Clinical study of different liniments to prevent mastitis in cows

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Abstract

Mastitis is one of the most frequent and costly diseases in the dairy industry. The sickness has been treated with antibiotics for decades, resulting in undesirable consequences for processors and consumers. Hence, it is vital to find a cheap and efficient solution for farmers to treat the cows before mastitis occurs or in early stages of the disease.

The main purpose of this study was to understand the effect of liniments on cows and find one with high efficiency to prevent mastitis. Agricam is the first company working with early detection of mastitis. Although it is known that the use of cameras serves as a useful tool for an earlier attention to the problem and potential treatment, no studies have been done before on liniment effect with this kind of technology until now.

However, by analyzing currently existing data on a farm using Agricam liniment, it is possible to make conclusions if the liniment has had any significant effect. A normal distribution curve was performed followed by two-tailed t-test to compare the significant difference before and after treatment. The data was obtained on 100 individuals during a one-month period. Software programs Python 3.6 and Spyder 3.1.2 were used.

The results showed that the data was normally distributed for all the data that was analyzed. The t-tests showed significant effects before and after treatment with liniment A and Agricam liniment for sick and healthy cows. Similarly, the test showed a significance in non-treated cows when analyzing the temperature differences. The significance indicates an increase of temperature in healthy cows and a drop of temperature in sick cows as a long-term effect.

Same result is seen in treated cows as in non-treated cows (drop of temperature). With the current test analysis, it is concluded that there is no significant difference between non-treated, Agricam liniment treated and Liniment A treated sick cows. Same result was held for healthy cows treated with Agricam liniment and Liniment A, i.e. no significant difference.
Contents

1. Introduction ........................................................................................................................................ 1

   1.1. Purpose of the study .................................................................................................................. 1

   1.2. Expected impact of study ........................................................................................................... 1

   1.3. Agricam AB ................................................................................................................................. 2

   1.4. Objectives of the work ............................................................................................................... 2

2. Theory and methodology .................................................................................................................. 4

   2.1 Scientific background .................................................................................................................. 4

   2.1.1. How infection occurs .......................................................................................................... 4

   2.1.2. Treatment of mastitis ........................................................................................................... 7

   2.1.3. Cost of mastitis ..................................................................................................................... 7

   2.1.4. Factors contributing to mastitis .......................................................................................... 7

   2.1.5. Liniment use and effect ..................................................................................................... 9

   2.1.6. The lactation cycle ............................................................................................................. 10

   2.1.7. Thermal cameras ................................................................................................................ 11

   2.2. Methodology ............................................................................................................................ 12

   2.2.1. Abnormality and aggregated abnormality ......................................................................... 12

   2.3. Models ....................................................................................................................................... 16

3. Methods ............................................................................................................................................ 18

   3.1. Data method .............................................................................................................................. 18

   3.2 Experimental method ............................................................................................................... 18

4. Results ............................................................................................................................................... 19

5. Discussion ......................................................................................................................................... 26

6. Future studies ................................................................................................................................... 29

7. Conclusion ........................................................................................................................................ 30

Acknowledgment .................................................................................................................................. 32

References ............................................................................................................................................ 33

Appendix A ............................................................................................................................................ 35

Appendix B ............................................................................................................................................ 36
1. Introduction

1.1. Purpose of the study
Mastitis is one of the most frequent and costly diseases in the dairy industry. Moreover, mastitis affect milk by increasing the number of somatic cells, watery milk appearance forming flakes and clots. Changes in protein, fat and calcium (among others) content is normally the consequences. Since mastitis is caused by bacteria and the inflammation causes pain, it needs to be treated with antibiotics. Treatment with antibiotics has undesirable consequences for producers and consumers.

The aim of this project study was to understand the effect of liniment on cows and find a potential one to prevent infection to occur. This was achieved by doing a literature study and a clinical trial.

1.2. Expected impact of study
It is vital to find alternative methods to prevent infections in cows. Not only for the animal health and welfare benefits but also because antibiotics become increasingly ineffective against certain illnesses because of their ability to produce toxic reactions and due to the emergence of drug-resistant bacteria. Another significant aspect is that antibiotics in dairy cause economic losses to the cheese and fermented milk industries, because of all the milk that needs to be discarded.

A statistic from 2013 shows top milk yield in the Nordic countries whereas Sweden is on the second place, an amount of 8341 kg of milk per cow and year. Different studies show different costs, one study from the U.S. showed that antibiotic treatment cost per cow per year vary from 17-261 EUR between farms where another study, also in the U.S. shows a range from 158-483 EUR.

Therefore, it is important to investigate alternative solutions where antibiotics should be excluded to avoid the appearance of antibiotic-resistant bacterial strains. The available method Agricam is using is a liniment which has yet not been verified by their current system, the thermal cameras, to verify its results. However, earlier studies have shown that liniment has an effect to prevent the infection when measuring the level of Somatic Cell Count, SCC.

By understanding the effect of the liniment on cows, more precise conclusions can be drawn of its impact and how it helps to prevent the early infection to develop into a manifest action of clinical mastitis with inflammatory symptoms like pain, swelling and reduced production. Moreover, correct liniment can be chosen with its active
substances and their uptake by the skin. By observing its effect, its consistency, easy application and distribution, the results will be certainly more reliable for the customer and the health security of the animal will increase. As the milk yield will decrease, less suffering for the cow will lead to better profitability in the long run when mastitis is prevented before it shows clinical symptoms and will reduce animal suffering, farmers labour and milk loss.

1.3. Agricam AB
The project is done at Agricam AB, a software company that was established in 2010. The company develops software systems, thermal image cameras, to be used in the agriculture sector for detecting infections in an early stage before they become severe. By identifying the changes in the udder in an early stage, mastitis can be prevented, a costly disease for the dairy industry.

This project is mainly directed to the evaluation of the liniments, to invest in documentation and analysis of data, so that it is clear where action is required. Moreover, this will be beneficial for improvements in animal health and welfare thanks to regular checks of how effective a treatment is. Finally, it is a valuable feature for the company, i.e. more reliable customer service and qualitative products proven by clinical trials. This in return would give rise to increased clients and higher net sales for the company by being aware of which investments that can be expected to be financially feasible.

1.4. Objectives of the work
The aim of the project was to identify if the liniment currently provided by Agricam has any significant effect on cows and compare it with other alternative liniment to study which one is better.

The specific objectives of the project are to
1. Analyze data on non-treated cows. Some data measurements are on cows that have increased temperature which has not been treated.
2. Analyze significance on liniment treated healthy cows. By analyzing some selected healthy cows that have been treated with liniment, to see if liniment has had any impact on the cows (in case of increase or decrease of temperature occur).
3. Analyze significance with current liniment treatment. Since a lot of data on treated cows already exist, it is vital to study its effect on the temperature differences.
4. Start a clinical trial, testing a new liniment. Having an already existing liniment as a reference, it is worthwhile to investigate another liniment to compare. By
choosing a liniment and implement a clinical trial during two-week period to apply on healthy and sick cows.

5. Compare use of different liniments by collecting all data on treated cows, using t-tests in Python. Use statistics to identify significance between the treatments.

6. For future studies; for those that the liniment does not have any affect, use a complementary treatment and consider alternative methods to prevent mastitis.

A second liniment other than the currently used by Agricam was chosen by the company. The clinical study was performed on a farm in Dalsland region with a herd of 253 cows. The liniments were applied during two-week period on the selected cows. The cows that had higher number than the threshold, an alarm was triggered which identify the sick cows that were selected automatically by the system to be treated with liniment. The healthy cows that had lower number than the threshold was selected manually in the system to be treated with liniment. The temperature of the cows was measured before and after the application of both liniments with thermal cameras to analyze significance. All the collected data was imported from the Agricams’ system to Python, where the statistical models were performed, and results obtained.
2. Theory and methodology

2.1 Scientific background

2.1.1. How infection occurs
Mastitis is the result of a bacterial infection of the bovine mammary gland and the host response to the infections. The three main bacterial species causing bovine mastitis is *Escherichia coli* (*E.* coli), *Streptococcus uberis* (*S.* uberis) and *Staphylococcus aureus* (*S.* aureus).

Mastitis can occur in two different forms; acute and chronic forms. In the acute form, the onset is usually sudden, and it can be recognized by swelling of the gland, fever and changes in the milk. This form can be seen at any time during lactation. However, in the chronic form, the feverish manifestations are usually absent. The glands show fibrous induration in the milk cistern and the milk shows small clots.

Mastitis can also occur in clinical and subclinical form. Clinical mastitis is mastitis in which abnormalities of the udder are observed. It can be mild, moderate or severe. In mild form, it can show abnormalities in milk such as clots and flakes with little or no swelling of the gland whereas cows with severe clinical mastitis have a sudden onset of udder inflammation, abnormal milk and systemic signs such as fever. Subclinical mastitis is a form of mastitis in which the udder is normal, and the disease is not expressed but the bacterial infection is present. In order to be able to detect subclinical mastitis, microorganisms can usually be cultured from the milk and the changes can be detected by measuring the SCC.

Innate immune response differs significantly between gram-negative bacteria such as *E. coli* and gram-positive bacteria such as *S. uberis* and *S. aureus*. The different types of bacterial species alter in speed and effectiveness of immune response. These specific responses can be recognized in the SCC patterns in milk relative to IMI (Intramammary Infections), milk production losses and risks of culling and death. SCC is the most widely accepted criterion for indicating the udder health status of individual cows within dairy herds. Typically, no clinical symptoms are shown when a cow has elevated SCC although the inflammation can occasionally change into the clinical form, showing visible milk changes and animal pain reaction. SCC is used to determine the likelihood that the individual cow or quarter is infected. Mastitis can only be proven by bacteriological culture of obtained milk samples. BTSCC (Bulk Tank Somatic Cell Count) is the number of white blood cells, secretory cells and
squamous cells per milliliter of raw milk. It is a measure of milk quality and as indicator of overall udder health. The cell count number is overall increased in infected cows; however, the number is lower in samples with gram-negative bacteria than gram-positive bacteria. This can be explained by the gram-negative bacteria’s ability of expressing endotoxin LPS on their surface provoking stronger increase in TNF-α and IL-1β expression and secretion than gram-positive pathogens.

SCC includes the count of somatic cells such as neutrophils, leucocytes, lymphocytes and can be divided in different infection phases.

- 0-99,000 somatic cells/ml is undisturbed/no infection,
- 100,000-200,000 somatic cells/ml has a possibility for infection,
- 201,000-300,000 somatic cells/ml has a high probability of infection,
- 300,000-700,000 somatic cells/ml are considered indicative of the presence of inflammation and
- 700,000 somatic cells/ml and higher has no treatment.

Somatic cell counts are widely used for evaluating milk quality and to define milk price. Therefore, many farmers consider this as an indicator of milk value, evaluating the relationship between intramammary infections and changes in milk composition. Mastitis influences cheese yield and composition by reducing milk yield, constituents used to produce cheese and by shifting milk attitude to coagulation.

The presence of a pathogen is a mandatory step in immune defense against the invading pathogen. Biopsies of the parenchyma of udders with E. coli mastitis show increased mRNA abundance of TLR2 and TLR4 genes (figure 1). Cytokine expression in cows with mastitis has been shown to correlate with NF-kB activation, which may enter nucleus and bind to target promoters. They act as a main switch to orchestrate a whole battery of immune defense genes. In absence of functional TLR4 the infecting E. coli P4 invades the epithelial cells with high efficiency, forming intracellular microcolonies. E. coli has a mammary epithelial invasive potential, limited by alveolar macrophages using a process dependent on TLR4 signaling. S. aureus is commonly assumed the result of transmission between cows where other infected animals in the herd is spreading the organism.
Figure 1. Different bacterial species cause an infection of the host response\(^6\). The largest contrast is between \textit{E. coli} and \textit{S. aureus}, activating different signal pathways for inducing infection (figure copied from Schukken YH., et al., 2011 with permission).

Colonization of the bovine mammary gland by pathogenic bacteria lead to changes in the four major components of milk; fat, protein, lactose, somatic cells and other minor components such as enzymes\(^14\), as can be seen in table 1 below. The changes in the milk composition caused by mastitis are shown by injury of udder cells which reduces the synthesis of milk components and by changes in permeability of membranes which permit increased leakage of materials from blood to milk\(^14\). Increase in proteolytic activity due to the inflammatory response may be the cause for the changes in the milk protein fraction, hence mastitic milk has more proteolytic activity than normal milk\(^14\).

Table 1. Different factors are changed in the production and composition of milk caused by mastitis with main focus on somatic cell count in most analysis\(^16\) (modified from the original source).

<table>
<thead>
<tr>
<th>Decrease</th>
<th>Degree of change</th>
<th>Increase</th>
<th>Degree of change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quarter milk yield</td>
<td>-(--)</td>
<td>Somatic cell count</td>
<td>+++</td>
</tr>
<tr>
<td>Fat</td>
<td>-</td>
<td>Immunoglobulins</td>
<td>+++</td>
</tr>
<tr>
<td>Lactose</td>
<td>-</td>
<td>Bovine serum albumin</td>
<td>+</td>
</tr>
<tr>
<td>Long-chained fatty acids</td>
<td>-</td>
<td>K casein</td>
<td>+(+)</td>
</tr>
</tbody>
</table>
2.1.2. Treatment of mastitis
The most common therapeutic treatment or prevention of mastitis for decades have been antibiotics. In a study of a farm with a total of 20,577 cows, they received 127,172 daily doses of a medical preparation over one year of which 69% of the doses applied were antibiotics used during lactation and 12% were antibiotics for the dry period. Other treatments used were homeopathy (natural substances) which accounted for 7% of the doses applied, 4% for anti-inflammatory drugs and finally 2% used supportive preparation like enzyme-based products. Less than 1% were phytotherapy such as camphor ointments or garlic intramammary.

2.1.3. Cost of mastitis
The total cost of mastitis varies between farms and countries. Moreover, the results of these calculations change with time owing to changes in milk quality regulations and changes in market circumstances. The calculations on the cost of mastitis varies between different studies depending on the used methodology and the population size used in the analyzes.

Drugs to treat infected animals cause an economic damage, depending on the legislation and the infrastructure of the country. The associated costs can be due to the following factors; milk production losses due to subclinical mastitis are considered to be a direct log-linear relationship between SSC and test-day records. The milk production per cow depends on the structure of the farming business, like payment based on kg of milk or kg of milk components (such as fat and protein). Veterinary services include besides delivering drugs, also time spend on diagnosis of a clinical mastitis case. Labour between farms where external labour is the time that has been used to prevent mastitis which is easy to calculate compared to if it comes from the farmers free time. Product quality includes a decreased milk quality in mastitis, causing less efficient processing of milk with less valuable properties. Factors part of the milk payment system are bacterial count and SCC, which change due to mastitis of a cow. Culling which is a decision of the dairy farmer; a cow is culled when replacement is the optimal decision whereas cows with mastitis have a higher risk of being culled.

2.1.4. Factors contributing to mastitis
There are multiple factors involved in affecting certain microorganisms that can cause mastitis such as hygiene, housing, climate, milking machines, feed and genetics. Klastrup et al. (1987) estimated that 25% of susceptibility to infection is attributable to environmental factors, 20% to genetic factors and 50% to herd management.
**Environmental factors**

Climate, housing, quality of indoor air, bedding and stress are all environmental factors contributing to mastitis\(^1\). Rapid temperature (like excessive humidity, to which bacteria can react differently) changes can encourage mastitis caused by biting insects that contaminate the udder with different bacteria\(^1\). Other conditions such as mud outdoor caused by abundant rainfall increase the number of microorganisms and the risks of infection. Indoor kept cows have a higher risk of udder injuries and the microbes are easily transmitted from one cow to another. Different materials used as bedding may affect the growth of different microorganisms. Sawdust and shavings if heated encourage rapid development of coliforms in general.

When a pregnant cow delivers a calf and starts producing large quantities of milk almost every day, it becomes more sensitive to the surrounding environment. Since the cow will be in a transition, she suffers from an enormous hormonal and metabolic change and thereby stress. Stressed animals have less efficient immune system and are less resistant to microbial infections\(^1,27\). Stress can be caused by noise, stray voltage, irregular management and/or microbial exchanges in tense relationship between animals\(^1\). Therefore, the more stressed, the greater risk of mastitis since it affects the integrity of intramammary cells.

**Genetic factors**

It has been shown that high-producing cows are more likely to be affected. Hereditary factors account for 12-20% of susceptibility to mastitis in a single breed\(^1\). The more a cow produces fat milk, the more it will be susceptible to mastitis. Genetically there is a correlation between percentage of milk and incidence of clinical mastitis\(^1\). Furthermore, there are highly strong genetic correlations of clinical mastitis with SCC and udder depth\(^29\). A significant factor in mastitis is the different patterns of leukocyte population of healthy gland. When analyzing the heritability of a trait and its correlation with udder health (by examining the leukocyte populations of uninfected mammary glands) was found that the effect of cow trait is significant for neutrophil, macrophage and T-lymphocyte-bearing CD4\(^{+}\)\(^29\).
Physical and ethological factors

Other factors are the needs of a calf, herd hierarchy, uterus-mammary and rumen-mammary glands\(^{11}\). When mastitis appears within the two months following calving, it is often linked to uterus that are not properly cleaned causing udder contamination\(^{11}\). When acidosis occurs in the rumen, it creates conditions that foster bacteria which eventually yeasts, the toxic substances travel through the system and favor gram-positive bacteria that attack the udder\(^{11}\). Some studies show that the probability for mastitis (clinical and subclinical) to occur is increased with number of lactation and age as presented in figure 2 below\(^{12}\).

![Figure 2](image)

**Figure 2.** The frequency of mastitis is increased with number of lactation and age\(^{12}\) (figure copied from Fadlelmula et al., 2009 with permission).

Human factors

Integrated human factors to consider is the poor farm hygiene\(^{20}\). Hygiene management is with high probability related to teat cleaning process and the udder health. This can be observed by the SCC variable, where high SCC and the rate of clinical mastitis is positively related to the proportion of cows with dirty teats before milking.

2.1.5. Liniment use and effect

Herbal medicines have been used since ancient times, having a rich history of traditional use and considered as a valuable ingredient in analgesics for treatment of minor muscle aches and pains\(^{15}\). Some of the ingredients have also antimicrobial activities. The most commonly occurring and among oldest herb used for medicinal use is menthol. Menthol is extracted from *Mentha piperita* (peppermint), which is a plant used a long time back, where its botanical extracts has been used to treat diseases. Mentha in liniment use has topical analgesic and anti-inflammatory properties\(^{21}\). The peppermint plant is also known to be chemopreventive and
antimutagenic\textsuperscript{21}. It is used as an analgesic and to treat headache. The essential oil in peppermint is dominated by monoterpens (menthol, menthone and their derivates). The oil improve blood flow by dilatation of the capillaries and the application of the mint oil cream can enhance the transportation of white blood cells to the udder and act as a prophylactic measure to prevent mastitis\textsuperscript{15,21}.

According to an in vitro study by Saeed et al., 2006, the peppermint that was examined against different species of bacteria exhibited high antibacterial activity, same results were seen in another study by Sartoratto et al., 2004\textsuperscript{22,23}.

One study analyzed the differences in SCC between treated and untreated cows by testing uddermint\textsuperscript{15}. The data showed SCC in all farms were systematically lower in the uddermint treated cows compared to untreated cows, as shown in figure 3 below.

![Figure 3](image.png)

**Figure 3.** Mint-oil cream treated cows (green color) has lower number of SCC compared to untreated cows (blue color). In x-axis in figure a) is the SCC results shown for 4 different farms\textsuperscript{15} (figure copied from Z. Konstantinos, 2015 with permission).

### 2.1.6. The lactation cycle

The lactation cycle is the period between one calving and the next. Every cow calves in average every 12 months\textsuperscript{17}. During this period, different phases of lactation are found; the early (when the body reserves are used for milk production), mid (dry matter intake and milk production), late lactation (body reserves for next lactation) and the dry period (ensuring cows to have feasible body reserves for early lactation). There are two major factors determining total lactation yield; peak lactation (which sets up the potential milk production) and the rate of decline from this peak. Total milk yield can be calculated by multiplying peak yield by 200. A cow peaking at 20 L/d should produce 4000 L/lactation. The rate of decline from peak depends on peak milk yield, nutrient intake following peak yield, body condition at calving and factors such as disease status and climatic stress\textsuperscript{17}. The four phases are shown in figure 4.

During the lactation, changes in the activity of genes in the mammary gland and in the liver are expressed\textsuperscript{24}. Fat and glucose synthesis are increased from pregnancy to early
lactation to provide fatty acids and blood glucose for milk production. In adipose tissue however, the fat synthesis is decreased, and the expression of transporter genes is reduced for the uptake of blood glucose into somatic cells to ensure availability for milk production.

![Figure 4](image)

**Figure 4.** Four lactation phases with regard to month and milk production\(^\text{17}\) (figure modified from the original source). The cross represents the peak milk yield.

2.1.7. Thermal cameras

Thermal imaging cameras have been long used to differentiate temperature in materials and living organisms. It was originally developed as a surveillance and night vision tool for military use but has recently been applied in broader fields like animal monitoring, agriculture, detection, tracking and more\(^\text{16}\). It is a device that forms a heat zone image using infrared radiation to form visible light. All objects emit a certain amount of black body radiation as a function of their temperature; the higher an object’s temperature the more infrared radiation is emitted.

The thermal camera Agricam is using has a wavelength of 7.5-13 µm range and the temperature is between -20 to +120°C. The camera has a built-in memory for image storage and a wide-angle lens which is well suitable for working in crowded spaces.

Warm-blooded animals try to maintain a constant body temperature, while cold-blooded animals adapt their temperature to their surroundings\(^\text{16}\). The temperature distribution in the warm-blooded animals can be unevenly distributed over the body surface depending on blood-circulation and respiration. Hence, thermal imaging is useful for diagnosis of diseases and thermoregulation, control of reproductive processes, analyzes of behavior and detection. Since the disease will affect the general body temperature, injuries caused by e.g. inflammation can be visible at specific spots. By these means, when an animal becomes ill, this can be detected in thermal images.
prior to clinical detection of the disease.

2.2. Methodology
Below is a picture of the system (figure 5) and how all the data is detected and analyzed for treatment in this project. The detecting process starts off with cows going through a milking parlour where the thermal cameras are placed on each sides of the passage (figure 5). The image of the udder is divided into four parts front-left, hind-left, front-right and hind-right, hence it is necessary to detect both sides to have a complete picture of the udder. An image analysis of all data is collected for further measurements. A temperature measurement is done on the image to see how the value of the cow is deviated towards the normal body temperature and finally make a weighting of the deviations over the past 48 hours to evaluate if the cow has a trend of increased temperature. The measurements show different deviation, whether it is a “normal” deviation or an aggregated deviation above the threshold and action need to be taken (figure 6).

2.2.1. Abnormality and aggregated abnormality

With the thermal cameras, temperature can be measured for the entire herd. In order to identify the deviations, abnormality and aggregated abnormality is calculated to set the critical value for alarm (which is a signal for the farmers that a cow has an increased temperature and is in need of treatment). 

Every herd has a critical value, so called threshold for triggering the alarm. Depending on the size and the capacity of treating the cows with liniment, the threshold is different on different herds. If the herd is big (having many cows) and the capacity is low (few staff available to apply with liniment), then the threshold is increased. This leads to treatment of fewer cows. However, if the herd is smaller and the capacity is high, then the threshold is lower (or optimal threshold is set). Abnormality is based on camera variations, the cows’ individual variation and the herds’ variation. It is linked to the temperature, but the value is compensated for not measuring differently because of left and right camera variations, that the individual cow is always warmer or that the herd is warmer. The formula is explained in appendix A.

When a cow under the last 48 hours has a temperature deviation which is a trend of increased temperature, then aggregated abnormality is calculated by taking the mean of the temperature deviations during that time. If aggregated abnormality is less than the threshold then no action is taken and if above the threshold, then an alarm is sent to the farm for liniment treatment.
Figure 6 shows a flow chart of the system from when a cow passes the thermal cameras set in the milking parlour as in figure 5 where all the cows pass by for image analysis. The collected data is a measure of the entire herd, giving a daily deviation (as shown in figure 7). If the data shows an aggregated deviation larger than the threshold, then an alarm is triggered automatically. However, for this study, an alarm was manually triggered for the healthy cows to be treated with liniment.

Figure 5. Milking parlour where the cows that pass by are imaged by the thermal cameras placed on each side (green boxes).
Figure 6. Flow chart showing the analysis and decision making of data from clinical study. From when image analysis of detected cows are measured and aggregated deviation is detected. The aggregated deviation can be normal (less than the threshold) or larger than the threshold, then a decision is made upon that result to either treat the cows with liniment (healthy cows are selected manually) or no treatment with liniment is done (for the sick and healthy cows).

All data measurements are registered for each and every cow as shown in the example in figure 7 below, an illustrated example for one cow, air marked 3746. The system registers all measurements and alarms are illustrated as vertical green columns, and the cow is then treated with liniment. Figure 7 is representing cow number 3746 (all cows are air marked with a certain number, the oldest cow in the herd has the lowest air mark number), during the time frame of 2018-10-16 to 2018-12-31. Daily measurements are the yellow dots and the blue dots are the mean for every two days (two yellow dots). The green horizontal line is the threshold for the herd and the green vertical line indicates when the blue dots have passed the threshold and an alarm is triggered for liniment treatment.
Figure 7. Output from registered data for one cow. The yellow dots show abnormality for each and every measurement. The blue dots are aggregated abnormality, mean value for 2 earlier measurements of the yellow dots. If the aggregated abnormality is above the threshold 1.3 (horizontal green line), then the cow is alarmed (vertical green stack) and need to be treated with liniment.

Six different groups were formed to study the liniment effect on cows.

i. Healthy cows treated with Agricam liniment
ii. Healthy cows treated with liniment A
iii. Healthy cows not treated
iv. Sick cows treated with Agricam liniment
v. Sick cows treated with liniment A
vi. Sick cows not treated

The methodology of this study was performed step-by-step as shown in Figure 8 below. The process shows for planning, proceeding and analyzing the results of the clinical studies demonstrated. In the first step, a literature study was made followed by selection of a liniment to compare Agricams’ liniment to. From here on, the selected liniment is referred to as liniment A. In the second step, material was ordered, and a test protocol prepared for the staff to follow, which was the regular, only informing about being extra aware of the application procedure and the amount of liniment to be used on each application. In the third step, the material was sent to the farm and all cows where imaged by the thermal cameras. In the fourth step, a data measurement was done on all cows. The sick cows were automatically chosen by the system whereas the healthy cows were manually chosen for liniment treatment. In the fifth and final step, a t-test was performed to analyze all data before and after the treatment and conclusion drawn from the results.
2.3. Models

In this study, a two tailed t-test was used to test the significance between different comparisons\(^\text{18}\). The alternative comparisons were done for before and after treatment with Agricam liniment and liniment A, alarmed non-treated cows and healthy cows. One-Way-Anova was used to analyze the statistical differences between the three groups mentioned (Agricam liniment, liniment A and non-treated)\(^\text{25}\).

A t-test’s significance indicates whether or not the difference between two groups’ averages most likely reflects a real difference in the population from which the groups were sampled. The statistical significance is determined by the size of the difference between the group averages, the sample size and the standard deviations of the groups.

In Python, stats.ttest_rel() SciPy function was used to calculate the significance. From the t-test, a statistical t is calculated which could be compared with the standard t-value (critical value). If \(t < \) critical value, then there is no significant difference (don’t reject \(H_0\)) and if \(t > \) critical value, then there is a significant difference (reject \(H_0\)).

Higher t-values indicate that a large difference exists between the two sample sets and the smaller t-values, the more similarity exists between the two sample sets.

The same argument can be concluded when calculating p-value. If \(p < \alpha\), then there is a significant difference and if \(p > \alpha\) then there is no significant difference. \(\alpha\) (alpha) is the value of probability as a criterion for acceptance, which normally can be assumed as 5%. If the null hypothesis qualifies to be rejected, it indicates that data readings are strong and are not by chance.
The t-test model was chosen since the data followed a normal distribution which allowed the testing to be applicable and the required results could be accomplished with this simple method.

As for the t-test, the comparison between before and after treatment with liniment, an increase or decrease in abnormality above or under the threshold should be predicted to understand its effect. Alarmed non-treated cows where chosen to be analyzed in order to identify any significant abnormality changes before and after the cows were supposed to be treated. Moreover, 10 healthy cows were chosen to be treated with liniment to distinguish if the liniment had any significant effect overall.

One-Way-Anova uses statistic F, which is the ratio of between and within group variances. In the comparison of the means of three groups that are mutually independent and satisfy the normality and equal variance assumptions, when each group is paired with another to attempt three paired comparisons. The same argument as for t-test can be done for significance. If F < critical value, then there is no significant difference (don’t reject H₀) and if F > critical value, then there is a significant difference (reject H₀).

With the different experiments explained above, giving observations from different angles, a better understanding of liniment effect was studied, hence the results could be explained more thoroughly.
3. Methods

3.1. Data method
All the data was detected and registered by the thermal cameras. The methods that were used are paired t-test for the each and every comparison that was made in Python. A package from python was imported to implement the t-test. Before implementing the t-test, a normal distribution curve was performed to ensure normality. One-Way analysis of variance (ANOVA) was used to determine whether there were any statistically significant differences between the groups in excel.

3.2 Experimental method
A clinical trial was done during two weeks in a farm in Dalsland. The farm possesses 253 cows in total. All the cows went through a milking parlour where they were detected with two thermal cameras, one on each side of the cow. Thermal cameras are heat sensors that registers different temperature levels and convert them into a film or video image. The higher the camera’s resolution, the more precisely the camera can pinpoint the heat sources. For the chosen thermal cameras, the IR-resolution was 320 x 240 pixels, minimum focus distance 0.4 m, image frequency 3 Hz and object temperature range -20 to +120℃. The cameras have built-in memory for image storage and were directly connected to PC. All the images are in 2D matrices with temperature for every pixel, which are finally analyzed.

The cows that were detected with an abnormality of 1.3 or more were alarmed and treated with liniment. If the abnormality was less than 1.3 then no action was taken. The treatment was carried on for 3 days of every alarmed cow. 15 ml liniment was applied on the alarmed cows 2-3 times a day.

From the experimental groups explained in section 2.2.1., study groups i. and ii. were treated with each liniment (Agricam liniment and liniment A), 10 cows were chosen in each group. The liniments were likewise applied on 10 chosen healthy cows to observe the temperature differences in the udder, the same procedure as the alarmed cows was done on the healthy cows, with the same threshold, 1.3. That is, in total, 20 cows were chosen to be applied with the two liniments.
4. Results

For a t-test to be valid, it is essential to perform a normal distribution curve. The test showed a bell shape curve which indicate normal distribution.

The following figures shows the results from two different farms. Farm 1 had a threshold of 1.3 in abnormality, the herd had a total of 253 cows and farm 2 had a threshold of 1.45 in abnormality with a total of 350 cows. Figure 9 is showing no treated cows from farm 2 and Figure 10 is showing treated cows with Agricam liniment from farm 1.

On the Y-axis, abnormality for left- and right front of the udder of the cow is measured and the maximum value is chosen, which is crucial for the alarm to be triggered when above threshold. The x-axis is showing all the individuals that are alarmed and the treatment period before (abnormality above the threshold) and the mean value of 10 days after the last day treated with liniment. The red horizontal line represents the threshold for every farm, where farm 1 has threshold 1.3 and farm 2 has threshold 1.45. The black line slope is calculated mean value of individuals that were alarmed before and after the treatment, illustrated with ‘x’.

Figure 9 represents farm 2 with a threshold of 1.45 where the cows were not treated. The data was analyzed to observe how the temperature varies without treatment. A decrease in abnormality could be seen, where the mean value before is 1.64 and abnormality after is 0.76.
Figure 9. The figure represents no liniment treated cows. Every dot represents one cow. Average abnormality of 2 days before the alarm was triggered and average abnormality 10 days after the supposed treated period with liniment is represented. The black cross is the average deviation for all the cows before and after supposed treatment. The red horizontal line is the threshold for the farm 2, 1.45.

Figure 10 represents sick cows that were treated with Agricams’ liniment on farm 1 with a threshold of 1.3. The mean value of abnormality before was 1.64 and mean of abnormality after was 0.84, which gave a decreased slope (black line) as seen in the figure.
Figure 10. The figure represents treated cows with Agricams’ liniment. Every dot represents one cow. Average abnormality of 2 days before the alarm is triggered and average abnormality of 10 days after the treatment period with liniment is presented. The black cross is the average deviation for all the cows before and after treatment. The red horizontal line is the threshold for farm 1, 1.3.

The same process and selection were applied when testing liniment A on sick cows. The clinical study was applied on farm 1 with threshold 1.3 and the results showed a mean abnormality value of 1.40 before and 0.79 after the treatment (black line slope).
Figure 11. The figure represents treated cows with liniment A. Every dot represents one cow. Average abnormality of 2 days before the alarm was triggered and average abnormality 10 days after the treatment period with liniment is represented in the figure below. The black cross is the average deviation for all the cows before and after treatment. The red horizontal line is the threshold for the farm, 1.3.

Ten healthy cows were selected to be treated with liniment A and Agricam liniment during a three day period. The trial was done on farm 1 (threshold 1.3). Figure 12 and 15 shows the abnormality differences before and 10 days after the treatment whereas the black cross indicates the mean value of abnormality before and after the treatment.

Figure 12. Ten different cows with an abnormality less than 1.3 were alarmed for treatment with liniment A and analyzed after treatment. An increased mean value of abnormality could be seen when looking at the black cross before (0.52) and after treatment (0.70).
Figure 13. Ten different cows with an abnormality less than 1.3 were alarmed for treatment with Agricam liniment and analyzed after treatment. An increased mean value of abnormality could be seen when looking at the black cross before (0.52) and after the treatment (0.95).

Table 2 shows the output from the clinical trial; type of liniment, p-value, significance and average abnormality before and after treatment. It can be seen that there was significance in all the methods. As mentioned in section 2.3 Models, t-test was calculated to determine significant difference as could be noticed in all the studies done. The significance was based on the statistical outcome of t- and p-value, both showed a rejection of null hypothesis, meaning there is a significant difference.

Average abnormality is the mean value of all the individual’s abnormality before and after treatment. As can be seen in the table, average abnormality is decreased for sick cows when no liniment is applied (0.88 in difference). Sick cows treated with Agricam liniment shows bigger decrease (0.68 in difference) than liniment A (0.61 in difference). Applying the liniment on healthy cows shows an increased average abnormality, where healthy cows applied with Agricam liniment showed a higher increase (0.43 in difference) than liniment A (0.18 in difference).
Table 2. Types of liniment, if there are any significance, p-values for different average abnormality before and after treatment. Results for no liniment shows average abnormality before supposed to be treated whereas average abnormality after is 10 days after when supposed to be treated.

<table>
<thead>
<tr>
<th>Liniment type</th>
<th>P-value</th>
<th>Significance (Yes/No)</th>
<th>Average abnormality before</th>
<th>Average abnormality after</th>
</tr>
</thead>
<tbody>
<tr>
<td>No liniment, sick cows (fig.9)</td>
<td>0.002</td>
<td>Yes</td>
<td>1.64</td>
<td>0.76</td>
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<tr>
<td>Agricam liniment, sick cows (fig.10)</td>
<td>0.004</td>
<td>Yes</td>
<td>1.52</td>
<td>0.84</td>
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<tr>
<td>Liniment A, sick cows (fig.11)</td>
<td>0.0077</td>
<td>Yes</td>
<td>1.40</td>
<td>0.79</td>
</tr>
<tr>
<td>Agricam liniment, healthy cows (fig.12)</td>
<td>0.001</td>
<td>Yes</td>
<td>0.52</td>
<td>0.95</td>
</tr>
<tr>
<td>Liniment A, healthy cows (fig.13)</td>
<td>0.0017</td>
<td>Yes</td>
<td>0.52</td>
<td>0.70</td>
</tr>
</tbody>
</table>

One-Way-Anova was done to test the equality between “No liniment”, “Agricam liniment” and “Liniment A” for sick cows. One-way-Anova results in no significance between the treatments. P-value=0.9352 is bigger than α=0.05 so the null hypothesis is not rejected and there is no significant effect between groups. Same conclusion can be said about statistical F; F=0.067 < F_{crit}=3.0265, means no significant difference.

Table 3. One-Way-Anova table between “No Liniment”, “Agricam liniment” and “Liniment A”. SS is the Sum of Squares, df is Degrees of Freedom, MS is Mean of Square. F is the statistical value and F_{crit} the critical value.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>SS</th>
<th>Df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F_{crit}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>0.0643</td>
<td>2</td>
<td>0.0322</td>
<td>0.067</td>
<td>0.9352</td>
<td>3.0265</td>
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<tr>
<td>Within groups</td>
<td>141.28</td>
<td>294</td>
<td>0.4806</td>
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<tr>
<td>Total</td>
<td>141.35</td>
<td>296</td>
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</table>
A second One-Way-Anova test was performed to test significance between “Agricam liniment” and “Liniment A” on healthy cows. The results are shown in table 4. P=0.0588 is bigger than $\alpha=0.05$ so the null hypothesis is not rejected and there is no significant effect between the groups. The F statistic results in $F=4.069 < F_{\text{crit}}=4.4139$, meaning no significant difference.

**Table 4.** One-Way-Anova table between “Agricam Liniment” and “Liniment A”. SS is the Sum of Squares, df is Degrees of Freedom, MS is Mean of Square. F is the statistical value and $F_{\text{crit}}$ the critical value.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>SS</th>
<th>Df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>$F_{\text{crit}}$</th>
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</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>0.9239</td>
<td>1</td>
<td>0.924</td>
<td>4.069</td>
<td>0.0588</td>
<td>4.4139</td>
</tr>
<tr>
<td>Within groups</td>
<td>4.0872</td>
<td>18</td>
<td>0.2271</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5.0112</td>
<td>19</td>
<td></td>
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</table>
5. Discussion

Healthy cows treated with liniment A and Agricams’ liniment in Figure 12 and 13 showed an increase in abnormality. An increase in abnormality is related to increase in cows’ individual temperature and the herds temperature (Appendix A), that in return dilates the capillaries and raise in blood flow. When analyzing Figure 12 when applied with liniment A, 5 out of 10 healthy cows acquired a decreased abnormality and the rest gained an increased abnormality. In comparison, Figure 13 illustrates application with Agricams’ liniment, 9 out of 10 acquired a decreased abnormality and only 1 was increased. However, the statistical One-Way-Anova test showed no significance between Agricams’ liniment and liniment A (table 4).

There are several factors to consider which could lead to incorrect results. One of them is that, although the staff were informed on the procedure to apply the liniment thoroughly, there is no documentation to assure this has been done. Therefore, some cows could have been ignored or missed, hence not treated at all. That could explain the results in Figure 12 where only 5 out of 10 cows had an increase in abnormality.

Finally, the cows might have had a normal temporarily temperature raise because of the climate, or that the cows became sick during that specific time period because of other reasons. Climatic conditions should have been recorded systematically. Taking these factors in consideration, it is difficult to interpret the results in any meaningful way.

The sick cows show a decrease in abnormality after the treatment with both Agricams’ liniment and liniment A (Figure 10 and 11). However, Figure 10 (treatment with Agricams’ liniment) illustrated a larger difference in decrease (as can be seen in table 2). The same pattern was seen in Figure 9 (decrease in abnormality), when the sick cows were not treated with any liniment. When doing a One-Way-Analysis test on significance between the three groups of sick cows “no treatment”, “Agricam liniment” and “Liniment A”, the difference showed no significance (table 3). The thermal cameras could be another cause. The thermal cameras usually become dirty because of the mud, dust or other particles that get stuck on the glass lens, making it difficult to detect the object (the udder). Because of that, the temperature sensed might be incorrect and wrong data is then held for analysis. This is usually avoided by cleaning the cameras every now and then. In the cases of this is missed, if a higher temperature than actual is detected, then application of liniment is unnecessary, leading to higher staff and liniment costs. If a lower temperature is sensed, then there is a high risk that the cows that are supposed to be alarmed and treated are instead neglected, leading to undetected cows which could be in the risk-zone of becoming sick. Moreover, the risk for mastitis is increased, leading to high costs of treating the
cow using antibiotics\textsuperscript{9}.

The non-treated sick cows had a decrease in abnormality, Figure 9. The sick cows that were treated with Agricam liniment and Liniment A both had a decrease in abnormality, Figure 10 and 11. The healthy cows treated with Agricam liniment and Liniment A both had an increase in abnormality, Figure 12 and 13. The decrease in Figure 9 was bigger than the treated cows in Figure 10 and 11. That can be explained by the absence of liniment that can keep the temperature level higher. In subclinical mastitis cases, the cows could be sick but not always show symptoms in terms of increased temperature. It can go from high temperature to low, but still be sick and therefore, show a bigger decrease in abnormality as in the figure. Another aspect could be that, the inflammation might be starting in a specific place of the udder where the thermal cameras can miss detection and therefore, showing a lower temperature than actual. The decrease can be a normal behavior of cows when the infection shows a signal and go back to its stage due to its own immune response that has defeated the bacteria. Another explanation could be that the data is random and the reason for this behavior is unknown and would require further data analysis.

Figure 10 and 11 both showed a decrease in abnormality after treatment, where one possible explanation could be the long-term effect the liniment has i.e. the time it takes for the liniment to work. Another reasonable explanation can be that the generally high body temperature of the cows. If the liniment gives a slight temperature raise as in figure 12 and 13, then a higher temperature in Figure 10 and 11 is not possible and the long-term effect is decrease in temperature as a result. It is reasonable to think that the cows get an increased temperature in the udder for that specific point where the inflammation might be starting and with the help of liniment, the heat will be spread around the area for the blood flow to the infected area\textsuperscript{19}.

It cannot be concluded if the cows had mastitis after liniment treatment or not. An analysis of this kind of data would be required to draw conclusions if the liniment treated cows developed mastitis or not and compare that number with the amount of untreated cows. From earlier studies SCC level were detected and could conclude the liniment effect which resulted in a decreased number of SCC\textsuperscript{11}. Similar detection could be done here, counting the SCC levels for each and every application on every cow.

It can be concluded that liniment has an increased temperature effect, which can be seen on healthy cows in Figure 12 and 13 (Agricams’ liniment and liniment A) and the formula explained in section 2.2.1. There is no significance between Agricams liniment and Liniment A (table 4). However, more data would be preferable to make
this conclusion reliable since these temperature differences could be more random variables than actual in this limited study.

It can also be said that liniment has a significant effect before and after treatment in all experiments when looking at p-value (table 2) which is smaller than $\alpha=0.05$ (the null hypothesis can be rejected). The t-test shows a decreasing temperature effect on sick cows, which is desired. Comparing the sick cows that were treated with the sick cows that were not treated, no significance was shown between these analyses, table 3.

Different studies show different results regarding different factors and their effect on cows, with no clear indications of what it depends on. Some studies showed that lactation number has an effect whereas others showed that factors such as lactation number, stage of lactation and season of the year have minor influences. Therefore, limiting the selection upon only certain lactation number (the ones with highest risk for mastitis) to be treated with liniment would be a worthy investigate.

From earlier literature studies, the production of milk with low SCC must be achieved for economic benefits because low SCC means higher milk yield and quality and therefore higher cheese quality and yield.

As a summary, Figure 9, 10 and 11 shows significant decrease in temperature in sick cows, which is anticipated. Figure 12 and 13 shows a significant increase in temperature for healthy cows, which can be explained by characteristics of liniment to increase the blood flow in the udder.

As a conclusion it can be said that the liniment has an effect in healthy cows (it increased the temperature). The long-term effect of liniment decreased the temperature on sick cows. In the non-treated sick cows, same pattern as treated sick cows could be seen (decrease in temperature). One explanation could be that the sick cows did not show temperature changes because it is subclinical effect (which cannot be seen as a temperature change) or that the cows treated defeated the infection and went back to their normal stage.

One-Way-Anova results in table 3, showed no significance between the three groups so the population means are all the same. Treating it with or without the liniments have no significant effect. Same conclusion can be drawn from table 4, when analyzing the differences in healthy cows with Agricam liniment and Liniment A.
6. Future studies

For future studies, it would be beneficial to follow a group of sick cows that are not treated with any liniment and investigate if they get mastitis and compare with the cows that were treated.

Different studies has shown that the lactation number and stage of lactation has an impact, others showed those factors have minor influences. Clinical studies could be done based on those factors and grouping the cows to analyze the differences.

Moreover, different SCC numbers are given on different studies as an indicator of infection. A study following the different cell count number before and after treatment indicating variation in dairy herd would be a suggestion since it might vary in different farms.

Some cows have more active immune response than others, leading to triggering of alarms more frequently. Another suggestion to foresee the infection could be by implementing predicting models that can alarm on cows that might have a risk of getting an increased temperature and alarm before they reach that stage so they can be treated beforehand.
7. Conclusion

The aim of this project was to identify if the Agricam liniment had any effect on cows, compare the Agricams’ liniment to another selected liniment and conclude which one gives better results. To show this, t-test and One-Way-Anova analysis were performed.

As explained in the discussion, healthy cows get an increased temperature when applied with both tested liniments (Agricams’ liniment and liniment A). The sick cows that are treated with both liniments gets a decrease in temperature. Agricams’ liniment showed a higher temperature increase in healthy cows compared to liniment A. However, with this analysis, it is not possible to conclude if there are any significant differences between the liniments since both showed significant effect of a decreasing temperature.

Since the study could not show any conclusions of which liniment is more likely to prefer, it is suggested to continue using the Agricams’ liniment. Agricams’ liniment is cheaper, which is more beneficial for the farmer to consider buying the liniment. More tests are suggested to run between the treatments to analyze data for more reliable results.

Conclusions are difficult to come to regarding untreated sick cows which had a considerable decrease in temperature, since no data of development of mastitis is given. It is possible that the cow healed itself or have subclinical mastitis.

One proposition for further analysis of data is to follow a group of sick cows that are not treated with liniment to investigate if mastitis has occurred and compare with sick cows that are treated and investigate if they developed mastitis and in such cases, how large the difference seemed.

Because of similar pattern of the results from the three tests (non-treated, treated sick cows with Agricams’ liniment and Liniment A), it is difficult to conclude which liniment effect is better. The statistical One-Way-Anova test showed no significance between the three treatment groups. The results question if a liniment should be used at all if the cows can heal themselves within the same timeframe.

One of the objectives in this project was to write a test protocol for the clinical trial and upcoming field studies. However, this part was at first thought not necessary since the experiment was done on the same way as with the Agricams’ liniment but it turned at the end of this project that it would have been valuable to consider some pitfalls like which exact cows that were treated with liniment to assure that the application has been done. Instead, the staff were informed of the procedure of application to be done thoroughly and be aware not to miss any cows. However, there are no documentation that this is the case. Finally, instead of comparing two liniments with the one from Agricam, only one was compared because it was too time consuming to proceed more
clinical studies and not the most important in this study. Instead as mentioned before, the focus was mainly on the data analysis of the effect of liniment in general on cows.
Acknowledgment

I would like to thank all the people that have supported me in this project. First, a thank to my supervisor Robert Gustavsson, who was willing to help me out when times of crisis and for my examiner, Jordi Altimiras who had all the good feedback for a better work. A big thank for my supervisors at the company, Ellinor Eineren, for allowing me to do my thesis at Agricam, Johan Waldner for his excellent expertise about cows, curiosity and encouragement in this project, Martin for all the data collection and software help and his easy-going and helpful mindset. My amazing supportive friend at the company, Erika Andeskär, for all the statistical help and moral support and all the lunch-walks and interesting discussions. Stefan Stenmark for reading and commenting on my report. Yousif Toma for all the report advises and help throughout the work process. Finally, for the farm who was willing to do this clinical study and make it possible to happen.
References

9. Doehring C., Sundrum A. The informative value of an overview on antibiotic consumption, treatment efficacy and cost of clinical mastitis at farm level. Preventive Veterinary Medicine. 2019;165(63-70).


Appendix A

Because of company restrictions, Appendix A is not included. Appendix A represent the formula for calculating abnormality.
Appendix B

Clinical study of different liniments on cows to prevent mastitis

For clinical mastitis in cows

Zen Dinha

Examiner, Jordi Altimiras
Supervisor, Robert Gustavsson
External supervisor: Johan Waldner
Abstract

The aim of this project study is to find a potential liniment that can be applied on cows by literature study and clinical trials.

The goal is to test different liniments with other characteristics than the current liniment Agricam is using. In order to reach the goal, literature study need to be done to understand the pharmacology of the ingredients the liniment is consistent with and choose the one with better fit. Two clinical trials will be proceeded with two different liniments and compared with the current liniment that is used. For a clinical trial to be proceeded, a test protocol will need to set up. The comparisons between the treatments will be done by statistical models with all the observations treatments in temperature and somatic cell count. Finally, an economic analysis of cost estimation will be done.

One hypothesis is that depending on the ingredients in the liniments; the results should be different. According to earlier studies on different ingredients, they will have different efficiency.

The methodology to investigate the hypothesis is to investigate the literature studies and compare it with the clinical studies that will be done. By comparing the temperature measures before, during and after applying the liniment it is possible to see the effects of the liniment, taking somatic cell count in consideration.
Purpose of the project

Mastitis is a disease considered to be one of the most frequent and costly diseases in dairy industry. The disease has been treated with antibiotics for decades, contributing with undesirable consequences for processors and consumer. Hence, it is vital to find cheap and efficient solutions for farmers to treat the cows before mastitis occur.

As for Agricam, finding and testing a liniment with better effects will give many valuable features, i.e. a more reliable service and products, which will give a better customer service, proven quality product by clinical trial results. This in return will bring more customers and higher net sales for the company.

Furthermore, the project has the aim to start a clinical trial that can continue over time, do more tests to find a higher quality and more reliable data collection will be proceeded. By acknowledging the trial and knowing the process for the first time, it will be easier to continue doing the trials in the future for testing new liniments.
Hypothesis of the project

It is possible to detect different temperatures by thermal image automation cameras installed in the farms\(^1\). By analyzing the temperature deviation in an early stage, it will be feasible to take a stand in treating with liniment.

The different liniment types containing different ingredients will give altered results because of their activity and efficiency.

Depending on the lactation number/age of the cow and the temperature rise, the somatic cell count should show a difference in different cows\(^2\).

By using the most efficient liniment, cost estimation should be lowered in the long term for farmers.
Objective

The objectives of the project is to:
1. Compare two different liniments with the current liniment that is used.
2. Provide a test protocol for the clinical trials and upcoming field studies.
3. Proceed an economic analysis for the different liniments.
4. For future studies; for those that the liniment does not have any affect, use a complementary treatment and consider alternative methods to cease mastitis.

Boundary conditions

Since the project is done at a company, it will not be possible to provide all the information about costs and how some methods/formulas are performed because of secrecy at the company. Therefore, some information will be excluded or might be estimated.
**Study model**

Two thermal cameras are placed on the both side of the cow to have a better picture of the udder of the cow which the udder is divided into four parts; front-left, hind-left, front-right and hind-right. The cows go through the camera detection and continue in either a circular motion or herring-bone milking parlour. All data is measured, collected and a camera module is set with an identification detector placed nearby for udder detection. Thereafter, an imagine analysis is done to make data measurements. The measurements will show different deviation, whether it is a “normal” deviation then no decision is made. However, if the deviation is above the threshold, either action will be taken by applying with liniment. If deviation is under threshold, no action is taken.

The focus in this system will be on the data measurements where the temperature will be measured to detect the deviation and on the type of liniment that will be used.
# Milestones for the project

Table 2. Milestones with short description, type of deliverable and date.

<table>
<thead>
<tr>
<th>Milestone number</th>
<th>Short description</th>
<th>Type of deliverable</th>
<th>Date (week)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Project description</td>
<td>Report</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>Planning report to supervisor and examiner</td>
<td>Report</td>
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<tr>
<td>3</td>
<td>Order material</td>
<td>Table, diagram</td>
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<tr>
<td>4</td>
<td>Results from mathematical simulation</td>
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<td>Results from 1&lt;sup&gt;st&lt;/sup&gt; clinical study (liniment type 1)</td>
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<td>8</td>
<td>Half-time report</td>
<td>PPT presentation</td>
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<td>9</td>
<td>Results from 2&lt;sup&gt;nd&lt;/sup&gt; clinical study (liniment type 2)</td>
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# Time plan

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<td>Data collection and evaluation of trial clinical study</td>
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<td>Clinical study</td>
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<td>Interim report</td>
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<td>Evaluation report</td>
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<td>Final report to examiner for final approval</td>
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Note: The table above shows the activities, their dependencies, and the corresponding weeks. The diagram visualizes the timeline and dependencies of these activities.
References