis* is likely to have elevated the transmission of this infection.

None of the known mastitis pathogens in our study demonstrated both an R_0 >1 during lactation and a significant effect of an existing subclinical IMI on transmission from infected to susceptible cows. This indicates that a major outbreak of mastitis cases due to these species of bacteria was unlikely. However, although transmission rates were low, an existing subclinical IMI was significantly associated with the number of new episodes of subclinical IMI for several Pat 2 pathogens, along with *Staph. aureus* and *Staph. epidermidis*. In such circumstances, the maintenance of a relatively constant prevalence of subclinical IMI is likely to depend on infected cows entering the lactation pen after calving (Reksen et al., 2012).

The EMR thresholds with 80 and 90% specificity for detection of Pat 1 subclinical IMI episodes showed both an R_0 >1 and a significant association between an existing pattern of OCC change and the occurrence of a new subclinical IMI episode in susceptible cows in our study. That is, the prevalence of cows with EMR over the threshold was significantly related to new episodes of EMR over the threshold in susceptible cows. In addition, spread of the patterns of OCC change apparently has the potential to be associated with an outbreak. This dynamic was observed despite no outbreak occurring during the study period; this implies, therefore, that this approach may be quite sensitive and useful for surveillance of the underlying udder health situation at the herd level. Our results also showed that the number of cows with an EMR over the threshold should be maintained at a low level to prevent an EMR over the threshold developing in other cows.

With low sensitivity and specificity for IMI, current application of sensors is of limited practical use for subclinical IMI management at the cow level (Norberg et al., 2004; Dalen et al., 2019). However, the current study showed that modeling transmission dynamics, based on patterns of OCC change as a proxy for subclinical IMI prevalence at herd level, may be useful for predicting the trend of new infections at the herd level. A forecast of an elevation in the proportion of infected cows in the herd may signal an increasing udder health problem in the herd. The usefulness of such a system depends on the defined threshold values for an alert. Application of predictions from the EMR-based transmission model with a specificity of 99% for Pat 1 subclinical IMI will result in relatively few alerts, but these will almost certainly be related to an ongoing subclinical IMI episode. This could prove useful for alerting the farmer of individual cows in need of attention. Lowering the specificity to 80% would result in more frequent but less specific alerts. These frequent alerts could be used in a surveillance of udder health on the herd level.

The duration of subclinical IMI episodes was significantly shorter for the 4 patterns of OCC change than the duration of the Pat 1 subclinical IMI episodes, as defined by culture results from QMS. A potential explanation for this is that the limited sensitivity and specificity for detection of Pat 1 subclinical IMI using the 4 patterns of OCC change results in a greater number of both false-positive and false-negative results. If so, this increases both the observed number of new episodes and cures, which, in turn, reduces the duration of each episode. Another possibility is that the duration of subclinical IMI episodes based on culture results is overestimated in our study, because milk sampling for bacteriological culture was performed monthly and the cows may have recovered from the infections in the period between the sampling events.

The basic reproduction number R_0 is a combined value affected by the number of contacts per unit time, transmission probability per contact, and duration of the infectious period (Anderson and May, 1991). Dalen et al. (2018) found that the cure rate for subclinical IMI differed significantly between 2 farms with the same mastitis pathogen, and that this difference affected the prediction of transmission dynamics of the same pathogen in each farm. With herd-specific knowledge of which transmission parameters have most effect on transmission dynamics, we can improve udder health management by focusing preventive actions on those management areas that are related to the transmission parameters of concern for each specific herd.

CONCLUSIONS

In the current study, we presented an investigation of transmission dynamics of mastitis pathogens, pathogen groups, and related alterations in EMR in a single herd. Forecasting changes in the herd prevalence of subclinical mastitis can be achieved using dynamic transmission models based on patterns of OCC change. The statistical analyses demonstrated transmission of patterns of OCC change as a proxy for subclinical IMI at specificity levels of 80, 90, and 95%, and new episodes of EMR over the threshold were influenced by patterns of OCC change exceeding the EMR threshold at specificity of 80, 90, and 99%. Although limitations were apparent, this study provides proof of concept that an EMR transmission model can be used at different levels of specificity for Pat 1 subclinical IMI episodes. This could be used for surveillance during lactation, depending on an individual farmer's herd-health man-

agement goals and tolerance for false positives. Future developments in sensor technologies and data analyses are likely to improve sensor-based transmission models.

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