THESIS

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Drying off of dairy cows without antibiotics

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List of abbreviations

ADCT – Antimicrobial dry cow therapy

CMT – California Mastitis Test

CNH – Casein hydrolysate

DCT – Dry cow therapy

DHIA – Dairy herd improvement association

DNA – Deoxyribo-nucleic-acid

FMO – Frequent milk-out

IMI – Intramammary infection

ITS – Internal teat-sealant

MIC – Minimum inhibitory concentration

SCC – Somatic cell count

SDCT – Selective dry cow therapy

1. Introduction

Mastitis is a common inflammatory disease afflicting the udders of dairy farm cattle, throughout the lactating as well as non-lactating period. The condition is caused by various pathogens, like environmental bacteria, entering into the udder (LIC AutomationTM, 2021). The disease can be expressed in various clinical forms, acute- or chronic clinical mastitis, and as a subclinical form. Different diagnostic methods are used for differentiating between these clinical mastitis forms. Differentiation is performed on an individual or a herd-level and is based on the expressed inflammatory symptoms as well as the somatic cell count level measured within the milk. Treatment and management of the condition is based on pathogen-based therapy (Klocke & Walkenhorst, 2007). Many different treatment methods can be used, but depending on the causative agent, these treatments may have varying rates of success (Vilar & Rejala-Schultz, 2020). Antimicrobial treatment is used for hard-to-cure cases of clinical mastitis, the antimicrobial drug used interacts with the causative bacteria in one of two ways, either the bacteria dies or it is unaffected by the drug. If an antimicrobial drug is ineffective against a bacterial species, it is frequently due to antimicrobial resistance. Bacterial resistance can be naturally occurring through generational evolution or it may be acquired from another bacterial species (Reygaert, 2018). However, mastitis caused by antibiotic-resistant bacteria is much more difficult to cure than udder inflammations caused by a non-resistant bacterial species (WHO, 2020). To decrease the antimicrobial resistance, alternative treatment methods to antibiotics are carried out by many practitioners. Some alternative treatment methods include, frequent milk-out, teat-sealants and early cessation of milk (Vilar & Rejala-Schultz, 2020).

2. Objectives

The purpose of this literature review was to gain a deeper understanding of the theory behind the antibiotic situation in relation to the problem of bovine mastitis, especially during the drying off period.

First and foremost, it is important to build up a broad spectrum of background data to understand the current state of the mastitis disease in Sweden as well as the prevalence of antimicrobial resistance to antibiotics. Alternative means of antibiotic mastitis treatment were sourced and evaluated for their potential as mastitis cures and as antibiotic replacements.

Secondly, field data was collected from a smaller scale dairy farm situated in the south of Sweden. The aim was to get an overview of the mastitis prevalence on a farm over a one-year period, and to compare the mastitis trend with that of a trend from four years prior.

Lastly, most of the base theories pertaining to mastitis research are more than five years old. However, several newer studies supporting these base theories, have been performed within the last five years. In the cases where older sources are used, they were screened to provide support for the newer ones. Older work is presented when more current data is not available.

3. Material and Methods

The data used within this thesis was obtained through medical literature, published scientific articles, and to a lesser extent from trusted internet domains. The search engines used for the purpose of gathering information relevant to this thesis topic were “Google Scholar” and “Science Direct”.

To collect articles, the following keywords were used in different combinations; “mastitis”, “intramammary infection”, “dry-cow”, “therapy”, “selective”, “protocol”, “teat-sealant”, “homeopathy”, “dry-off”, “antibiotics”, “resistance”, “Staphylococcus aureus”, among others.

Literature such as books and articles in both English and Swedish were used. A Swedish report, stating the dairy herd´s test milking result on Lyngens gård, was evaluated and interpreted into tables and a graph.

4. Literature review

4.1 Mastitis background

4.1.1 Overview

Mastitis is a process that occurs in the udder of a cow and may affect a minimum of one udder quarter of the mammary gland, it is prevalent during both the lactating and dry (non-lactating) periods of dairy cows (LIC AutomationTM, 2021). During this time the cows are milked with the intent to decrease the milk production before eventually ceasing it completely, since high milk yield at dry-off, the final milking at the end of lactation, increases the risk for intramammary infections (IMI). The milk yield at dry-off is important for the rapid involution of the udder, the process the mammary gland undergoes to return to its non-lactating state, which will stimulate the efficiency of the immune system to promote udder health. One of the most common methods used for dry-off dairy cows is the gradual cessation of milking towards the end of the lactation period, which physiologically indicates for the animal that it produces more milk than what it can get rid of and so milk yield decreases at dry-off. However, drying-off practices differ between countries as well as herds and may include various milk cessation methods. Examples of these methods are changes in feeding and milking frequency, the use of antimicrobial dry cow therapy (ADCT) and teat-sealants, and potential changes in housing (Vilar & Rejala-Schultz, 2020). There are studies proving that DCT reduces the instance of clinical mastitis in a within-cow comparison, where some cows were treated with dry cow antimicrobials and later compared to untreated control cows (Scherpenzeel et al., 2016).

After milking the teat-canals are physiologically open for a short period of time. During the time it takes the teat-canals to re-seal themselves, there is a window of opportunity for bacteria to enter into the udder. The teat-canals, therefore, work as a gateway through which the microorganisms may enter into the cow's udder and cause IMI. Studies have shown that teat-canal diameter, increased sphincter patency, and teat injuries have a strong positive association with the high somatic cell count (SCC) which indicates the presence of IMI. The physical appearance of the udder is also a factor to be taken into consideration, and plays a part in the overall mastitis prevalence of a herd. For example, cows with dish-shaped or well-attached rounded udders are less prone to get mastitis compared to cows with pendulous-shaped udders. A short teat-floor distance also increases the risk for both teat- and udder

injuries as well as for environmental contaminations. Furthermore, a short udder to ground distance is associated with a predisposition for mastitis (Woloszyn, 2007).

4.1.2 Mastitis pathogens

IMI is most frequently caused by bacterial pathogens, the disease can however be caused by yeast, algal or protozoal infections to a lesser extent as well (Royster & Wagner, 2015). Mastitis is a potentially fatal inflammation of the cow´s mammary gland, the udder, which is usually caused by bacteria entering the teat canal and moving into the udder tissue. Furthermore, a large number of white blood cells, leukocytes, will migrate into the mammary gland as a response to the invading bacteria, and thus activating the animal's immune system. The bacteria usually enter the teat-canal through contact with the environment or during the milking process. It may also be caused in case of an injury to the cow´s udder (LIC AutomationTM, 2021). The major mastitis pathogens such as *Staphylococcus aureus*, *Streptococcus uberis*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, and the coliforms are usually more virulent and damaging to the udder compared to the minor mastitis pathogens such as *Corynebacterium bovis* and the coagulase-negative staphylococci (Reyher et al., 2012; Royster & Wagner, 2015). The toxins released by the mastitis bacteria damage the milk-secreting tissue and ducts throughout the mammary gland, resulting in a reduced milk yield and quality. IMI can occur at any stage of lactation, including the dry period, regardless of the cow's age. However, it is most likely to occur in the first month after calving and in late lactation (LIC AutomationTM, 2021).

4.1.3 Clinical forms of mastitis

Mastitis is a disease that can be classified as acute clinical, depending on the visible characteristics of the IMI present, or as subclinical mastitis based on two laboratory diagnostics parameters, the milk´s microbiological profile and the somatic cell count (SCC) (Klocke & Walkenhorst, 2007). As the somatic cells contained in the milk derive from the blood, most of these are primarily white blood cells, leukocytes, and present as part of the udders´ protective mechanism, the immune system. Additionally, the presence of milk-producing cells, epithelial cells, can be found free-floating in the milk but to a much lesser extent than compared to the leukocytes. The presence of epithelial cells may range from 0% to 7% of the total somatic cell population found in the milk (Woloszyn, 2007).

Acute clinical mastitis can be diagnosed by checking for the following five cardinal inflammation symptoms, which can be divided into redness (latin *rubor*), heat (*calor*), swelling (*tumor*), pain (*dolor*), and lastly dysfunction, which can be expressed as secretory alterations like flakes, clots and aqueous milk secret (*functio laesa*). In cows suffering from acute mastitis, all five symptoms may not be detected. However, the most frequently observed symptoms are *calor* and *tumor*, and the acute type of mastitis therefore more or less cause a decrease in the general health condition of the affected cow (Klocke & Walkenhorst, 2007). The swollen udder is in many cases a very painful condition, which in turn influences animal welfare negatively. In order to decrease the discomfort and to better the general state of the animal, fluids can be given in addition to anti-inflammatory drugs (Roberson, 2012). There are, besides the acute type of mastitis, cases where only secretion symptoms are expressed, these are classified as chronic mastitis cases and can be considered less severe types of mastitis (Klocke & Walkenhorst, 2007).

The subclinical type of mastitis is as previously described a very problematic disease with few to no symptoms visible, as the milk looks normal and the udder shows no signs of *rubor* nor *tumor*. The cow looks healthy in general, with no signs of fever, depression nor any dramatic drop in the milk yield of the infected quarters. Yet, there is a large overall reduction in milk yield caused by the subclinical mastitis and any undetected cows may contribute to the infection spreading within the herd when the mastitis is caused by bacteria (Woloszyn, 2007). It is therefore very important to identify these animals through laboratory methods. One of the two control parameters is the SCC, which in case of increase indicates the presence of an IMI in the udder. The SCC is a measure of leukocytes such as macrophages, lymphocytes and segmented neutrophil granulocytes in the milk, and SCC is, therefore, a parameter that indicates activation of the immune system and is present in all forms of mastitis. An SCC threshold is set, by the governmental regulations and dairy companies, for distinguishing between healthy and affected milk, and this threshold may vary between countries. In a healthy udder the SCC is more or less stable, with a relatively constant number of cells secreted into the milk throughout lactation. The threshold for differentiating between a healthy or a mastitis diseased udder is commonly accepted as an SCC level of 200,000 cells/ml for an affected udder (Klocke & Walkenhorst, 2007).

4.1.4 Mastitis detection

Mastitis is a very painful condition causing suffering and potential death if untreated. The detection and treatment of mastitis in cows are critical for managing herd health, milk-quality, and maximizing production capacity of the herd. Cow mastitis detection and treatment will help you optimize your milk quality and pay-out, as well as reducing antibiotic treatments and culling expenses. A common treatment method is the use of antibiotics, a type of antimicrobial substance active against bacteria (LIC AutomationTM, 2021). A common diagnostic tool used for detection of mastitis based on the SCC is the California Mastitis Test (CMT), and according to the Scandinavian scoring system udder disturbances are present at a score of 3 or higher, where SCC exceeds 300,000 cells/ml (Woloszyn, 2007). In Sweden, a healthy and lactating udder should have an SCC of <100,000 cells/ml. Subclinical mastitis is suspected when the SCC is higher than normal. Clinical mastitis is suspected at >100,000 cells/ml for primiparous cows, first-time calvers, and >150,000 cells/ml for multiparous cows, cows that have calved at least twice (Persson Waller, 2018).

Table 1. Mastitis type definition and SCC standards

Mastitis type	Definition	Swedish SCC standard range
Subclinical mastitis	A non-visible inflammation of the mammary gland, requires diagnostic tests for detection. Frequently diagnosed with SCC. Most prevalent form of the disease.	≥100,000 cells/ml
Mild clinical mastitis	Little or no signs of swelling of the mammary gland with abnormalities observed in the milk, generally flakes or clots.	>150,000 cells/ml - <250,000 cells/ml
Moderate clinical mastitis	Swelling of the infected mammary gland with obvious abnormalities observed in the milk, however, no other systemic signs of illness.	>250,000 cells/ml - <350,000 cells/ml
Severe clinical mastitis	Characterized by sudden onset of inflammation with grave systemic and local symptoms.	≥350,000 cells/ml

This can be compared to the American milk recording association DHIA, Dairy Herd Improvement Association, which has set their threshold value to DHI score no 5. This is equivalent to an SCC of approximately 283,000 cells/ml of milk. Furthermore, it is important to distinguish between SCC measurements done on the whole udder and those done on an udder quarter level. Due to the anatomical structure of the udder, which comprises four separate milk-producing glands independent of each other, only one-quarter of the udder can

be infected. Thus, elevated SCC in one affected quarter may be masked by the dilution effect from the other healthy quarters in a composite milk sample, especially since the affected udder quarters with a high SCC decreases in milk yield (Woloszyn, 2007).

4.2 Antibiotic resistance

4.2.1 Overview

Antibiotics are medicines used for the prevention and treatment of bacterial infections, and antibiotic resistance occurs when bacteria change in response to the use of these medicines (WHO, 2020). When antibiotics were first introduced in the medical field in the 1900´s, it was thought that the war against microorganisms was won. It was soon discovered however, that the microorganisms were capable of adapting and developing resistance to any of the drugs that were used at that time (Reygaert, 2018).

Antibiotics have a long history of saving millions of human and animal lives, as well as contributing to improving health and welfare. However, during the last decades, there has been a steadily growing concern for antimicrobial resistance harming human and animal health, as well as on food safety. Antimicrobial resistance is therefore a global public health concern and provides a major challenge for the future of society. The use of antibiotics on animals and humans benefits the already resistant bacteria, at the same time as it kills off the sensitive bacteria. The resistant bacteria may work as pathogens inducing sickness in the patient, or the bacteria may just be a part of the natural bacterial flora of the host, causing dysbacteriosis if the balance is disturbed (Emanuelson et al., 2018). It is important to note that it is the bacteria that develop resistance to antibiotics, not humans or animals. When these antibiotic-resistant bacteria then infect humans and animals, the infections they cause are harder to treat than those caused by non-resistant bacteria (WHO, 2020).

The use of antibiotics in the field of the livestock industry is a contributing factor for the development of antimicrobial resistance to antibiotics, and it is therefore an important goal to decrease the need and use of such antibiotic treatments in animal production. A big part of the total use of antibiotics in Swedish animal husbandry is the dairy industry. In Sweden many organic dairy farmers are of the opinion that prevention, rather than treatment, plays a key role in the decrease of the antimicrobial resistance, including in the use of DCT (Scherpenzeel et al., 2016; Emanuelson et al., 2018).

The world needs to urgently change the way it prescribes and uses antibiotics. Even if new medicines are developed, without a change in our behaviour, antibiotic resistance will remain a major threat. Especially, since in some parts of the world it is possible to buy and use antibiotics without a prescription, which makes the emergence and spread of antimicrobial resistance worse. Similarly, in countries without standard treatment guidelines, antibiotics are often over-prescribed by both health workers as well as veterinarians and as a result are often over-used by the public. This leads to the forced development of new antibiotics, however none of them are expected to be effective against the most dangerous forms of antibiotic-resistant bacteria. The World Health Organization endorsed a global action plan in 2015 in response to the rising problem of resistance, the "Global action plan on antimicrobial resistance", which consists of the following objectives. Increase the understanding and awareness of antimicrobial resistance, strengthen surveillance and research, and reduce the incidence of infections. As well as, optimizing the use of antimicrobial medicines and ensuring sustainable investment in countering antimicrobial resistance (Reygaert, 2018; WHO, 2020).

Reygaert (2018), also states that the transmission of antimicrobial resistance from animals to humans may occur in various ways, where the most common route is the direct oral route. Which includes consumption and ingestion of antibiotic-treated meat and faeces contaminated food or water. Another common route of transmission is through direct contact with the animals and animal keeping. In order to stem the increases in resistance various methods of antimicrobial stewardship have been suggested, where one method suggests the diversity in antimicrobial use. This refers to various components such as not only giving a single drug, but instead use two or more drugs, either through alternation or in combination and where the drugs used preferably have different mechanisms of action (Reygaert, 2018).

4.2.2 Microbial activity

The main mechanisms of antimicrobial resistance are: limiting uptake of a drug, inactivation of a drug, modification of a drug target, and the active efflux of a drug from the system.

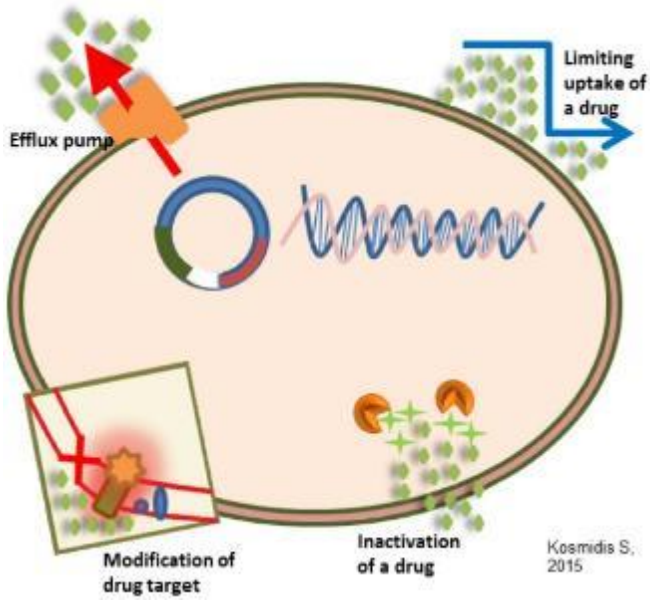

Figure 1. General antimicrobial resistance mechanisms (Kosmidis, 2015)

These mechanisms may be native to the microorganisms, or they may be acquired from other microorganisms and where the understanding of these mechanisms could lead to better treatment options for infectious diseases. As well as, the development of antimicrobial drugs that can withstand the microorganisms attempts to become resistant. Antimicrobial agents can, depending on their mechanism of microbial activity, be divided into groups. Where the main 5 groups and drugs that work on these are: agents that inhibit cell wall synthesis (e.g., β-lactams and glycopeptide agents), inhibit protein synthesis (macrolides and tetracyclines), inhibit nuclei acid synthesis (fluoroquinolones and rifampin), inhibit the metabolic pathway (trimethoprim-sulfamethoxazole), and depolarize the cell membrane (polymyxins and daptomycin) of the bacteria (Tenover, 2006; Reygaert, 2018).

The first group, the agents that interfere with the cell wall synthesis includes the β-lactams, such as penicillin and cephalosporins, and glycopeptides, including vancomycin and teicoplanin. In short, β-lactams agents interferes with the enzymes required for the synthesis of the peptidoglycan layer, which makes up the bacterial cell wall, and thus inhibits the cell wall synthesis resulting in the death of the bacteria. Vancomycin and teicoplanin also interfere with the synthesis of the cell wall. They do so however, by preventing the cross-linking step of the peptidoglycan chain, which is required for a stable cell wall synthesis. The second group of antimicrobial agents that inhibit protein synthesis includes macrolides,

tetracyclines and chloramphenicol. They inhibit the protein synthesis by selectively binding to the 30S subunit of the ribosome for macrolides and tetracyclines, respectively the 50S subunit for chloramphenicol and thus inhibiting bacterial growth. The third group, fluoroquinolones will inhibit nucleic acid synthesis by causing lethal double-strand deoxyribo-nucleic-acid (DNA) breakage during DNA replication. The fourth group inhibits the metabolic pathway for bacterial folate synthesis by the common antimicrobial drug combination of trimethoprim-sulfamethoxazole. Which blocks the enzymatic pathway of folic acid synthesis needed for DNA synthesis. The fifth and last, although less well characterized, mechanism of action deals with the depolarization of the bacterial cell membrane. Polymyxins increase the bacterial membrane permeability, causing the bacterial content to leak out. Daptomycin is a cyclic lipopeptide that inserts its lipid tail into the cell membrane of the bacteria, causing depolarization of the membrane and eventually bacterial death (Tenover, 2006).

4.2.3 Persistence vs resistance

Before the various aspects of antimicrobial resistance are discussed, it would be beneficial to distinguish between the resistance and persistence of bacteria. There are two possible scenarios, A and B when a bacterial cell culture is exposed to an antimicrobial agent.

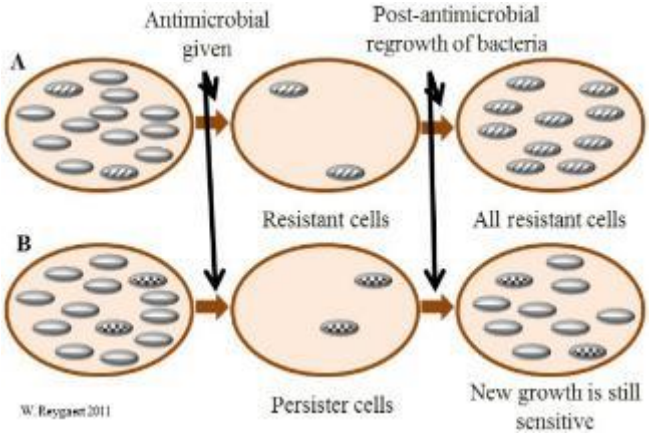

Figure 2. Persistence vs resistance (Reygaert, 2011)

In scenario A, a bacterium that is resistant to a certain antimicrobial agent will produce daughter cells that are also resistant to this agent, unless any additional mutations would occur in the meantime. The non-resistant cells will be killed by the drug, while the resistant cells will be left unharmed and these may later repopulate, resulting in all of the cells in the culture being resistant. In scenario B, persistence is used to describe those bacteria that are not susceptible to a certain antimicrobial agent and do not possess any resistance genes. The

persistence is therefore likely due to the dormant or stationary growth phase that some cells in the bacterial population undergo. Most antimicrobial agents are ineffective on cells that are non-dividing or not actively growing. In this scenario when the culture is exposed to a drug, the non-persister cells will be killed, leaving only the persister cells. When these persister cells mature and repopulate the culture, those cells that are not in a dormant state will still be susceptible to the antimicrobial agent that was used (Reygaert, 2018).

4.2.4 Innate vs acquired resistance

Levels of resistance to any particular antimicrobial agent may vary greatly within related bacterial groups, since not all bacterial groups or species are uniformly susceptible or resistant. Some bacterial species are innately resistant to at least one class of antimicrobial agents (Tenover, 2006). Therefore, the susceptibility and resistance are usually measured as a function of minimum inhibitory concentration (MIC), which is the minimal concentration of a drug required for inhibition of bacterial growth. The susceptibility for any drug given to the same bacterial species is expressed as a range of the average MIC. Should that average MIC for a certain bacterial species be within the resistant part of the range, then that species of bacteria are considered to have an innate resistance to the drug used. Natural resistance can be induced or innate, where innate resistance is a trait that is always expressed and shared universally within a bacterial species. It is not dependent on previous exposure to antibiotics. Induced resistance, however, is only expressed when the naturally occurring resistance genes are activated through exposure to an antibiotic (Reygaert, 2018).

Moreover, bacterial resistance may also be acquired through resistance genes from other related organisms, where the level of resistance may vary depending on the acquired genes and species used (Reygaert, 2018). The acquired resistance is of greater concern since initially susceptible bacterial populations will become resistant to a certain antimicrobial agent, which may later proliferate and thus spread further under the selective pressure of use of that agent. Many different bacterial genera have already attained a variety of resistance mechanisms and therefore adapted to a wide range of antimicrobial agents (Tenover, 2006).

4.3 Dry cow protocols with antibiotics

4.3.1 Overview

Drying off is a natural process in the cow´s yearly cycle. This is the intermediate stage between lactation and the dry period, which is the period where the cow is able to metabolically recuperate and prepare for the next years pregnancy. It is therefore important that the drying off of the dairy cow is done successfully and without delay. The most common problem that occur during this stage is the mastitis, which can be treated in various ways. For example, with antibiotics in dry cow therapy (DCT). An intramammary DCT formulation, which may contain a long-acting antimicrobial drug, is inserted into one of the four teats and the antimicrobial drug is thus infused into one of the four quarters of the cow's mammary gland (Royster & Wagner, 2015). The goal of the DCT can be stated as decreasing the instances of intramammary infections (IMI) by eliminating existing IMI at drying off, as well as preventing the occurrence of new IMI during the dry period. DCT that use dry cow antimicrobials (ADCT) has showed to reduce clinical mastitis, and the DCT has therefore a direct effect on the udder health during both lactation and dry period. In terms of curative use of dry cow antimicrobials, the cows need to be selected correctly. Which is done by identification of IMI based on different criteria, such as clinical mastitis history, bacteriological culture and somatic cell count (SCC). Herd-level parameters can also be taken into account, such as the bulk milk SCC (Scherpenzeel et al., 2016). The effectiveness of ADCT could be confirmed through a comparative analysis of 22 studies published before 2003, where the previously performed studies estimated that the cure rate was up to 78% successful for an antibiotic treated quarter, compared to a cure rate of 46% for spontaneous healing quarters. However, practitioners that utilize ADCT have to take the potential for violative drug residues into consideration and should to the best of their ability follow the on-label use of the prescribed antimicrobial drugs and furthermore, ensure protocol compliance from everyone involved in the ADCT (Royster & Wagner, 2015).

4.3.2 Treatment based on SCC

Since the antimicrobial resistance is of a global concern and is continuously changing, it propagates for prudent and restricted use of antimicrobial agents, and for this a selective dry cow therapy (SDCT) regimen can be used. A study was made, where SDCT was applied on the basis of withholding DCT for primiparous cows with SCC <150,000 cells/ml and for multiparous cows with SCC <250,000 cells/ml at the last recording of milk values before drying off. Primiparous cows are animals that have calved once, and multiparous cows have

calved at least twice. The participating herds bulk milk SCC varied in this study from 41,000 cells/ml to 387,000 cells/ml, with an average of 184,000 cells/ml. The evaluation of this study showed significant increases in the prevalence rate of clinical- and subclinical mastitis. The study resulted in a substantial decrease in antimicrobial usage, but with a potential negative impact on the animal welfare as well as on the economics. The treatments carried out in the SDCT applied in this study depend on the selection procedures sensitivity and specificity. A high sensitivity of selection results in more likely treatments of infected cows, whereas a high specificity results in less likely treatments of uninfected cows (Scherpenzeel et al., 2016).

4.3.3 Pathogen-directed therapy

There are considerable differences in cure rates and other outcomes among mastitis causing pathogens. Which in part can be explained by the virulence factors specific to the organism, for example immunosuppression, toxin production, varying degrees of tissue invasion and damage, or the difference may be due to a varying antimicrobial susceptibility. A pathogen example of this difference would be the lower cure rate of *Staphylococcus aureus* and *Streptococcus uberis*, compared to the cure rates of other mastitis causing species of staphylococci and streptococci. The antimicrobial susceptibility of *Staphylococcus aureus* species varies depending on if the isolated bacteria are positive for β-lactamase, the enzyme that deactivates β-lactam antibiotics like penicillin, are known for having much lower cure rates compared to bacterial isolates that are negative for β-lactamase, thus do not possess the deactivating enzyme. The cure rates are therefore known to be low, even when the treatment may be with a non β-lactam drug.

Another example of a pathogen showing resistance more frequently compared to other bacterial species within its group is the *Enterococcus spp,* which is commonly referred to as environmental streptococci. Although the response therapy undoubtedly is affected by the antibiotic susceptibility, there is little evidence of a correlation between treatment outcomes in cows and the in vitro susceptibility testing. Treatment and management decision of mastitis should therefore take into consideration the different pathogen and potential outcomes of the chosen drug treatments. It is therefore important to combine the knowledge of the infection causing pathogens with the mastitis and SCC history of a cow, so as to select a proper treatment method or management action for a cow with clinical mastitis. Proper record-keeping practices are required, where individual cow records should include mastitis

case history with severity score, number of affected quarters, date, and test-day SCC. Typically, the knowledge of the infection causing pathogen has not always been available for a cow-side decision making process, since the result for a mastitis culture taken at the farm need to be processed and evaluated at a laboratory, which in turn may take several days (Royster & Wagner, 2015).

The hardest part with culturing bacterial samples is the evaluation of the result. It is easy to contaminate samples, since neither human, animal nor the sample site is sterile in case of mastitis sampling. In case of cultures from these non-sterile sample areas, it is difficult to decide what has a significant importance and what can be ignored on the culturing disc. For every bacterial strain that needs to be isolated, identified and have a resistance evaluation made on it, the result to the veterinarian or farmer will be delayed. This delay may lead to a worsening state for the patient before the correct treatment may be given. In order to save time, the veterinarian may ordinate an antibiotic empirically, a treatment with an antibiotic that through previous experience has been proven to be effective, or as local treatment method recommendations pending the laboratory result. When the results are received, a more appropriate antimicrobial drug may be chosen depending on the causative agent. The choice of an appropriate antimicrobial drug is based on the resistance and specificity result. However, in order to start treatment a bacteriological sample must have been taken before any antibiotic insertion is made. A correct sampling methods and good clinical data evaluation is very important in order to facilitate the microbiological laboratory work (Melhus, 2019).

Through the increase of on-farm culturing programs however, which categorize cases of mastitis into broad diagnostic groups depending on a selective bacterial growth media, a more correct choice of antimicrobial drugs can be made. These results are usually available within 18 to 24 hours, and where the broad diagnostic groups can be classified as no growth, Gram-positive, or Gram-negative bacteria. In approximately 10% to 40% of the mastitis cases no bacteria growth on the culturing media could be found, no bacteria were isolated from the milk culture, in those cases it is not justified to use intramammary antimicrobials. Furthermore, some farmers may choose not to use intramammary antimicrobial drugs for mild or moderate cases caused by Gram-negative bacteria, since this type of cases has shown promising results of healing on their own. For a good general practice, the choice of intramammary antimicrobial drugs should be chosen with consideration for the target

pathogens origin, Gram-negative or Gram-positive, and thus the product should have the appropriate activity and spectrum (Royster & Wagner, 2015). If a pathogen-based therapy is used based on the severity level of the clinical mastitis expressed, then severe cases of mastitis with highly specific pathogens should be treated while mild- to moderate cases of mastitis with less specific pathogens may be treated with antimicrobials as the veterinarian see fit. In severe cases of clinical mastitis, it may also be valid to use fluids to rehydrate the animal and to give anti-inflammatory drugs to decrease the swelling, and indirectly the pain caused by the inflammation, of the affected mammary gland quarters (Klocke & Walkenhorst, 2007).

4.3.4. Field data

Field data were collected from, Lyngens gård, a midscale dairy farm with 90-110 actively milking Swedish red and white dairy cows. The farm is located in the southern part of Sweden. Data collected was per oral agreement with owner Magnus Uhlin. The data collected from this operation were used to determine the prevalence of clinical mastitis on the farm over a one-year period, and to use these findings in a comparison with data collected four years prior (2016-01-05 to 2016-12-28). Field data was recorded in a monthly table format, one for each year for comparison. In order to clearly visualize clinical mastitis trends through the sampling period, a graph depicting the different years was included (Individram, 2021). For reference, clinical mastitis is suspected for multiparous cows with a SCC of >150,000 cells/ml in accordance with Swedish standards (Persson Waller, 2018).

Table 2. Average SCC on Lyngens gård, Sweden 2020

Date	Total no. of cows on the farm	No. lactating cows	Sum of 24h milk collection (kg)	Average SCC ($\times 10^3$ cells/mL)
20-12-03	111	90	3003,4	47
20-11-04	105	90	3081,3	144
20-10-12	105	96	3239,6	99
20-09-23	101	95	3081,0	85
20-08-24	102	90	2861,3	93
20-07-27	103	91	3047,8	130
20-06-30	105	98	3287,3	114
20-06-01	106	102	3663,0	106
20-04-28	110	106	3760,9	111
20-03-30	114	105	3825,6	106
20-02-03	111	100	3619,3	120
20-01-08	111	99	3498,9	77

Table 3. Average SCC on Lyngens gård, Sweden 2016

Date	Total no. of cows on the farm	No. lactating cows	Sum of 24h milk collection (kg)	Average SCC ($\times 10^3$ cells/mL)
16-12-28	109	96	3351,3	77
16-11-30	111	95	3181,3	44
16-10-11	106	93	3042,6	64
16-08-31	105	95	3218,5	94
16-08-03	108	97	3323,3	110
16-07-07	111	99	3419,2	89
16-06-08	112	100	3357,7	71
16-05-11	112	101	3597,9	71
16-04-05	113	103	3662,4	100
16-03-02	111	111	3672,7	80
16-02-03	109	109	3611,6	62
16-01-05	107	107	3218,3	67

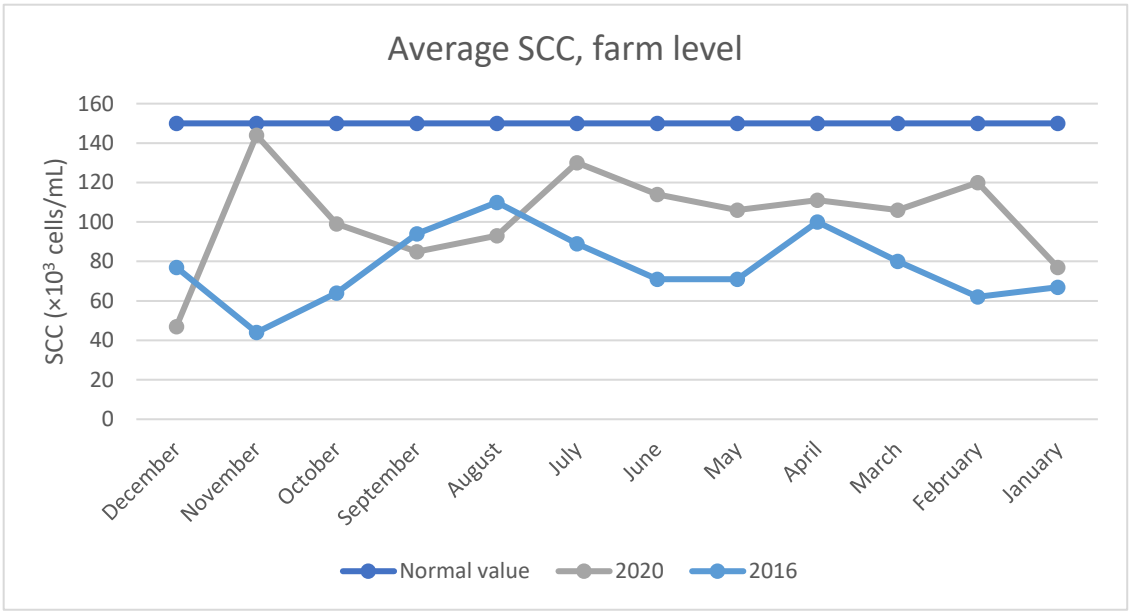

Figure 3. Graph on average SCC, Lyngens gård

4.4 Dry cow protocol without antibiotics

4.4.1 Overview

Due to the different causative agents of mastitis, there are cases that are more or less likely to benefit from antibiotics, antimicrobial drug treatment should therefore be reserved for those cases where no other treatment method is effective. When a cow is selected or screened to see if it is eligible for treatment, there are some cases that will not benefit from antibiotic treatment. The producer should take certain cow-level factors into consideration, factors that will affect the likelihood of a cow remaining in the herd on a long-term basis, before a decision is made whether to treat the mastitis or not. Some of these determining factors are nonpregnant cows in late lactation, low milk yield, sick cows with lameness or other current illnesses. These types of cows should, rather than being considered as treatment candidates, instead be considered as potential culling candidates. Likewise, if a cow is infected with a difficult-to-cure pathogen or in case of a chronic infection that does not improve despite repeated treatment, these cows are unlikely to benefit from further antimicrobial drug therapy and should be either managed in other ways or culled. Such alternative management options may be segregation of the herd into different milking groups, drying-off of the infected udder quarter, or in general an early dry-off of the cow (Royster & Wagner, 2015).

4.4.2 Milk cessation

Drying-off of an infected mammary gland quarter is not without its risks, and should be reserved to those individual cases where the cow is otherwise healthy and productive. It can be done physiologically by decreasing the milk-out, cessation of milking, or by chemically damaging the milk producing glands (Royster & Wagner, 2015). Such udder quarters suffering from therapy-resistant IMI and chronic mastitis cases poses a serious problem for dairy farms, since the bulk tank milk quality decreases due to the high SCC. However, if these udder quarters are dried-off and thus removed from the milking process, it would give an opportunity to keep the cow in lactation without lowering the bulk tank milk quality or losing out on the whole milk yield for that cow lactation period.

In a German study from 2016 performed on high yielding dairy cows, the effectiveness of casein hydrolysate (CNH), which is an endogenous substance and an enzymatic fission product of casein, as a dry-off technique for downregulating of the milk secretion in chronically infected udder quarters was tested. The goal was to use CNH to dry-off these single quarters without inducing clinical mastitis, where each udder quarter was treated with

an infusion of intracisternal CNH six times over three treatment days. On day three, after the last infusion of CNH, milking was no longer performed on the treated udder quarters. A successful drying-off was achieved in 21 out of 24 (87,5%) udder quarters, and where only three of these showed signs of clinical mastitis. During the study there were no signs of pain during the intracisternal application, nor any signs of a defence response from the cows. However, during the treatment period an increase in the average SCC could be seen and a decrease could be seen in 11 out of 16 conventionally milked quarters. It could therefore be shown that CNH infusions was both an effective and a gentle method during lactation for downregulating milk yield, as well as for drying-off therapy-resistant and chronically infected single udder quarters (Tho Seeth et al., 2016).

4.4.3 Homeopathy

There are currently no well-designed studies published which clearly indicate the efficacy of homeopathic mastitis treatment, despite there being more clinical trials made in recent years. In a comparative study made in 2012, it was concluded that the use of a homeopathic complex, used to treat clinical mastitis cases in buffaloes, was lacking. The results gained from this study reported cure rates of 80% for fibrosed, thickened and scarred connective tissue, and 97% for non-fibrosed mammary gland quarters. Despite the improper use of a control group, non-specified cure and the lack of a bacteriological milk culture study.

However, the same authors performed a similar study a year later on dairy cows in India, where they reported that a homeopathic combination medicine was used with great effectiveness. The resulting cures were successful in 86,6% of the cases for acute non-fibrosed mastitis and in 59,2% of the cases for a historical antibiotic group. The cures were defined as normal udder confirmation and milk let down with a normal California Mastitis Test (CMT) result. Just as the previous study, this one has some concerns when it comes to the lack of bacteriological milk cultures, the lack of a nontreated control group, and the use of a historical antibiotic group. Moreover, cows that did not respond to the homeopathic treatment within the first few days of the study were ultimately excluded from the study.

From a third study made on 180 lactating dairy cows, which compared homeopathy with placebo and antibiotic treatment of clinical mastitis, showed no evidence of homeopathic therapy being effective beyond having a placebo effect. Suboptimal bacteriological cures of 60% to 81% were seen for treatment with antibiotics. However, antibiotic treatments were still more effective than the individualised homeopathy, 33% to 43%, whose effect appeared

little different to that of a placebo effect, 45% to 47% (Robertson, 2012; Keller & Sundrum, 2018).

4.4.4 Teat-sealants

During the dry period internal teat-sealants (ITS) can be used as a supplementary therapy or as a preventative strategy, in order to reduce the risk of new intramammary infections (IMI) occurring. The primary function of the ITS is that it stimulates the naturally occurring keratin plug´s purpose, and thereby prevent the mastitis inducing pathogens from accessing the mammary gland (Freu et al., 2020). If correctly used, the ITS can act, in the milk canal, as a persistent internal barrier against infections throughout the whole dry period. These products often contain a non-antimicrobial and inert substance, for example bismuth subnitrate, which is also insoluble in milk. The ITS can therefore be considered to provide an effective and non-antimicrobial treatment option for the prevention of new IMI.

In a comparative study from 2013, the efficacy of an ITS was evaluated as a standalone therapy with the efficacy of an in-combination therapy with ADCT. The result of this study made on lactating cows, reported that the ITS significantly reduced the risk for IMI and clinical mastitis in case of alone or in combination use with ADCT. However, the result of another comparative study showed that the estimates of effect differed for the studies that used an ITS alone and those who used it in combination with ADCT. The investigators of this study imply that the used estimate of efficacy is specific to the situation in which it is used, and whether ITS are used in combination or alone. Several of the studies that promoted the use of standalone teat-sealants, selected the treatment cows on the basis of SCC criteria, CMT, clinical mastitis history before dry-off, and on farm culturing systems. The cows selected for these studies were presumed to be unaffected by clinical mastitis (Royster & Wagner, 2015).

A study made in 2020 evaluated the effectiveness of a new ITS, which was infused at drying-off, as an alternative preventative treatment of IMI in a tropical country. The result collected showed that the new ITS alone had no effect on the treatment of subclinical mastitis cure, on the risk of bacteriological cure, or new cases of subclinical mastitis postpartum, after calving. However, it was proven through the study that the use of ITS in combination with an intramammary antibiotic was effective in preventing new IMI, as well as reducing the risk of clinical mastitis up to 60 days postpartum (Freu et al., 2020).

4.4.5 Frequent milk-out with oxytocin

There are some studies made on the relation between frequent milk-out (FMO) and mastitis recovery. Some studies say that there is no significant benefit of FMO as a therapy for clinical mastitis, and a study from 2004 rather state that it may be unfavourable in finding a cure for clinical mastitis caused by environmental streptococci. Another more recent study suggests that FMO, in this case milking four times a day, as a supporting therapy for mild-, moderate- and severe mastitis cases already treated with antimicrobials, is not recommended either.

There is one study made in 2010 that indicate that the concept of FMO significantly improved the clinical cure rate on the second day of naturally occurring cases of acute *Escherichia coli* mastitis. The study involved 90 cows, where 26 of these were frequently milked out with at least two additional milkings per day. From these 26 cows, 17 were treated with an antibiotic, and thus leaving a control group of 9 cows that only received FMO as a therapy. Just as the theory of FMO, the use of oxytocin is commonly recommended as a supportive therapy for clinical mastitis cases, however there is little evidence to prove its effectiveness.

In a study from 2002, clinical mastitis was experimentally induced with *Streptococcus uberis*, and where oxytocin was used as the sole treatment method. The result of this study showed minimal cure rates and where the mastitis instead increased in severity. A historic study from 1993, which were one of the first studies that practically used a non-treated control group, indicated that oxytocin alone did not have an effect on the mastitis agents, nor did it affect the inflammation directly. One of the prime functions of oxytocin is to assist the milk let-down, which can be beneficial in removing mastitis related components from the infected mammary gland quarters. Oxytocin was not proven to be very helpful for the milk let-down in cases with severely swollen quarters however, as the likelihood of the milk ducts being blocked by inflammatory products and debris like pus is high. For a better flushing out effect of the infected mammary gland a combination strategy of combined FMO and oxytocin may be beneficial as a supplement therapy, but not as a standalone therapy (Roberson, 2012).

5. Summary

Mastitis is a common mammary gland disease in cattle, frequently occurring on dairy farms throughout the lactating and dry, non-lactating, period. It is a condition caused by pathogens, like bacteria, entering into the cows´ udder and thereby causing an inflammation, intramammary infection (IMI) (LIC AutomationTM, 2021). It is important to decrease the milk production during the dry-off period, final milking at the end of lactation. A high milk yield at dry-off increases the risk for IMI, since the cows´ udders are still physiologically susceptible to many pathogens. One of the most common methods in use during the dry-off period, the drying-off of the dairy cows, is the gradual cessation of milking which results in the physiological involution of the udder. Involution is the process the mammary gland undergoes in order to return to a non-lactating state. The methods for drying-off varies between countries as well as dairy herds, some examples for such methods are changes in milk frequency, the use of teat-sealants or antimicrobials (Vilar & Rejala-Schultz, 2020).

In case of an active mastitis, the IMI can be diagnosed by the somatic cell count (SCC), inflammatory- and white blood cells from the udders immune system, which are secreted in the milk throughout the inflammatory process (Woloszyn, 2007). There are different forms of mastitis, acute- and chronic clinical mastitis as well as subclinical mastitis. The different forms can be differentiated based on the SCC level from the milk as well as the expressed inflammatory symptoms. Key management of clinical mastitis can be said to depend on pathogen-based therapy and severity levels of IMI. A proper evaluation and selection of cows is done to identify those individuals that are viable for antimicrobial treatment. This is done on an individual cow level with the aim to identify the presence of IMI based on several parameters, such as clinical mastitis history, SCC in the milk, bacteriological cultures, and with herd-level parameters such as bulk tank milk SCC (Klocke & Walkenhorst, 2007).

Antimicrobial treatment work on the basis of microorganisms interacting with the antimicrobial drug used in two different ways, either the microorganism dies or it survives. If the microorganism survives, it can be due to several reasons where the most feared one is due to resistance against the used drug. Microorganisms are capable of adapting to various scenarios and may develop resistance to any drugs used. Resistance can be classified as innate to the bacteria or as acquired, where the main mechanisms of resistance can be listed as: limiting uptake of a drug, inactivation of a drug, modification of a drug target, and the

active efflux of a drug from the system (Reygaert, 2018). Mastitis caused by antibiotic-resistant bacteria are much harder to treat than IMI caused by non-resistant bacteria (WHO, 2020).

Antimicrobial dry cow therapy (ADCT) should be considered for those mastitis cases where no other alternative treatment is possible and in order to reduce the likelihood for antimicrobial resistance increasing. Thus, alternative treatments are strongly advised in the case of hard-to-cure chronic IMI or gram-positive clinical mastitis. Mild- to moderate clinical mastitis that can be treated using alternative treatment methods, are therefore not the best candidates for antimicrobial therapy. In case of mastitis caused by non-bacterial pathogens, like yeast or fungi, or bacterial cultures with no growth isolates it is not warranted to use antibiotic treatment methods (Royster & Wagner, 2015). On the other hand, it can be said that antibiotic therapy, in addition to fluids and anti-inflammatory drugs, is warranted in practically all severe cases of clinical mastitis on the basis of animal welfare (Roberson, 2012). Some alternative treatment methods for mild- to moderate clinical mastitis cases can be said to have more or less successful cure rates, treatments that showed less effectiveness were frequent milk-out (FMO) and general homeopathic remedies. Alternative treatments with higher cure rates, were for example achieved with internal teat-sealant (ITS) and milk cessation with casein hydrolysate (CNH). The effectiveness would be greater and may cure more severe cases of clinical mastitis if the alternative methods were used as supplementary treatments in combination with ADCT (Vilar & Rejala-Schultz, 2020). In order to prescribe and treat a mastitis case with the most appropriate antimicrobial agent a bacteriological sample is taken from the infected udder, and processed in a lab on the basis of identification of the causative pathogen, antibiotic resistance and specificity (Melhus, 2019). Thus, the chosen antimicrobial drugs should have the appropriate activity and spectrum to act based on the target pathogens origin, Gram-negative or Gram-positive (Royster & Wagner, 2015).

Lastly, the monitoring of treatments success rates is very important to ensure that suitable farm-specific protocols are in place, since the pathogen profile may change with the seasons, when new cows enter the farm, or in case of new mastitis control strategies. Producers and practitioners that monitor their herds well and recognizes the mastitis signs early have a higher chance of making the right farm adjustment and to maximize successful mastitis cure rates (Royster & Wagner, 2015).

6. Conclusion

A comparison between 2016 and 2020 values can be observed. A peak in SCC can be seen in November 2020, although it does not reach the SCC level for clinical mastitis of >150,000 cells/ml milk. It is clear that there were some subclinical cases of mastitis, SCC >100,000 cells/ml milk was detected. There is no real explanation on why this peak occurred, the number of cows in the herd as well as cows in lactation were kept constant. However according to the data, six cows were removed from lactation between October and November 2020, but that is not reason enough for a 50% increase in SCC. The most likely reason is therefore new cases of environmentally induced subclinical mastitis. Over all, the dairy herd on Lyngens gård is very healthy with few cases of actual clinical mastitis, the occurrence of subclinical mastitis is not frequent but still the most prevalent form of mastitis seen on the farm. The SCC levels are kept relatively constant throughout the year, which is indicative of a good herd health practice and consistent hygienic practices. Lyngens gård is therefore a precedent for farms that could use SDCT and alternative DCT to, reserve the use of antibiotics for severe cases of clinical mastitis. It should be taken into account that the evaluation was done on a herd level, which means that one case of clinical mastitis can be masked through the dilution principle as a subclinical mastitis if the rest of the herd is healthy.

Antibiotics are still required for treatment of severe cases of clinical mastitis, since there are few treatment methods that are as effective and readily available on the market at the moment. The best cause of action would be to use antibiotics judiciously, which is already implemented in the SDCT. However, animal welfare should never suffer from withholding necessary treatments. Alternatively, DCT should be used in those cases where ADCT is not a viable treatment method. However, it should be taken into consideration that culling in some cases is the most viable option. The bacterial resistance will likely remain as the main problem with antibiotics for as long as they are in use, due to the microorganism’s great adaptability and short generational intervals. Further research is required in order to find new drug preparations and treatment methods.

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Appendix 1. Report: Besättningens provmjölkningsresultat

individram

Uhlin Magnus
5-10499

2021-04-11
Sida 1

Besättningens provmjölkningsresultat

Årsavkastning

	Mjölk kg	Fett	Protein	ECM kg	Okorr celler	Dagar	Medel-koantal
Senaste 12 månader:	11488	4,6 %	3,8 %	12622	85		
Föregående 12 månader:	11666	4,5 %	3,7 %	12736	94		
Hittills under kontrollåret:	6587	4,6 %	3,8 %	7299		213	106.9
Hittills under föreg kontr år:	6724	4,5 %	3,7 %	7341		214	

Antal kalvningar

Månad:	**Maj**	**Jun**	**Jul**	**Aug**	**Sep**	**Okt**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**
Senaste året:	5	3	4	14	11	6	8	16	9	5	6	
Kommande:	4	8	4	9	13	14	14	5				

Provmjölkningsuppgifter

						Samtliga månader									
Provn nr	Datum	Kor totalt	Mjölkande antal	kg	Dygnsmjölk summa kg	Mjölk kg	Fett %	Prot %	ECM kg	Ureahalt mmol/l	antal	Celler okorr	Juvh klass 0-2	3-5	6-9
202108	21-04-01	107	99	35,1	3471,6	32,4	4,6	3,8	35,7	3,8	99	55	79	11	3
202107	21-03-03	109	99	35,7	3539,1	32,5	4,5	3,9	35,7	4,3	99	62	82	6	6
202106	21-02-03	108	98	34,5	3385,6	31,3	4,7	3,8	35,1	4,0	98	41	77	8	4
202105	21-01-07	108	97	34,0	3295,4	30,5	4,7	3,9	34,3	4,3	97	55	65	12	4
202104	20-12-03	111	90	33,4	3003,4	27,1	4,7	3,8	30,2	3,4	90	47	63	16	3
202103	20-11-04	105	90	34,2	3081,3	29,3	4,5	3,8	32,0	3,6	90	144	63	15	7
202102	20-10-12	105	96	33,7	3239,6	30,9	4,6	3,8	34,1	3,4	96	99	65	14	10
202101	20-09-23	101	95	32,4	3081	30,5	4,6	3,7	33,5	3,6	95	85	55	17	9
202012	20-08-24	102	90	31,8	2861,3	28,1	4,5	3,8	30,6	4,6	90	93	57	17	9
202011	20-07-27	103	91	33,5	3047,8	29,6	4,3	3,7	31,2	4,0	91	130	63	16	10
202010	20-06-30	105	98	33,5	3287,3	31,3	4,6	3,7	34,3	3,6	98	114	64	20	10
202009	20-06-01	106	102	35,9	3663	34,6	4,4	3,7	37,4	4,3	102	106	71	17	10
202008	20-04-28	110	106	35,5	3760,9	34,2	4,6	3,7	37,7	3,9	106	111	75	17	7
202007	20-03-30	114	105	36,4	3825,6	33,6	4,5	3,8	36,6	4,3	105	106	79	14	8
202006	20-03-03	113	103	36,0	3707,9	32,8	4,5	3,7	35,7	4,0	103	97	74	12	6
202005	20-02-03	111	100	36,2	3619,3	32,6	4,4	3,7	35,1	4,1	100	120	73	11	6
202004	20-01-08	111	99	35,3	3498,9	31,5	4,6	3,7	34,5	4,3	99	77	60	13	6
202003	19-11-26	113	99	33,2	3288,1	29,1	4,5	3,8	31,9	4,6	99	86	64	21	7
202002	19-10-29	109	94	34,4	3236,2	29,7	4,6	3,7	32,7	3,9	94	95	59	17	5
202001	19-10-01	104	90	33,3	2996,6	28,8	4,6	3,7	31,5	4,8	90	73	56	18	3
201912	19-08-20	106	96	33,0	3167,5	29,9	4,6	3,7	32,8	5,4	96	89	67	18	4
201911	19-07-16	110	100	35,5	3553,9	32,3	4,4	3,7	34,6	5,3	100	83	72	17	6
201910	19-06-18	109	101	35,1	3548,2	32,6	4,5	3,7	35,4	4,4	101	93	75	12	7
201909	19-05-14	111	101	36,5	3691,5	33,3	4,6	3,8	36,8	3,8	101	86	70	15	9
201908	19-04-09	111	102	36,5	3722	33,5	4,6	3,7	36,8	4,1	102	78	75	18	6
201907	19-03-12	113	105	35,7	3745,5	33,1	4,4	3,8	36,0	4,2	105	71	77	16	8
201906	19-02-12	117	109	34,5	3759,4	32,1	4,6	3,8	35,5	4,3	109	88	70	15	8
201905	19-01-15	112	101	34,8	3513,6	31,4	4,5	3,8	34,1	4,8	101	65	63	15	9
201904	18-12-11	110	97	33,6	3262,4	29,7	4,6	3,8	32,9	4,8	97	86	60	13	11
201903	18-11-13	106	90	34,1	3067,1	28,9	4,6	3,8	32,2	5,8	90	88	56	9	11
201902	18-10-16	101	83	34,4	2851,1	28,2	4,6	3,7	31,0	4,6	83	91	61	7	8
201901	18-09-18	103			2965,2	28,8	4,5	3,8	31,5			79	64	9	5
201812	18-08-09	105	90	32,7	2946,7	28,1	4,2	3,7	29,3	3,8	90	52	73	8	4

Besättningens provmjölkningsresultat

Provmjölkningsuppgifter

Provn nr	Datum	Kor totalt	Mjölkande antal	Mjölkande kg	Dygnsmjölk summa kg	Mjölk kg	Fett %	Prot %	ECM kg	Ureahalt mmol/l	Ureahalt antal	Celler okorr	Juvh klass 0-2	Juvh klass 3-5	Juvh klass 6-9
					Samtliga månader										
201811	18-07-11	105	94	35,1	3300,2	31,4	4,5	3,7	33,9	4,4	94	66	71	9	7
201810	18-05-30	109	96	34,9	3418	31,4	4,4	3,6	33,4	4,2	98	80	73	8	8
201809	18-04-25	110	99	34,7	3437,9	31,3	4,6	3,8	34,4	5,0	99	78	76	8	11
201808	18-03-28	110	99	34,1	3375,2	30,7	4,5	3,8	33,6	4,4	99	57	77	9	8
201807	18-02-28	112	100	34,9	3491,2	31,2	4,7	3,8	34,7	4,1	100	74	76	10	6
201806	18-01-31	112	104	34,7	3608,5	32,2	4,6	3,8	35,5	3,7	104	71	80	15	2
201805	18-01-03	109	101	35,6	3596,4	33,0	4,5	3,8	35,9	4,1	101	72	68	16	3
201804	17-11-29	109	99	35,9	3549,4	32,6	4,5	3,7	35,2	3,9	99	44	69	16	2
201803	17-11-02	107	94	35,4	3331,4	31,1	4,4	3,7	33,2	4,2	94	186	62	13	6
201802	17-10-04	106	92	35,0	3224,2	30,4	4,4	3,6	32,3	4,6	92	128	63	10	6
201801	17-09-07	103	88	34,7	3054,4	29,7	4,3	3,6	31,4	4,4	88	99	61	8	8
201712	17-08-02	104	91	35,3	3211,4	30,9	4,3	3,6	32,7	4,3	91	84	74	7	7
201711	17-07-05	106	93	35,2	3270,4	30,9	4,4	3,6	32,6	4,6	93	79	69	10	5
201710	17-06-01	107	98	35,9	3513,5	32,8	4,5	3,6	35,6	3,8	96	98	73	9	7
201709	17-04-26	108	95	36,1	3426,5	31,7	4,4	3,7	34,0	3,9	95	91	71	8	9
201708	17-03-29	106	93	36,2	3368,4	31,8	4,3	3,6	33,6	3,8	93	82	64	9	10
201707	17-03-01	104	92	36,0	3315,2	31,9	4,4	3,7	34,2	4,3	92	124	70	6	12
201706	17-02-01	112	102	35,3	3601,7	32,2	4,4	3,7	34,3	3,6	102	125	71	11	7
201705	16-12-28	109	96	34,9	3351,3	30,7	4,5	3,8	33,4	4,7	96	77	68	9	7
201704	16-11-30	111	95	33,5	3181,3	28,7	4,4	3,8	30,9	3,8	95	44	66	10	6
201703	16-11-02	107	91	33,4	3040,9	28,4	4,4	3,8	30,7	5,3	91	93	63	12	6
201702	16-10-11	106	93	32,7	3042,6	28,7	4,4	3,7	30,9	4,8	92	64	64	10	7
201701	16-08-31	105	95	33,9	3218,5	30,7	4,3	3,7	32,6	4,8	95	94	61	13	14
201612	16-08-03	108	97	34,3	3323,3	30,8	4,4	3,7	32,9	4,8	97	110	62	18	11
201611	16-07-07	111	99	34,5	3419,2	30,8	4,3	3,7	32,8	5,4	99	89	65	15	10
201610	16-06-08	112	100	33,6	3357,7	30,0	4,2	3,5	31,2	4,9	100	71	77	6	11
201609	16-05-11	112	101	35,6	3597,9	32,1	4,4	3,6	34,0	4,7	101	71	76	9	7
201608	16-04-05	113	103	35,6	3662,4	32,4	4,5	3,6	34,8	4,4	103	100	78	5	9
201607	16-03-02	111	102	36,0	3672,7	33,1	4,5	3,7	35,7	4,7	102	80	77	6	6
201606	16-02-03	109	99	36,5	3611,6	33,1	4,4	3,6	35,4	4,0	99	62	72	7	6
201605	16-01-05	107	93	34,6	3218,3	30,1	4,4	3,7	32,0	4,8	93	67	64	10	6
201604	15-12-02	103	91	33,2	3019,8	29,3	4,3	3,7	30,9	4,3	89	69	59	13	7
201603	15-11-04	100	86	34,1	2934	29,3	4,5	3,8	32,1	5,3	85	74	57	15	5
201602	15-10-07	105	89	31,8	2828,3	26,9	4,6	3,7	29,5	3,4	89	77	52	16	5
201601	15-09-02	99	84	32,2	2704,3	27,3	4,4	3,5	28,8	5,1	84	93	59	12	6
201512	15-08-07	100	85	34,1	2902,1	29,0	4,4	3,6	30,7	4,6	85	81	63	11	5
201511	15-07-08	102	89	34,0	3023,6	29,6	4,3	3,5	31,1	3,9	89	102	62	12	11
201510	15-06-10	100	88	35,3	3102,5	31,0	4,4	3,6	32,9	3,3	88	78	70	11	6
201509	15-05-06	105	100	34,8	3482,9	33,2	4,6	3,7	36,1	4,0	100	112	69	16	8
201508	15-04-08	101	99	34,4	3402,9	33,7	4,4	3,7	36,1	3,8	99	71	69	14	5
201507	15-03-04	104	95	33,9	3216,9	31,0	4,6	3,7	33,9	4,2	95	86	69	11	8
201506	15-02-03	105	89	34,7	3087,3	29,4	4,4	3,6	31,4	3,2	89	56	63	12	6
201505	15-01-08	105	88	33,4	2943,4	28,0	4,5	3,7	30,4	4,5	88	78	56	13	7
201504	14-11-26	109	89	33,0	2933	26,9	4,2	3,6	27,9	5,2	88	76	62	10	7
201503	14-10-29	109	96	31,5	3020,3	27,7	4,6	3,6	30,1	5,3	94	96	63	11	9
201502	14-10-01	103	92	31,4	2887,4	28,0	4,5	3,6	30,0	4,0	92	86	58	9	9
201501	14-09-03	99	84	31,7	2660,1	26,9	4,4	3,6	28,7	3,7	83	100	58	8	10
201412	14-08-06	103	88	31,9	2806,9	27,3	4,4	3,6	28,9	4,4	88	103	66	9	8

Appendix 2. Thesis topic declaration form

University of Veterinary Medicine

Announcement of the chosen topic of the Thesis

Name of student (capital letters): Nelly Aronsson

I would like to ask for the permission of the Head of the Department of Obstetrics Department, to write my thesis in the following topic advertised and supervised by the Department.

Budapest, 2019-03-18 (date)

(signature of student)

Topic of thesis:

Drying off of dairy cows without antibiotics.

Title of thesis:

Drying off of dairy cows without antibiotics

Signature of supervisor:

I approve:

Signature of Head of Department

Appendix 3. Electronic License Agreement and Copyright Declaration

HuVetA

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Name: Nelly Aransson

Contact information (e-mail): nearansson@gmail.com

Title of document (to be uploaded): Drying off of dairy cows without antibiotics

Publication data of document:

Number of files submitted: 1

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- *facilitate professional relations and collaboration;*
- *support open access.*

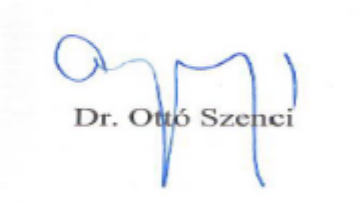
Appendix 4. Supervisor counter-signature form

I hereby confirm that I am familiar with the content of the thesis entitled: **Drying off of dairy cows without antibiotics.**

written by **Nelly Linnea Aronsson** (student name)

which I deem suitable for submission and defence.

Date: Budapest, March 31, 2021.



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Supervisor name and signature

University of Veterinary Medicine Budapest

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Department of Obstetrics and Food Animal Medicine Clinic

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