Describing patterns of mastitis indicators during a clinical mastitis episode

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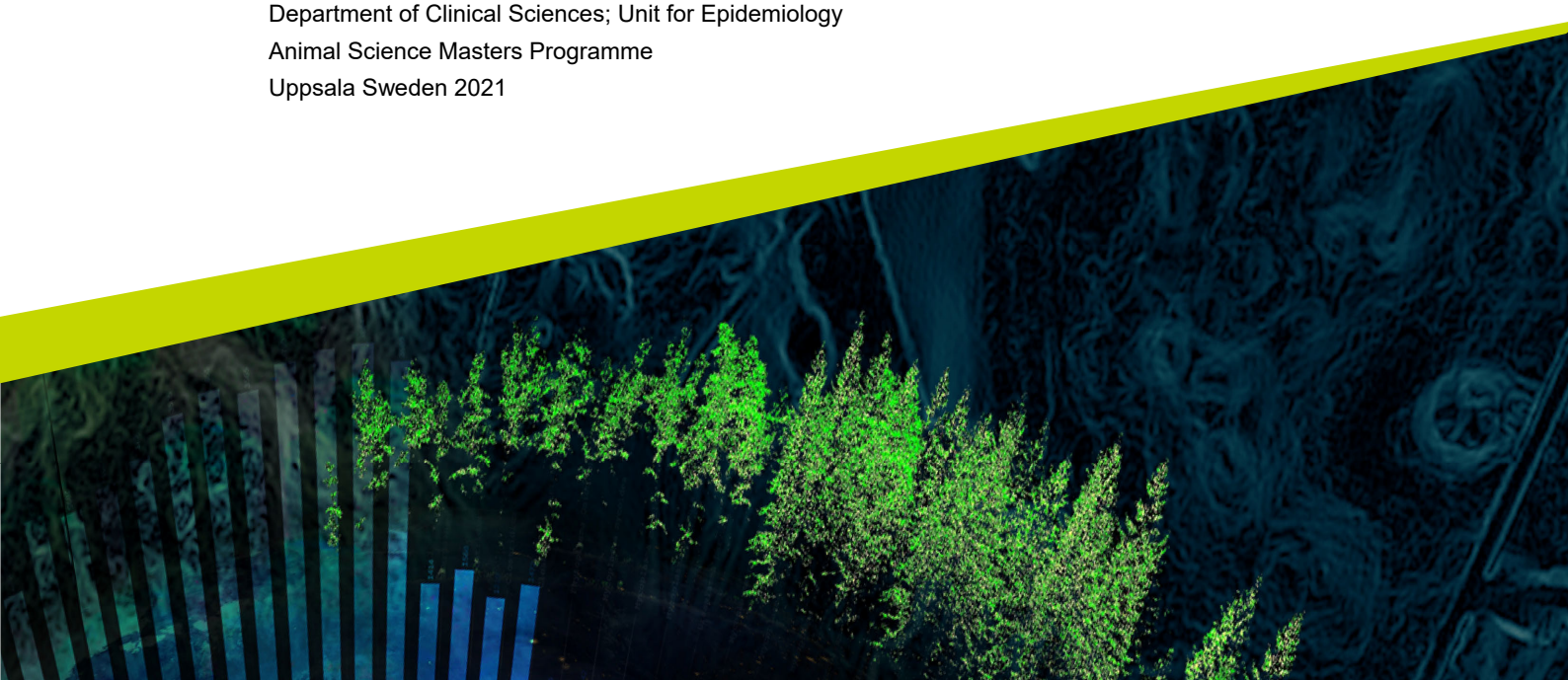

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Abstract

A total of three dairy farms, run on Automatic Milking System (AMS) and having on average 163, 177, and 99 lactating dairy cows respectively, were included in this study. Two of the farms were located in the Netherlands, and one in Canada. The data was retrieved from the database of DeLaval International AB (Tumba, Stockholm). The study aimed to analyze and describe the changes in patterns of mastitis indicators, recorded by sensors, before, during, and after a case of clinical mastitis (CM). In total, 149 cases of CM were identified in the study period, out of which 91 were a first case of CM during a lactation. Fifty-eight of these cases recovered from CM. Recovery was defined based on the somatic cell count (SCC) values being less than 200,000 SCC/ml during the end of the follow-up period. The parameters studied were the SCC, electrical conductivity (EC), and lactate dehydrogenase (LDH) levels of the milk for recovered and non-recovered cases. The statistical analyses were carried out on recovered cases with linear mixed models and results presented as estimated marginal means that were used to analyze the patterns of mastitis indicators for an episode of CM. Further, association analysis was also carried out to check the strength of the relationship between the individual mastitis indicator before and during the treatment initiation and the end of the follow-up period i.e., after 48 days of the treatment initiation. It was found that for recovered cases, the increase in SCC values started approximately 5-8 days before achieving a peak whereas the EC values began to increase relatively later, i.e., approximately 1-4 days before attaining a peak. LDH values, for both, recovered and non-recovered cases started to increase the earliest, that is approximately 9-12 days before attaining a peak value. Furthermore, for recovered cases, it took approximately 20 days for the SCC, EC, and LDH values to stabilize after achieving a peak value. For recovered cases, the SCC and EC values took 20-24 days to drop to the pre-CM level, whereas for LDH it took up to 28 days. No significant associations between the variation in the individual mastitis indicator before CM and the recovery phase were found. Further research with a larger dataset is needed to test whether a pre-treatment variation in SCC, EC, and LDH is of value to predict recovery.

Keywords: CM, SCC, EC, LDH, sensor patterns, recovery phase

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Abbreviations

AMS	Automatic Milking System
CM	Clinical Mastitis
CMT	California Mastitis Test
DIM	Days In Milk
EC	Electrical Conductivity
EMM	Estimated Marginal Means
IMI	Intra Mammary Infection
IMM	Intra Mammary
LDH	Lactate Dehydrogenase
OCC	Online Cell Counter
PMN	Polymorphonuclear Neutrophils
SCC	Somatic Cell Count

1. Introduction

Mastitis is a widespread and one of the costliest diseases of dairy cattle (van Soest et al. 2019) making it widely studied globally. It is the inflammation of one or more quarters of the mammary glands, mostly caused by various microorganisms. Based on whether gross changes in milk (such as watery, serous, or purulent milk, presence of clots, flakes, or blood) gross changes in the udder (such as painful or inflamed udder) and animal are seen or not, mastitis is categorized either into clinical or subclinical (Mdegela et al. 2009). For the scope of this master thesis, we shall concentrate on clinical mastitis (CM) and the possible recovery from the CM episode.

With the introduction of Automatic Milking Systems (AMS), the sensors can measure milk parameters every time the cow goes for milking. These measurements are then used to identify the changes in milk parameters. With the AMS being able to record the milking details, it has become easier to identify CM cases (Khatun et al. 2018). However, earlier diagnosis can be important to start the treatment earlier. Therefore, the patterns of the sensors are important to study to check the progression of CM. Finding patterns in these sensors can aid in earlier diagnosis and the possibility of predicting the course of the disease.

1.1. Aim

This study aimed to analyze and describe the changes in patterns of mastitis indicators, recorded by sensors, before, during, and after a case of CM. The indicators for doing so are somatic cell count (SCC), electrical conductivity (EC), and lactate dehydrogenase (LDH) levels.

2. Literature Review

2.1. Diagnosis of CM

As discussed by Ruegg (2021), one of the possible measures for the diagnosis of mastitis is based on the recognition of an inflammatory response, most commonly caused by an infection in the mammary gland, i.e. an intramammary infection (IMI). CM is diagnosed when the magnitude of the resultant immune response is sufficient to cause visible changes in either milk, udder, or the cow. The clinical signs observed are the presence of blood, clots in milk, curdled milk, and swollen and painful mammary glands. Rasmussen (2005) mentions that the detection of abnormal milk during foremilking can have a sensitivity of at least 70%. The scoring of foremilk run through a 0.1mm size pore filter is less subjective, but it does not yield the same results as visual scoring in a strip cup. The filter method does not give reliable results for watery, yellowish, or bloody milk, but it appears to be accurate and efficient in detecting clots in milk, similar to the visual scoring method. Furthermore, to detect CM the milk should only be scored based on homogeneity and not on color. For the milk to be counted as abnormal, clots should be visible on the filter. The advantage of this scoring is that both trained, as well as untrained people, can score normal and abnormal milk with high specificity (> 90%) and high sensitivity (>80%) (Rasmussen, 2005). Therefore, various manual and automatic systems can be tested against this method as a reference for the detection of CM.

The reporting of detected CM cases is potentially influenced by the reporting ability of the farmers and the willingness of the farmers to involve a veterinarian. A study by Wolff et al. (2012) on the completeness of disease recording system for dairy cows in the Nordic countries highlighted that the completeness of data for veterinary visited cases for CM, which theoretically should be 100%, was lower in all four countries. They further explained that this lack of completeness of registration of veterinary visited cases could be because one or more steps in the system do not work properly. A farmer having a lower threshold for detection and

not necessarily for treatment will notice many CM cases and not have them treated by a veterinarian, thereby decreasing the total completeness. Hence, the coverage is highly influenced by the farmers' threshold for consulting a veterinarian.

In addition to the clinical signs, various indicators of mastitis can also be used in the diagnosis of CM (refer to section 2.4). For laboratory examination to identify the causative microorganism, bacteriological culturing is done.

2.2. Treatment of CM

A systematic way of describing the decision-making for a CM case is based on mastitis symptoms. Ruegg (2021) describes CM cases as being mild, moderate, or severe based on the severity of clinical signs. The decision on treatment and other measures are made based on prognosis, animal welfare, and finances of the dairy farm. The type of treatment that is appropriate for a CM case is determined on a case-by-case basis depending on the symptoms and how long the cow has had mastitis. Depending on the severity of the symptoms, different supportive drug treatments and measures may be needed. The motivation to initiate the treatment of a cow suffering from CM usually starts with the appearance of clinical signs. Ideally, treatment of a case of CM starts with identifying the causative organism. Knowledge of the etiology is a key for initiating appropriate treatment and is critical to value the outcomes. The type of intervention strategy invariably depends on the causative organism within the herd, and hence, control needs to be herd specific (Gussmann et al. 2019).

Various pathogens have different virulence and hence possess differing abilities to initiate an immune response that may result in a spontaneous bacteriological cure. If not, then identification of the causative organism is then followed using the specific antimicrobial that targets the particular microorganism and the spectrum of which should be appropriate for the etiological organism. Most cases are treated with intramammary (IMM) antibiotics, but systemic antibiotics in combination with IMM antibiotics are also used for severe cases (Ruegg 2021). The treatment regimens could also differ between various countries and the production systems.

2.3. Effects of CM

2.3.1. Milk yield

Many studies have shown milk yield losses as a consequence of CM episodes. Rajala-Schultz et al. (1999) mention that the daily loss in the first 2 weeks post-diagnosis was between 1- 2.5kg and a total of 110 to 552kg of milk was lost over the entire lactation, depending on the parity of the cows and the time of occurrence of mastitis. Moreover, the cows failed to reach the pre-mastitis milk yield for the remainder of their lactation, depicting a long-lasting effect of mastitis on the milk yield. Fogsgaard et al. (2015) were one of the firsts to study the recovery period after a CM episode on the changes in milk yield, LDH, milking frequency, and the inter-quarter ratio (IQR) of EC post CM treatment. Their study mentions that the milk yield for primiparous cows had started to drop 3 weeks before the treatment was initiated and was nearly the lowest for the week when the treatment was initiated. This similar level of milk yield persisted for the remaining study period. In contrast, the highest milk losses occurred in multiparous cows when the milk yield was compared to the pre mastitis level and the drop in milk yield started 1 week before the initiation of treatment. Furthermore, milk yield levels were the least among multiparous cows during the week when treatment of CM was initiated. A week later, the milk yield improved relatively and remained almost at a similar level for the rest of the study period, but overall, the milk yield decreased when compared to the control group. For the span of the study period, neither primiparous nor multiparous cows were able to reach the pre-mastitis milk yield.

The highest milk yield losses occur in the initial weeks of lactation and gradually taper down to a constant value approximately 2 months post CM detection (Rajala-Schultz et al., 1999).

Adriaens et al. (2021) studied the milk yield dynamics when the cows showed perturbations across various lactations. They found highly significant associations between perturbation characteristics and parity, lactation stage, and their interactions. The developmental phase of perturbations was on average 1.5 days shorter than the recovery phase of the perturbations that lasted for 11.6 days on average. Of all the perturbations, a majority (82.2%) lasted for less than 30 days.

They further revealed that an average of 3.4 perturbations was detected per lactation with an average of 92.1 kg of milk loss. The relative losses per day when expressed in percentage were higher for early and late lactation perturbations irrespective of the parity. They explained that the high relative milk losses in late lactation were mainly linked to the recovery phase. Perturbations during peak lactation, i.e., in the mid-early lactation lasted the longest having a longer recovery phase for every parity. Early lactation perturbations had higher milk losses compared to the perturbations in later stages of lactation. Across parities, perturbations in first parity cows were less severe than perturbations in higher parity cows. Perturbations in higher parity cows had higher losses than first parity cows. Milk loss per perturbation increased with parity and was higher during peak lactation. The developmental rate was higher, higher milk losses, and a slower

recovery. As CM also results in milk losses, these perturbations could hold true for CM as well.

Furthermore, Ruegg (2021) mentions that recurrence of CM is strongly affected by additional risk factors such as the parity where older cows are at a greater risk of recurrence and also higher milk yield.

Milk yield losses are not just restricted to the current lactation where CM is diagnosed but could be carried forward to subsequent lactation. A cow with one or more CM episodes in previous lactations can produce less milk in the current lactation as compared to cows without any CM episodes (Bar et al. 2007).

2.3.2. Culling

The last resort as a result of the effect of CM is the culling of the animals. A study by Bar et al. (2008) mentions that CM significantly increased the risk of a cow being culled in all parities for at least 2 months after any CM case. After the third CM case, the odds of culling the cow were, even 2 months after CM had occurred, more than 4 times as high as the odds of a cow without CM. Cows with repeated cases of CM and the cows in higher parities (more than 4) are more likely to be culled than cows in their second lactation.

2.3.3. Economic losses

The major effect of CM is the economic loss incurred to the dairy farmer due to milk loss (DeGraves & Fetrow 1993). Furthermore, reduced milk quality, milk production losses, and increased veterinary costs are some other effects of CM (Halasa et al. 2007; Kossaibati & Esslemont 1997). Alteration in the normal milk constituents renders the milk unfit for human consumption if not pasteurized before use. And hence, mastitic milk is discarded. The economic losses are in the form of milk discard, reduced milk quality, and quantity (DeGraves & Fetrow 1993), and hence the dairy industry faces production-related challenges.

2.4. Changes in milk parameters

2.4.1. Somatic Cell Count

There is a direct link between the onset of mastitis and the rising of somatic cell count (SCC). Upon bacterial invasion in the mammary gland, an inflammatory

response is generated. Polymorphonuclear neutrophils (PMN), leucocytes, and phagocytes are attracted in large numbers to the damaged tissue because of the production of chemotactic agents. Many of these cells pass from the milk-producing cells to the lumen of the alveolus which damages the secretory cells and ultimately increases the SCC. These somatic cells comprise mainly white blood cells. Specific substances that draw more leukocytes to the region may also be released by the leucocytes in milk to combat the infection. Somatic cell count remains in high concentrations after the removal of bacteria before gland healing occurs. Clots formed by leukocyte aggregation and blood clotting factors prevent complete milk removal by blocking the ducts (Sharma et al. 2011). Therefore, an increase in the SCC and a decrease in milk yield are observed. SCC values more than 200,000/ml are indicative of mastitis, implying healthy and/or recovered cows will have SCC values less than 200,000/ml (Dohoo & Leslie 1991).

De Haas et al. (2004) mention how various pathogens affect the SCC values for cases of CM. The patterns of SCC also can distinguish between chances of risk for specific mastitis-causing pathogens. CM caused by *Escherichia coli* was significantly associated with the presence of a short peak in SCC, whereas *Staphylococcus aureus* was associated with long and increased SCC. *Streptococcus dysgalactiae* was not strongly associated with any of the defined patterns of peaks in SCC, and no single unambiguous pattern was found for *Strep. uberis.*

SCC values are studied to check the progression of CM. The values usually stabilize after 3 to 4 weeks of the initial inflammation, which can be treated as a cutoff point by farmers to differentiate between chronic and nonchronic cases of udder inflammation (Bonestroo et al. 2021).

Furthermore, California Mastitis Test (CMT) is used to check the elevated SCC in milk (Sargeant et al. 2001). It is a cow-side test in which milk from the suspected quarter is collected and allowed to react with the test agent in a four-welled paddle. The formation of a gel-like structure, as a result of elevated SCC, indicates a positive test.

2.4.2. Electrical Conductivity

When a cow suffers from CM, the concentration of Na+ and Cl- increases in the milk from the affected quarter, thus increasing the total EC (Kitchen 1981). Another parameter used in the detection of CM is thus EC of the milk, which is calculated by determining the concentration of the cations and anions present in the milk. The normal EC values of milk from healthy quarters vary between 4.0 to 5.0 mS at 25˚C and are influenced by the udder temperature. It is a useful parameter to judge udder

health (Norberg et al. 2004). Bonestoo et al. (2021) studied the progression of EC values along with SCC for a case of CM. They reveal that these values stabilized within 3-4 weeks of initial inflammation, but they were above the pre-onset levels.

2.4.3. Lactate dehydrogenase enzyme

The PMN covers the bacteria at the infection site and releases enzymes that can kill organisms (Jones & Bailey, 2009). One of these enzymes is LDH (Viguier et al. 2009) which is an inflammatory indicator. Kato et al. (1989) reported that mastitic milk has higher LDH activity than normal milk, and they attributed this to specific leucocyte fractions and other prominent fractions in mastitic milk. Hence, LDH is an important milk parameter for the early detection of bovine mastitis (Chagunda et al. 2006; Friggens et al. 2007). The mean LDH value for normal milk is 296 IU/L (Bogin et al. 1976).

As mentioned earlier, Fogsgaard et al. (2015) also analyzed the progression of LDH levels during the recovery phase of CM in their study. They found that LDH was at a maximum level for both primiparous and multiparous when the treatment was initiated. Further, they also mention that the LDH levels for primiparous cows returned to baseline at week 7 of the recovery phase, although for multiparous cows, LDH remained higher than the baseline throughout the study period.

2.4.4. Other milk parameters

Other milk parameters also change because of CM. These mainly include the lactose, fat, and protein composition of the milk. In general, during mastitis, there is an increase in the total milk protein which is attributed to an influx of blood-borne proteins, but a lower concentration of fat and lactose (Auldist et al. 1995)

2.5. Other implications of CM

In addition to the milk parameters, other parameters also change as a result of CM. Siivonen et al. (2011) mention that mastitis can cause motivational conflict in the behavioral priorities of a cow. Unlike typical sickness behavior, the cows do not increase their lying time, instead spend more time standing to avoid lying on the side of the inflamed udder quarter. Additionally, there is a negative bearing on the

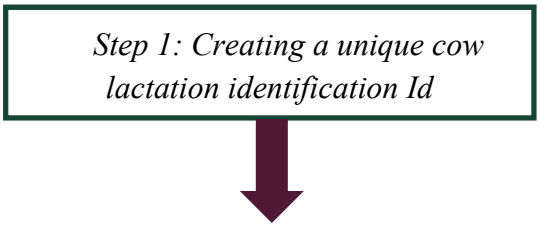
health and welfare of the animals because of the pain and swelling, a consequence of intramammary inflammation (Petersson-Wolfe et al. 2018).

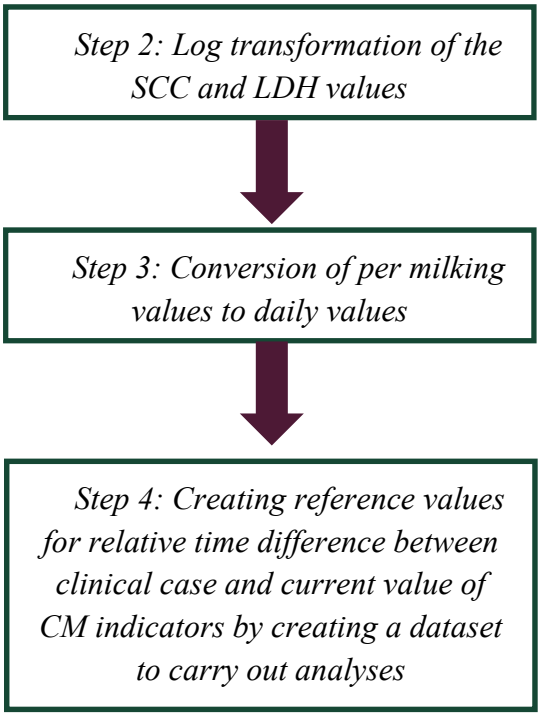
Further, the services per conception can increase based on the time of CM occurrence. Additionally, the reproductive efficiency of a cow can decrease due to CM as cows with CM took a longer time to get pregnant (Ahmadzadeh et al. 2009). Moreover, mastitis results in lower fertility due to the deterioration in the ovarian follicular response (Wolfenson et al. 2015).

3. Methods

Data from 3 dairy farms, two of which located in the Netherlands and one in Canada, were retrieved from a database of DeLaval International AB (Tumba, Sweden). The dairy farm in Canada had, on average 177 lactating cows, while the ones in the Netherlands had, on average, 163 and 99 lactating cows, respectively. These dairy farms used DeLaval VMS^{TM}, Online Cell Counters (OCC; DeLaval International AB), and HerdNavigator^{TM} (DeLaval International AB). The farmers identified clinical mastitis and treated mastitis by using their personal methods and their own treatment protocol. This was mostly collected via digital copies (1 Dutch farm) as well as paper copies (1 Dutch and 1 Canadian farm) of their treatment records. The cases of CM were identified by the OCC and the HerdNavigator^{TM} systems by raising an alarm when the values of milk yield, SCC, EC, and LDH deviated from the normal for 3 days consecutively The data was collected from January 2019 to December 2019 and comprised the quarter level milk yield, SCC, quarter level EC, LDH, days in milk (DIM), cow identification, and parity of the dairy cows. These values were reported per milking. Due to mainly maintenance issues of the DeLaval VMS^{TM}, the data had missing values of milk yield, SCC, EC, and LDH. Additionally, the LDH values were taken by the HerdNavigator^{TM} and were not available on one of these dairy farms. The decision to initiate the treatment of CM was made by the respective dairy farmer and the data on the type of treatment offered were not available on any of these farms. The statistical analyses were carried out using R (*R: The R Project for Statistical Computing*).

3.1. Data cleaning





Step 1: Creating a unique cow identification Id

To facilitate easier identification and data analyses of each cow lactation, a unique cow identification Id was created considering the herd number, cow number, and parity. Starting with this set of data, there were a total of 160,219 observations comprising per milking values of each cow. The per milking values included information on the start date of lactation, SCC, herd number, LDH, EC of each quarter, parity, DIM, the time interval between successive milkings, and the number of times the cow was milked in a day.

Step 2: Log transformation of the SCC and LDH values

All the SCC and LDH values were then log-transformation with the natural logarithm to have an approximately normal distribution of the values and to facilitate further analyses of the data. These values were named LnSCC and LnLDH, respectively.

Step 3: Conversion of per milking values to daily values

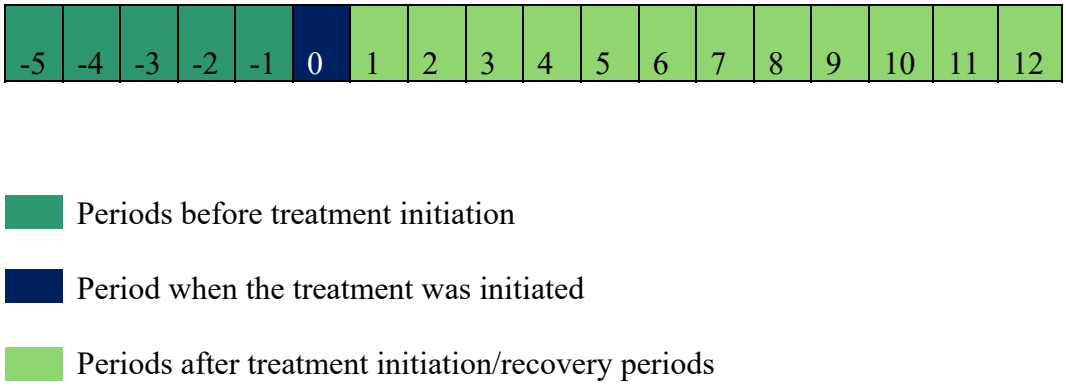
The per milking values were converted to daily milking values for all the cows in the study. The grouping in this step was done based on the unique cow id created

in Step 1 and the starting date of the lactation. The daily milking values were calculated as the mean values of the LnSCC and LnLDH values for the milkings during the day. Similarly, the mean values of EC of each quarter and milk yield of each quarter were calculated.

For all the above-mentioned variables, missing values from the data were omitted by not including those values in mean calculations for the particular cow, for the particular milking. This was done to carry out further analysis without errors.

Step 4: Creating reference values for the relative time difference between clinical case and the current value of CM indicators by creating a dataset to carry out analyses

Firstly, a dataset of all cows that suffered from CM during the data collection period i.e., from January 2019 to December 2019 was made. A total of 149 such cases were identified. Only the first cases of CM within parity were included in this study. Thus, secondly, a new dataset was created including only the first case of CM within the lactation period for each cow, and hence 91 such first cases of CM were identified. For every DIM in each cow lactation, the time difference between the current DIM and the DIM of the first CM case was calculated. This time difference was divided by 4 days to obtain a new variable called the relative time period. The four-day relative time period was considered as 0 for when the treatment of a cow diagnosed with CM was initiated. Records with relative time periods between -5 and 12 were kept for analysis. The periods preceding the four days where CM treatment was initiated were identified as period -5 to period -1. Similarly, the periods succeeding period 0 were recovery periods starting from period 1 till period 12, implying 48 days post-treatment initiation.



Furthermore, 2 new variables were created namely, SDConductivity and MaxConductivity. SDConductivity was the standard deviation of the natural log of the summed conductivities of each of the four quarters (LF, RF, RR, and LR). MaxConductivity was the maximum value of EC among all four quarters. The

observations outside the relative time periods (period -5 to period 12) were not included in the analyses. As a result, 91 first cases of CM having 5621 daily observations remained out of the initial 160,219 observations.

The threshold for recovery based on SCC values was taken as 200,000 cells/ml (Dohoo & Leslie 1991). Recovery was defined as the mean of LnSCC at period 12 being less than the natural log of 200,000/1,000 i.e., 5.298 cells/ml.

Three individual cases: Case Id 43, 53, and 54 were also studied to check if their CM indicators followed a similar pattern to the CM indicators of all the recovered cases. These cases were selected based on having the least number of missing values from period -5 to period 12.

3.2. Statistical Analysis

3.2.1. Linear Mixed Model

A linear mixed model was used to analyze the CM indicators according to the following formula:

$$Indicator = intercept + \sum_{i=a}^{b} Time\ period\ relative\ to\ CM\ treatment\ initiation_{\ i} + Parity + DIM + DIM^2 + cow\ random\ intercept$$

Where Indicator could be LnSCC, LnLDH, or MaxConductivity, and

a = period no. before detection (-5)

b= period no. in the recovery phase (+12)

The model included the relative time period, starting from the period no. before CM treatment was initiated to the last period no. in the recovery phase of CM. Additionally, the parity and DIM were included in the model as these also affect the level of the CM indicator. Lastly, the cow random intercept was also included as it handles the repeated observations within each cow.

The maximum value of conductivity was used here.

Recovered cases and non-recovered cases were analyzed separately in the linear mixed model to evaluate if the indicator patterns were different in the two cases. The R package lme4 was used for the linear mixed model analyses.

3.2.2. Estimated Marginal Means

Estimated marginal means (EMMs), sometimes referred to as least-square means, for each relative time period were calculated with the R package emmeans (Lenth et al. 2019) to improve the interpretability of the results of the linear mixed model. The EMMs were plotted in graphs to aid in checking the level and relation of each mastitis indicator with the recovery phase.

3.2.3. Variation and Association Analysis

The association between the variation in the CM indicator before or at CM and the level of the indicator at the end of the post-CM period was analyzed with a linear model:

$$\begin{aligned} &Mean\ indicator\ at\ period\ 12 \\ &\quad = intercept \\ &\quad + the\ standard\ deviation\ of\ indicator\ at\ period\ x \end{aligned}$$

Where, indicator could be LnSCC, LnLDH, or SDConductivity, and

period x = period -5, -1, or period 0

SDConductivity was used here unlike MaxConductivity in the previous analysis since the aim was to capture variability.

4. Results

The study analyzed 91 first cases of CM. The herd in Canada had the least no. of CM cases (22) as compared to the two herds in the Netherlands (38 and 31), although it was the largest herd. Out of these 91 cases, 58 cases of CM recovered. The herd located in Canada had 15 recovered cases out of the 58 total recovered cases. The herds in the Netherlands had 20 and 23 recovered cases, respectively. Various statical analyses were carried out on the 58 recovered cases to check the patterns of CM indicators and how they varied concerning the relative time period. Separate graphs were plotted for the progression of each CM indicator for recovered as well as non-recovered cases.

4.1. Individual cases

The individual CM cases were studied to ascertain the progression of sensor values of the three mastitis indicators studied. These cases were selected based on having the least number of missing values, still enabling having enough values to study the progression of CM indicators. The cases are shown below:

4.1.1. Case Id 43

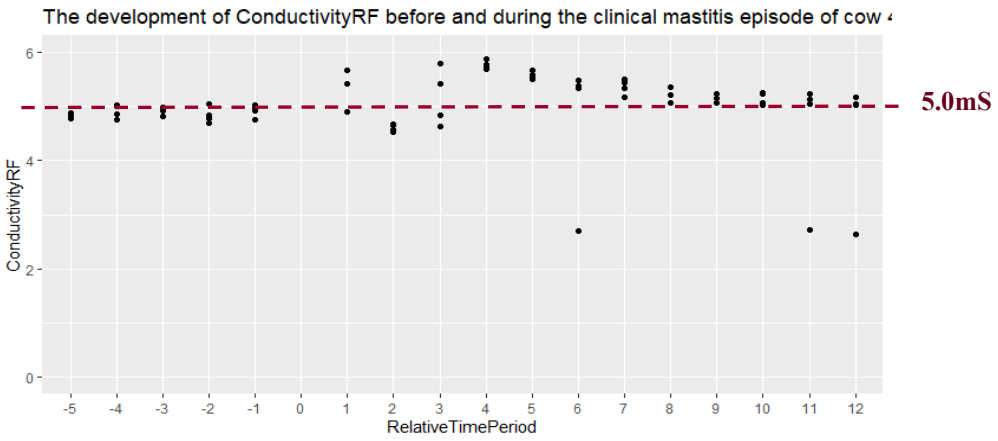


Figure 1. Mean Conductivity of the right front quarter vs. Relative time period for case Id 43 (Normal EC values = 4.0 to 5.0 mS)

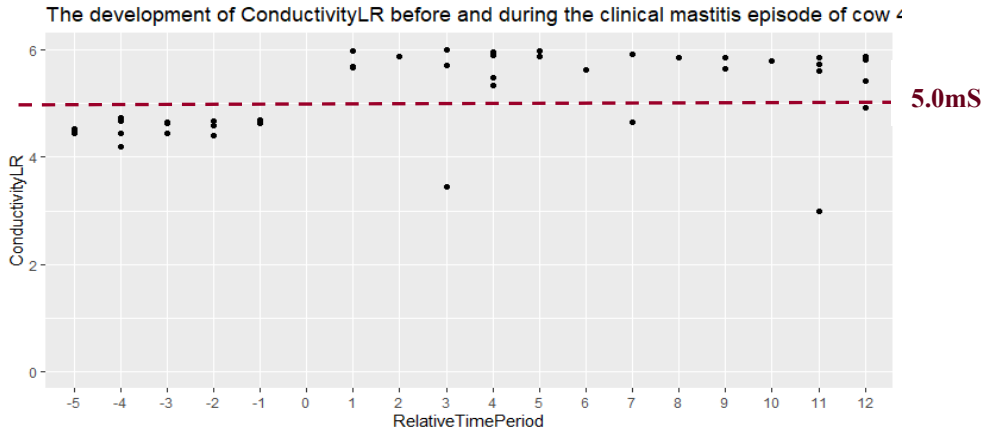


Figure 2. Mean Conductivity of the left rear quarter vs. Relative time period for case ID 43 (Normal EC values = 4.0 to 5.0 mS)

The progression of the values of the EC of the right front quarter and the left rear quarter for case id 43 are shown in Figure 1 and Figure 2, respectively. The four dots seen in each period indicate the mean values of the mastitis indicator for each day in the period as each period consists of 4 days (as mentioned earlier). Periods that do not show four dots each imply missing values for those days. Despite the missing values in period 0 for both quarters, it can be interpreted that both quarters were affected by CM as period 1 in both these quarters has higher than normal values as compared to period -1, i.e., before the initiation of treatment. Additionally, for the left rear quarter, the EC values continue to remain elevated till period 12,

whereas the right front quarter does show a decline in the values from period 5 as the study period progresses depicting better recovery for the right front quarter than the left rear quarter. Further, the EC values of the right front quarter fall back to pre-CM levels by period 12, whereas for the left rear quarter, the EC values do not attain pre-CM levels. Hence, it can be ascertained that the left rear quarter could be chronically affected, while the right front quarter recovered.

The progression of SCC values could not be checked for this case due to many missing values.

4.1.2. Case Id 53

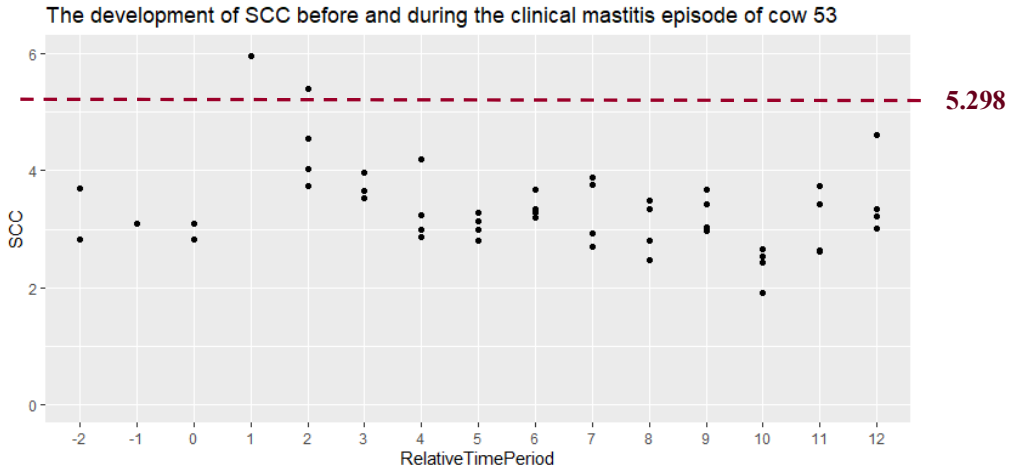


Figure 3. LnSCC vs. Relative time period for case Id 53 (Ln 200,000/1,000= 5.298)

Figure 3 shows the progression of LnSCC values for case Id 53. Studying the recovery periods, i.e., period 1 onwards, a decline in the LnSCC values can be noted. By period 3, the values drop to non-mastitic values. Hence, it took approximately 12 days after treatment initiation to attain non-mastitic values for case 53. By period 5, i.e., 20 days after the initiation of treatment the LnSCC values drop to pre-CM values and are stabilized. Additionally, the increasing values of LnSCC from period 10 onwards could indicate possibly a second exposure to CM.

4.1.3. Case Id 54

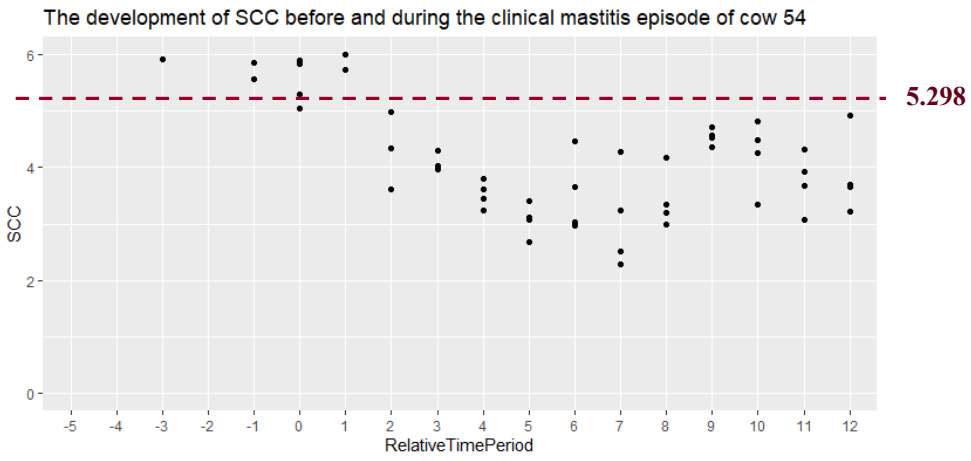


Figure 4. LnSCC vs. Relative time period for case Id 54 (Ln 200,000/1,000= 5.298)

Figure 4 shows higher values of LnSCC. Similar to case Id 53, considering the recovery period for this case the LnSCC values dropped to non-mastitic levels (< 200,000 cells/ml) by period 2, i.e., 8 days after treatment initiation. These values continued to drop till period 5. Period 6 onwards, there was an increase in the LnSCC values, but it was within the non-mastitic level. LnSCC values, in this case, stabilized after period 6, i.e., 24 days after the treatment was started.

Comparing the 3 cases, LnSCC values for case Id 54 took longer to get stabilized. This could indicate a higher bacterial load or a stronger infection in the udder.

4.2. Linear Mixed Model

The linear mixed models to carry out regression analysis for all the indicators showed that the coefficients for period 0 were of the highest significance implying the largest changes in mastitis indicators, followed by period 1 and then by period 2. The coefficients in period 12 for all the mastitis indicators had a negative value indicating a lower level of the indicator in period 12 of recovered cases. The results of the regression analysis can be found in the Appendix.

The distribution of the variables and the homoscedasticity of the residuals were checked for all the indicators. There was a slight deviation from the normal distribution of all the indicators included in this study. The residual plots for the indicators were similar and enough homoscedastic (see Appendix).

4.3. EMM Plots

To check the respective levels and relation of each mastitis indicator with the recovery phase, EMM plots were analyzed.

4.3.1. Patterns of LnSCC values for recovered cases

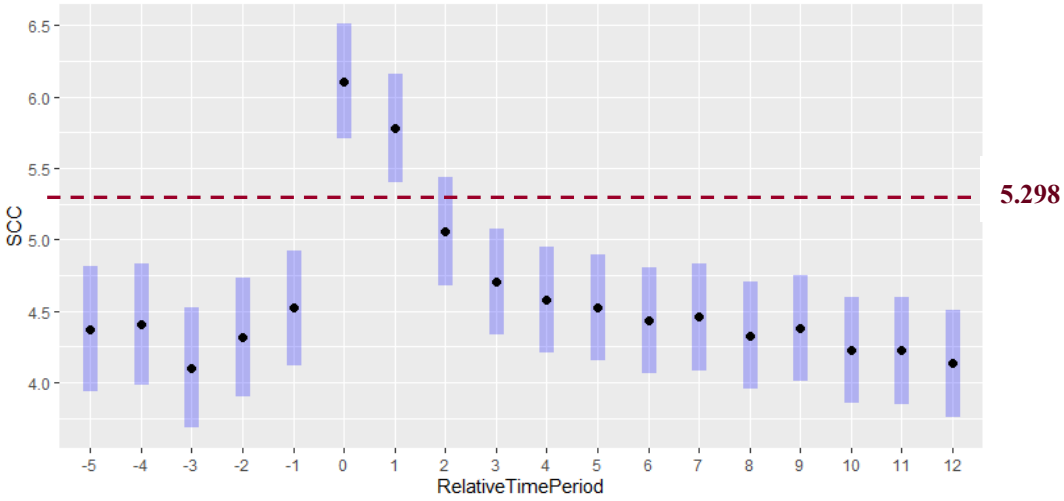


Figure 5. LnSCC vs. Relative time period for the recovered cows from period -5 to period 12 (Ln 200,000/1,000= 5.298)

The LnSCC values for a CM case were analyzed from period -5 to period 12 (Figure 5). The x-axis depicts the natural log of SCC values (ln 200,000/1,000= 5.298) and the y-axis depicts the relative time period. The plots show that an increase in the LnSCC values started from period -2, implying an early increase, i.e., approximately 5-8 days before reaching the peak values. However, these values still were within the non-mastitic value. LnSCC values attained a peak at period 0, i.e., when the treatment was initiated. A gradual decline in the values during periods 1 and 2 depicted the success of the offered treatment. The LnSCC values dropped back to non-mastitic values by period 2 i.e., 5-8 days after treatment initiation. These values continued to decline till period 6. Hence, it took approximately 20-24 days for the LnSCC values to stabilize. Pre-CM values were reached by period 6 i.e., 20-24 days after treatment initiation (Figure 5). These values further decreased from period 10 and continued to decrease till period 12.

As a general pattern, it can be ascertained that the values of SCC began to increase approximately 5-8 days before reaching a peak and then stabilized after period 5, i.e., 20 days after the day of treatment initiation. Hence, for SCC values, a minimum of 20 days was required to attain relatively stabilized values and a minimum of 24 days to fall back to the pre-CM level.

4.3.2. Patterns of EC values for recovered cases

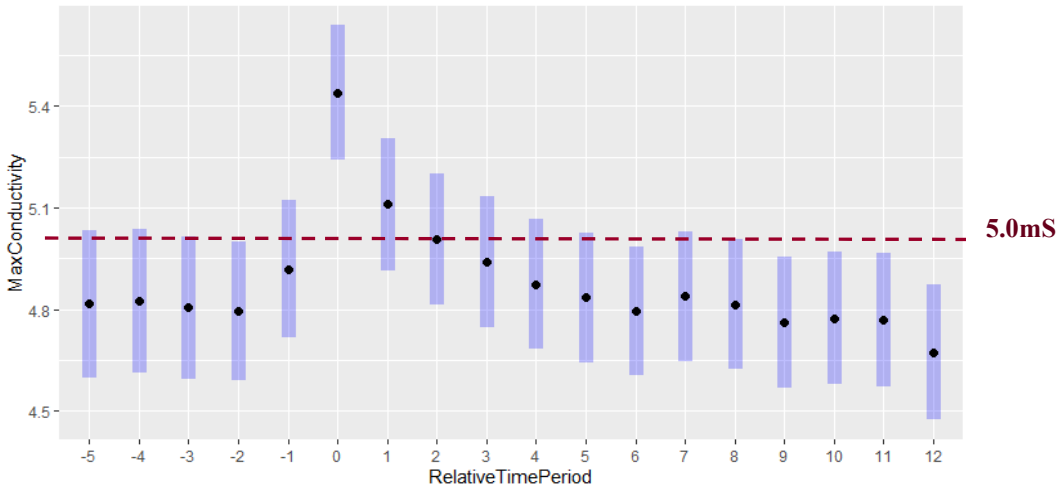


Figure 6. Maximum EC value vs. Relative time period for the recovered cows from period -5 to period 12 (Normal EC values = 4.0 to 5.0 mS)

Figure 6 depicts the maximum EC values from period -5 to 12 for the entire udder. The normal range of EC values is 4.0 – 5.0 mS.

The values of EC started to increase from period -1, i.e., 1-4 days before the treatment initiation. This was followed by a sharp increase and a peak during period 0. Following periods starting from period 1 and going till period 5, EC values were following a decreasing pattern. The values did not show much variation in this decrease. By period 5, the EC values stabilized and by period 6 were similar to pre-CM values.

Therefore, for the case of CM, the EC values began to increase 1-4 days before attaining a peak value. This was then followed by a continuous and gradual decrease till 20 days post-treatment. The values stabilized by 20 days and were back to pre-CM values by 20-24 days after starting the treatment.

4.3.3. Patterns of LnLDH values for recovered cases

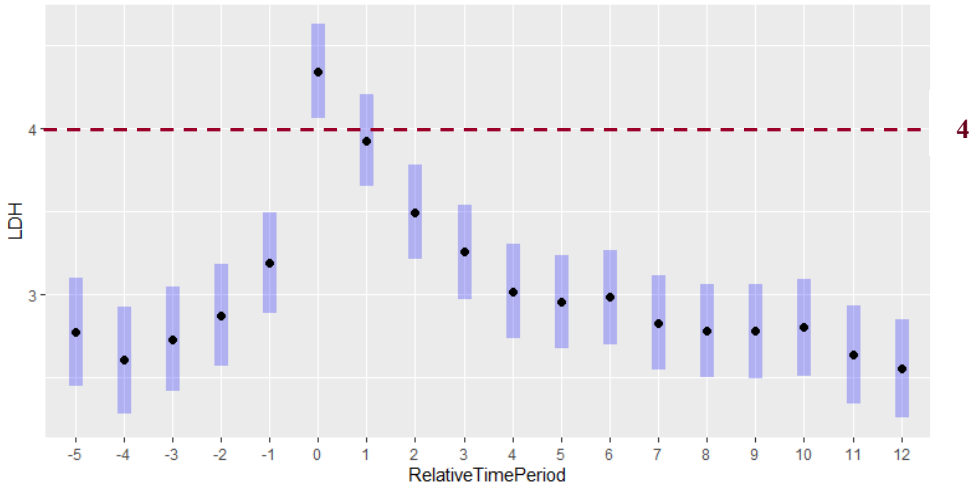


Figure 7. LnLDH vs. Relative time period for the recovered cows from period -5 to period 12 (Ln 296 IU/L = 2.47, normal LnLDH range = 2-4)

Figure 7. shows the natural log of LDH values (normal range of LnLDH = 2-4) against relative time period from period -5 to period 12.

The increase in mean LnLDH values began from period -3 (Figure 7). Following periods showed a gradual increase till period -1. A sudden and sharp increase of the values was observed at period 0 where the LnLDH values attained a peak. Period 1 onwards, similar to SCC and EC, LDH values too started declining gradually till period 4, after which they stabilized. Periods 4, 5, and 6 depict relatively stable values of LnLDH. These values decline further till period 7 and are again stabilized till period 10. The last two periods show a further decline.

Hence, the LDH values as a general pattern began increasing 9-12 days before the initiation of treatment, attaining a peak when the treatment was started. These values continued to decrease till 16 days after the treatment initiation and stabilized by 20 days. It took approximately 25-28 days for the values to be similar to the pre-CM values.

4.3.4. Non-recovered cases

The CM cases that had more than 200,000 SCC/mL milk at the end of the follow-up period, i.e., period 12, were counted as non-recovered. The following graphs depict the EMM plots of non-recovered cases:

LnSCC patterns for non-recovered cases

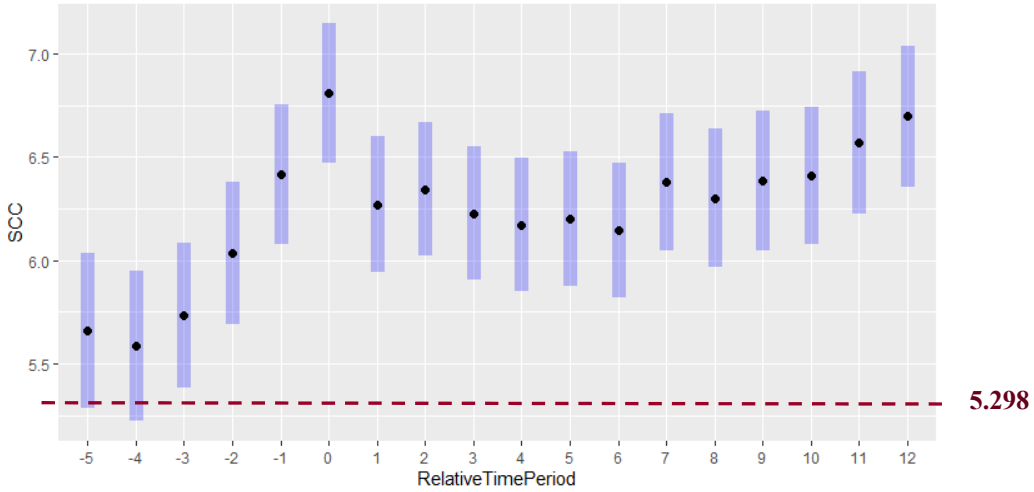


Figure 8. LnSCC vs. Relative time period for non-recovered cases from period -5 to period 12 (SCC values > 200,000/ml in period 12, Ln 200,000/1,000 = 5.298)

Comparing Figure 5 with Figure 8, it is observed that the LnSCC values of non-recovered cases were higher and beyond the non-mastitic value (more than 200,000 SCC/ml) for the whole study period. The peak at period 0 for non-recovered cases was higher (approximately 6.75) than the recovered cases (approximately 6.1).

EC patterns for non-recovered cases

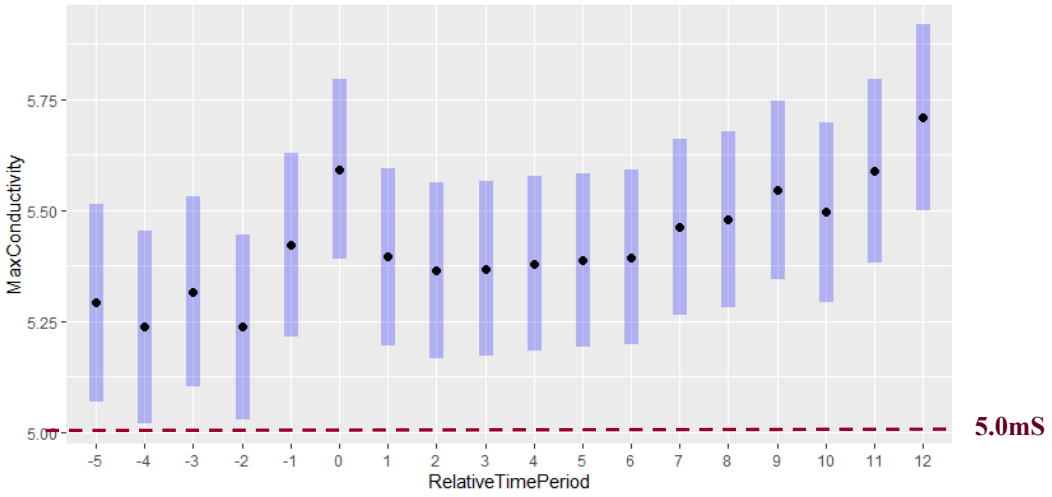


Figure 9. Maximum EC value vs. Relative time period for non-recovered cows from period -5 to period 12 (Normal EC values = 4.0 to 5.0 mS)

Similar to LnSCC values, the EC values of non-recovered cases remained higher than recovered cases from period -5 to period 0. The EC value at period -5 for non-recovered cases was approximately 5.3mS while the EC value of recovered cases at period -5 was approximately 4.8mS. The peak attained at period 0 too was higher for non-recovered cases (approximately 5.6mS) than recovered cases (approximately 5.5mS).

LnLDH patterns for non-recovered cases

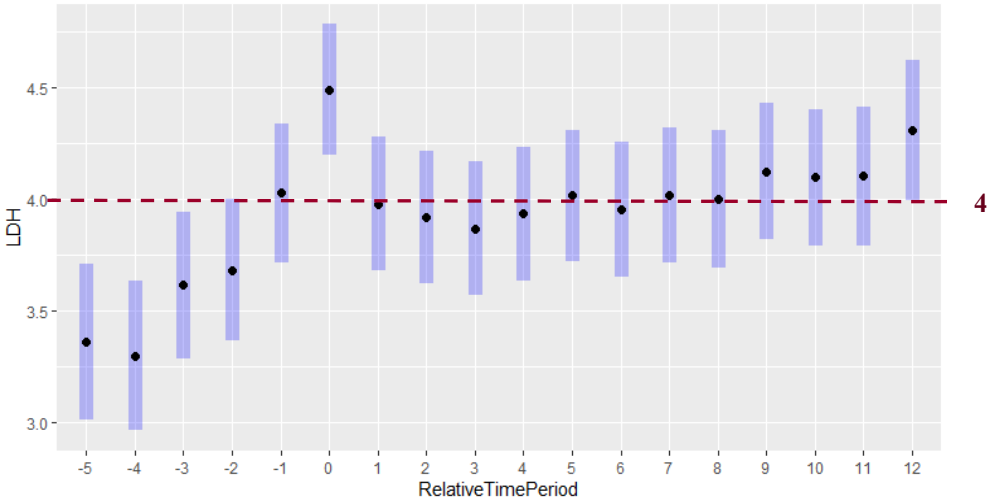


Figure 10. LnLDH vs. Relative time period for non-recovered cows from period -5 to period 12 (Ln 296 IU/L = 2.47, normal LnLDH range = 2-4)

The LnLDH patterns unlike the LnSCC and EC patterns for non-recovered cases were not beyond the non-mastitic range at period -5 (approximately 3.3). The pattern for non-recovered cases had a similar pattern as the recovered cases where the values started to increase from period -3 onwards and attained a peak at period 0. The peak values at period 0 differed for recovered cases (approximately 4.3) and non-recovered cases (approximately 4.5)

4.4. Association Analysis

An association analysis was carried out for recovered cases at periods -5, -1, and 0 to check how the variation in the mastitis indicators before and during the commencement of treatment was associated with the values of the same indicators in the last period (period 12) of the recovery phase. (p-value > 0.05)

Table 1. Association analysis for recovered cases at period -5, -1, and 0 for mastitis indicators

	Regression coefficient		
Mastitis indicator	Period -5	Period -1	Period 0
LnSCC	-0.078	0.056	-0.134
SDConductivity	-0.019	0.038	-0.086
LnLDH	-0.149	-0.112	0.113

In Table 1, as indicated by the values for period -5, the variation in mastitis indicators are all negatively correlated with recovery. For period -1, regression coefficients show positive values of SCC and EC whereas a negative value for LDH. Lastly, period 0 depicts negative SCC and EC values, while the LDH is a positive value. This implies that the variation in SCC and EC are negatively correlated to the recovery. The reason behind a positive LDH value could be because of a very small number of observations included in the calculation.

5. Discussion

Mastitis is one of the most widely studied diseases and yet the sensor-based investigation of the recovery phase of CM has not received much attention. So, this study was aimed to analyze and describe how the sensor patterns change before, during, and after an episode of CM. We included only the first case of CM within a lactation, and cases that recovered from CM were included in the statistical analyses. To define recovery, we used the SCC values dropping to less than 200,000 cells/ml as a reference to define the cow having been recovered, similar to the study by Bonestroo et al. (2021). The EC or LDH values were not used as a reference as these are typically a range of values, instead of a single threshold value that non-mastitic mammary glands and milk possess. Hence, having a single reference value to define recovery based on EC and LDH would not be possible. Additionally, LDH values were also not available for all the farms.

The study period was divided into smaller periods of four days each, contrasting to studies by Bonestroo et al. (2021) and Fogsgaard et al. (2015) where the sensor values were analyzed based on week-in-milk. The data included in these studies were much larger than the dataset used in the present study, therefore they used weekly study periods. However, using fewer days in a period could aid in defining a more precise phase for when the values of the CM indicators change, and hence in the present study, the study period was divided into smaller periods of four days. An even shorter period would have been better, for instance daily, but the amount of data did not allow for such parsimoniousness. The parameter to define a CM case in the present study was the initiation of treatment, while Bonestroo et al. (2021) used milk diversion as the case definition, which may explain differences in results. Furthermore, the present study did not include any information on the type and kind of treatment offered to the animals suffering from CM.

Individual cases that were included in this study were selected based on having the least number of missing values and could also be used to analyze the particular case. In addition, the cases were also selected considering the progression of the indicators and how they align with the sensor pattern of respective indicators observed in this study.

To describe the sensor patterns of mastitis indicators, the mean of natural log values of SCC and LDH were used whereas for EC, maximum values were used as EC values are generally a range and not a threshold value like the somatic cell count. So, the highest and the lowest values of EC could average out each other,

giving a value within the normal range, therefore the progression of EC could have yielded an erroneous pattern. Additionally, the maximum is more expressive. No other studies are known to have used the maximum values of EC for analysis of recovery patterns.

In the present study, observing the patterns of SCC, EC, and LDH for recovered cases of CM, all the indicators peaked at period 0. This could be indicative of a maximum level of infection at period 0 as the SCC comprising PMN, leucocytes, and phagocytes are released in response to inflammation (Sharma et al. 2011). The PMN cover the bacteria and release inflammatory indicators like LDH enzymes (Viguier et al. 2009), thus elevating the LDH levels. This inflammatory response would then alter the milk composition as the influx of ions such as Na^+ and Cl^- (Kitchen 1981) and the influx of blood-borne proteins would increase (Auldist 1995), ultimately increasing the EC values. Further, observing the progression of these values, the LDH values started increasing the earliest i.e., from period -3 which is 9-12 days before treatment initiation. Contrastingly, Fogsgaard et al. (2015) mention that for both primiparous and multiparous cows, LDH started to increase 3 weeks (21 days) before reaching a peak at week 0. The increase in LDH was followed by the SCC values that started to increase from period -2, which is 5-8 days before treatment initiation. While EC values started to increase relatively later from period -1 indicating SCC and LDH increased earlier as compared to EC for recovered cases. However, this period of time is heavily dependent on the respective farmer and the farm management strategies employed by the farmer that could differ between various dairy farms. Moreover, it is also dependent on the farmer's decision-making on when to initiate treatment for a CM case. In addition, the etiology could also impact the values of the mastitis indicators.

For non-recovered cases, the SCC and EC values were much higher and beyond the non-mastitic limit from the start of the study period. Whereas the LDH patterns for non-recovered cases were slightly higher than recovered cases at period -5, but still within the non-mastitic range. A similar pattern of increase in LDH value from period -3 was seen in non-recovered cases as well as recovered cases. Hence, LDH values could be a better indicator for earlier detection of CM.

Since the SCC and EC values of non-recovered cases were high and beyond non-mastitic levels from the start of the study period, it could be possible that these higher values were the reason behind the cows not recovering from CM or the other way around. It could also indicate the sustained presence of the pathogen in the mammary gland.

For the subsequent follow-up periods, SCC and EC values of recovered cases followed a similar pattern. Both indicators stabilized by 20 days after the treatment was started. For recovered cases, SCC values dropped to pre-CM values by approximately 24 days, whereas the EC values dropped to pre-CM values by 20-24 days. The LDH values like SCC and EC stabilized by 20 days. Whereas in the study by Fogsgaard et al. (2015) for primiparous cows, the LDH values returned to

baseline levels at week 7 (49 days), but for multiparous cows, LDH values remained elevated throughout the study period. It is noteworthy that in our study, LDH values took up to 28 days to drop to pre-CM levels. Therefore, comparing these three indicators based on the results obtained in the present study, the LDH values increased the earliest and took the longest to drop back to pre-CM levels. Hence, LDH can be used for the early detection of mastitis. This is in line with Friggens et al. (2007) who mention that LDH can be used for early identification of mastitis as it could accurately detect significant differences between mastitic and healthy cows 4 days before treatment in their study. More studies need to be carried out to better understand the progression of LDH values for a case of CM as it is difficult to draw any further results from this study due to the lack of availability of LDH values from one out of three farms.

The results obtained in this study, in particular, the SCC and EC patterns of recovered cases are in line with those described by Bonestroo et al. (2021). They mention that the SCC values of cows having udder inflammation stabilized within 3 to 4 weeks after initial inflammation, closer to the pre-onset values. In the present study, we found that the SCC values stabilized within 20 days and in approximately 24 days they were the same as the pre-onset levels or in this case, pre-CM levels. However, a contrasting result in the EC values was observed. In the present study, the EC values of recovered cases did fall back to pre-CM values within 20-24 days, which was not the case in Bonestroo et al. study. Moreover, the EC values stabilized by 20 days after the peak value which is within the range described by Bonestroo et al. (2021).

The present study has certain limitations as the dataset is quite small and only based on three herds. Further, there are missing values in the data which makes the analysis more difficult. A similar study can be carried out on a larger dataset with data collected from more herds which includes LDH values from all the cows included in the study to facilitate getting significant results, but it can be more expensive. Moreover, other factors influence the recovery phase of CM as well. One of these factors includes the farmer's decision on when to start the treatment. As mentioned by Wolff et al. (2012), a farmer's threshold for diagnosis and treatment initiation holds an important place in the completeness of the disease recording. Further, the type and kind of pathogen that caused the particular CM episode, the treatment offered, and lastly if the cow was exposed to mastitis previously (outside the study period) also influence the recovery from CM. In the present study, these factors were not known, hence a deeper study including these factors could facilitate a better understanding of the sensor patterns during the recovery phase. For instance, the knowledge of the type of pathogen that has caused CM can aid in a better understanding of the patterns of SCC values as *E. coli* is associated with a short peak in SCC, whereas *S. aureus* is associated with a long and increased SCC (De Haas et al. 2004). Hence, the pathogen can affect the patterns of mastitis indicators during recovery.

Critically studying the sensor patterns is of paramount significance as it can aid in recognizing the progression of the disease, which can facilitate further research to detect CM at the earliest possible time and hence aid in reducing the recovery period to a minimum possible limit. As seen in this study, a CM episode can last for approximately a month in total. This can amount to huge economic losses incurred by the farmer in the ongoing lactation (Rajala-Schultz et al. 1999) or could also be forwarded to subsequent lactation (Bar et al. 2007).

As future research, the progression of the mastitis indicators can be thoroughly studied that includes all the factors that affect the recovery period. Additionally, the effect of other production or metabolic diseases on the recovery of CM can also be checked. Lastly, AMS can be upgraded to a system that can predict the course of a CM case in advance based on the sensor values of the indicators, hence facilitating easier and earlier detection of CM by the farmers. This system could work well for farmers worldwide.

6. Conclusion

On average, for recovered cases, the increase in SCC started approximately 5-8 days before achieving a peak whereas the EC values began to increase 1-4 days before attaining a peak. On average, LDH values for both recovered and non-recovered cases began to increase the earliest that is 9-12 days before attaining a peak value. Further, it usually took approximately 20 days for the SCC, EC, and LDH values to stabilize after achieving a peak value for a case of CM on average. For the recovered cases, all the values of the indicators dropped to pre-CM levels. SCC and EC values took 20-24 days to drop to the pre-CM level, whereas LDH took up to 28 days for the same. There was a negative association between variation in the mastitis indicators at the period with the peak values and the value of the indicator at the last period of the recovery phase, but the association was not significant. Further research with a larger dataset in more herds is needed to test whether a pre-treatment variation in SCC, EC, and LDH is of value to predict recovery.

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Appendix

Table 2. Regression analysis of LnSCC

	Estimate	Standard error	T value
Intercept	4.07125	0.39614	10.277
Relative time period -4	0.03426	0.18996	0.180
Relative time period -3	-0.26934	0.18972	-1.420
Relative time period -2	-0.05279	0.19083	-0.277
Relative time period -1	0.14666	0.18549	0.791
Relative time period 0	1.73577	0.18883	9.192
Relative time period 1	1.40524	0.18089	7.769
Relative time period 2	0.68324	0.18142	3.766
Relative time period 3	0.33232	0.18283	1.818
Relative time period 4	0.20664	0.18409	1.123
Relative time period 5	0.15184	0.18715	0.811
Relative time period 6	0.05891	0.18959	0.311
Relative time period 7	0.08348	0.19473	0.429
Relative time period 8	-0.04427	0.19739	-0.224
Relative time period 9	0.01075	0.19967	0.054
Relative time period 10	-0.14588	0.20105	-0.726
Relative time period 11	-0.14880	0.20495	-0.726
Relative time period 12	-0.24109	0.20733	-1.163
Parity	0.08647	0.10161	0.851
DIM	-0.19365	0.16212	-1.195
I (DIM^2)	0.08928	0.06453	1.384

Table 3. Regression analysis of MaxConductivity

	Estimate	Standard error	T value
Intercept	4.6718360	0.2168994	21.539
Relative time period -4	0.0103750	0.0615487	0.169

Relative time period -3	-0.0111069	0.0612177	-0.181
Relative time period -2	-0.0205817	0.0612274	-0.336
Relative time period -1	0.1039115	0.0605972	1.715
Relative time period 0	0.6242433	0.0601071	10.386
Relative time period 1	0.2942457	0.0614435	4.789
Relative time period 2	0.1903810	0.0630964	3.017
Relative time period 3	0.1241681	0.0649562	1.912
Relative time period 4	0.0584321	0.0670016	0.872
Relative time period 5	0.0191705	0.0692127	0.277
Relative time period 6	-0.0206815	0.0715711	-0.289
Relative time period 7	0.0226119	0.0740599	0.305
Relative time period 8	-0.0006103	0.0766642	-0.008
Relative time period 9	-0.0549196	0.0793706	-0.692
Relative time period 10	-0.0412748	0.0821674	-0.502
Relative time period 11	-0.0462362	0.0850445	-0.544
Relative time period 12	-0.1428999	0.0881173	-1.622
Parity	0.0469126	0.0597145	0.786
DIM	0.0190698	0.0909299	0.210
I (DIM^2)	-0.0113328	0.0224489	-0.505

Table 4. Regression analysis of LnLDH

	Estimate	Standard error	T value
Intercept	2.449693	0.305011	8.031
Relative time period -4	-0.170823	0.153232	-1.115
Relative time period -3	-0.043958	0.153220	-0.287
Relative time period -2	0.099304	0.147873	0.672
Relative time period -1	0.417711	0.150211	2.781
Relative time period 0	1.567541	0.143137	10.951
Relative time period 1	1.152803	0.138063	8.350
Relative time period 2	0.721345	0.145781	4.948
Relative time period 3	0.479687	0.145579	3.295
Relative time period 4	0.242458	0.149193	1.625
Relative time period 5	0.178632	0.149818	1.192
Relative time period 6	0.208327	0.152779	1.364
Relative time period 7	0.054607	0.153145	0.357
Relative time period 8	0.009375	0.0766642	0.061
Relative time period 9	0.004608	0.156667	0.029

Relative time period 10	0.025974	0.160787	0.162
Relative time period 11	-0.139814	0.163857	-0.853
Relative time period 12	-0.220192	0.166759	-1.320
Parity	0.100267	0.077344	1.296
DIM	-0.044392	0.105896	-0.419
I (DIM^2)	0.076220	0.048114	1.584

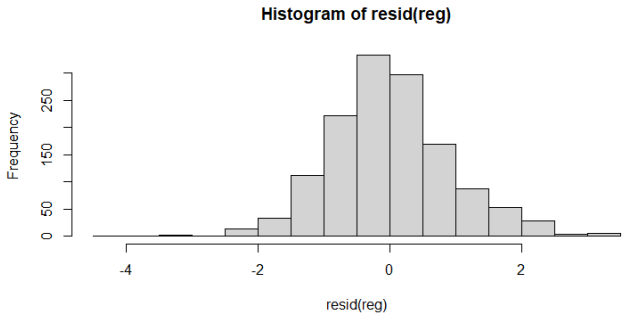

Figure 11. Distribution of LnSCC

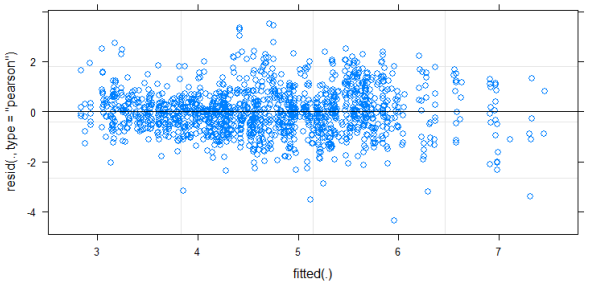

Figure 112. Homoscedasticity of LnSCC

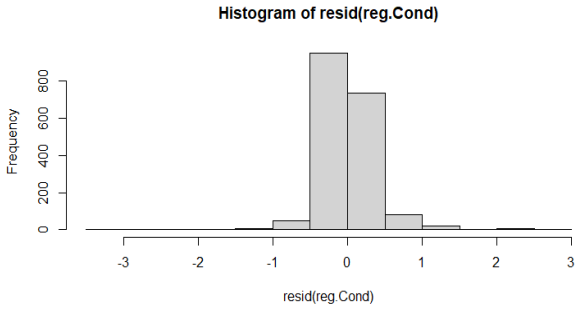

Figure 13. Distribution of MaxConductivity

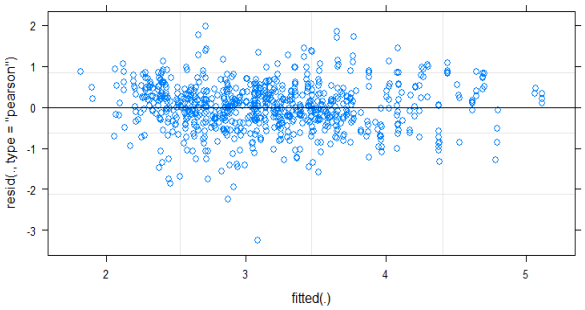

Figure 14. Homoscedasticity of MaxConductivity

Figure 15. Distribution of LnLDH

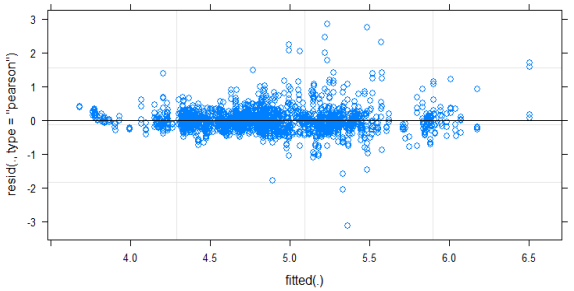

Figure 16. Homoscedasticity of LnLDH

Popular Scientific Summary

Mastitis is the inflammation of the mammary glands, usually caused by various microorganisms. Based on the appearance of gross changes in milk and udder, mastitis is classified into clinical and subclinical. It is an important production disease of dairy cattle where among other cow health and milk parameters, the milk quality and quantity are adversely affected. These milk losses along with the treatment and other additional costs cause economic loss to dairy farmers all over the world. So, it is important to study this disease and the associated mastitis indicators. Cases of clinical mastitis (CM) were studied in this project. The data was collected from the database of DeLaval International AB (Tumba, Stockholm) comprising 3 dairy farms run on an automatic milking system. Two of the farms were located in the Netherlands and one in Canada. The data was collected from January 2019 to December 2019. The mastitis indicators that were studied in this project were the somatic cell count (SCC), the electrical conductivity (EC) of milk, and the enzyme lactate dehydrogenase (LDH). These indicators increase when a cow suffers from mastitis. Hence, the study aimed to analyze and describe the changes in patterns of mastitis indicators, recorded by sensors, before, during, and after a case of CM.

To start, 149 cases of CM were identified from all three herds, followed by the identification of 91 first cases of CM. Further, these 91 cases were then divided into recovered (58) and non-recovered cases (33). The statistical analyses were carried out on the recovered cases. The mastitis indicators of recovered cases were analyzed using a linear mixed model and their graphs were plotted using their estimated marginal means. The patterns of the indicators for non-recovered cases were also checked.

It was found that LDH started to increase earliest i.e., approximately 12 days before attaining a peak for both recovered and non-recovered cases. This implies that LDH could be used for earlier detection of CM. Additionally, the SCC, EC, and LDH did fall back to pre-CM values for the recovered cases implying complete recovery from CM.