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Mastitis in dairy cattle with emphasis on strategies to reduce antibiotic use

Szarvasmarhák tőgygyulladása, különös tekintettel az antibiotikumok használatának csökkentésére irányuló stratégiákra

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Abstract

Mastitis is a significant concern in dairy farming, noted for its economic and animal health consequences such as reduced milk production, decreased milk quality and increased culling rates. This thesis investigates the prevalence and management of mastitis in a selected number of Norwegian dairy herds, focusing on antimicrobial resistance (AMR). Five farms using automatic milking systems (AMS) were selected and investigated via structured interviews and milk sample analysis from the Norwegian mastitis laboratory in Molde, identifying the bacteria responsible for the infection and existing prevention methods from the farmers. Findings identified *Staphylococcus aureus* as the primary pathogen in the investigated farms, suggesting a need for improved epidemiological measures and better farm hygiene practices.

The research emphasises the Norwegian and European prudent use standards as an important approach to reducing antimicrobial usage while maintaining good udder health. On the other hand, the deficiencies in farmers' understanding of disease transmission and the non-antibiotic interventions such as internal teat sealants were recognised. Recommendations on these specific farms include increased farmer education regarding biosecurity and pathogen profiles.

Absztrakt

A tőgygyulladás jelentős probléma a tejelő tehenészetekben, gazdasági és állat-egészségügyi következményei miatt, mint például a tejtermelés csökkenése, a tej minőségének romlása és a selejtezési arány növekedése. Ez a dolgozat a tőgygyulladás prevalenciáját és kezelését vizsgálja néhány norvég tejelő állományban, az antimikrobiális rezisztenciára (AMR) összpontosítva. Öt robotizált fejési rendszert (AMS) használó gazdaságot választottam ki és mértem fel kérdőívek és a moldei mastitis laboratóriumban elvégzett tej mikrobiológia vizsgálatok segítségével, azonosítva a fertőzésért felelős baktériumokat és a gazdálkodók által használt megelőzési módszereket. Az eredmények a Staphylococcus aureus-t azonosították az elsődleges kórokozóként a vizsgált gazdaságokban, ami arra utal, hogy jobb járványvédelmi, megelőző intézkedésekre és jobb farmhigiéniai gyakorlatokra van szükség.

A kutatás hangsúlyozza az antibiotikumok felelősségteljes felhasználásának norvég és európai uniós előírásait, amelyek fontos lépést jelentenek az antimikrobiális szerek használatának csökkentésében a tőgy jó egészségének megőrzése mellett. Másrészt azonosítottam a gazdálkodók változó mértékű tájékozatlanságát a betegségek terjedésével és a nem antibiotikumos beavatkozásokkal kapcsolatban. Ilyenek voltak például a szárazra állításkor alkalmazott tőgyzáró készítményekkel kapcsolatos ismeretek, illetve azok nem kielégítő volta. Az ezekre a konkrét gazdaságokra vonatkozó ajánlások közé tartozik a gazdálkodók fokozottabb oktatása a tőgypathogén kórokozók típusaival és az ellenül való hatékony védekezéssel kapcsolatban.

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1. Introduction

Mastitis is commonly ranked as the most important disease of dairy cattle, causing huge economic repercussions due to poor milk quality, reduced milk yield, discharge of milk containing antibiotic residues and increased culling rates for uncurable cows. Additionally, the treatment of mastitis accounts for the most significant proportion of antibiotics used in dairy cattle farming, and it is essential to address this in conjunction with drug expenses, veterinary services and treatment failures. Estimating the total cost of mastitis is frequently challenging due to the numerous factors contributing to the loss [1–4].

Addressing mastitis through effective management practices is important, not only to improve the productivity of the farm but also as a concern for public health [5, 6].

Norway implemented strict rules for antibiotic use years ago, and in this thesis, five Norwegian farms were investigated with a main focus on prevalence, farm profile of bacteria and preventative measures.

2. Literature review

2.1 Mastitis and aetiology of mastitis

Mastitis is caused by different mechanisms, including physical damage, chemical irritation, or, most commonly, infectious origin, mainly bacteria but also algae, fungi or yeast. The clinical signs and reservoir of the pathogens are used to classify mastitis as well as the affected area of the udder. [7]

Epidemiologically, mastitis can be divided into contagious and environmental depending on the reservoir and form of transmission. Contagious mastitis pathogens consist of bacteria such as *Staphylococcus aureus, Streptococcus agalactiae, Mycoplasma* spp. and *Corynebacterium bovis*. These pathogens can be transmitted from cow to cow with equipment, contaminated hands, and inadequately cleaned materials [8, 9]. Environmental bacteria constitute the bacteria found in faeces, soil, bedding material and water sources, such as *Escherichia coli, Streptococcus uberis and Klebsiella* spp [10]. There is also literature about bacteria being both contagious and environmental, in particular *Streptococcus dysgalactiae* which can be persistent on a farm, or spread from cow to cow. [11–13]

Based on clinical signs and severity of the infection mastitis can be divided into clinical and subclinical mastitis. Clinical mastitis is used to classify disease with visibly abnormal milk with one or more of the fundamentals of inflammation “tumor, rubor, calor, dolor et functio laesa” which is the canonical description of the visual changes seen. In case of mastitis this manifest in changes of colour, the presence of clots and flakes, or other changes in the udder such as swelling, heat, pain or redness [14, 15]. The principal microorganism responsible for clinical mastitis are *S. aureus, E. coli, Klebsiella spp., S. uberis* and *S. dysgalactiae* [16]. Clinical mastitis can be further categorised based on the severity of the inflammation as sub-acute, acute, peracute or chronic.

Sub-acute mastitis is characterized by a mild and gradually developing inflammation. Variations from the healthy udder are small and often results solely in decreased milk production and minor microscopic alterations of the milk. Flaky particles may be seen during fore-stripping. [17]

Cases of acute clinical mastitis are characterized by prominent symptoms of udder inflammation, including swelling, redness along with alterations of milk such as changed consistency and appearance. Furthermore, fever is common, and the sensitivity of the udder skin and tissue are increased. [18]

Per-acute clinical mastitis on the other hand is the more serious form of mastitis often associated with anorexia, shock and death. This form commonly destroys large parts of the udder tissue leading to pain, fever, depression and eventually death. [12, 19]

Chronic mastitis is usually a result of inflammation lasting several weeks to months caused by inadequate treatment of acute mastitis. In case of chronic mastitis, the udder typically have fibrous indurations and may become firm. Treatment of these cases are particularly challenging due to a combination of factors related to the biology of the disease and the nature of pathogens involved. Bacteria such as *S. aureus* and *S. uberis* are capable of forming biofilm, a protective layer of polysaccharides and proteins shielding bacteria from the immune system and antimicrobial treatment. [20, 21]

On the other hand, subclinical mastitis does not exhibit any systemic symptoms or visible local inflammation. Furthermore, it is thought to cause more economic losses for the farmer than clinical mastitis aggravated by the lack of symptoms making the diagnosis more difficult [22]. The treatment of subclinical mastitis also differs from clinical mastitis as this is not treated during the lactation, but rather at drying off with selective dry cow therapy. Prudent use guidelines have been developed in veterinary medicine and the key elements in the EU is to identify the pathogen causing mastitis to avoid unnecessary treatment both during lactation and at drying off, as well as implementing strategies and good farm practice to decrease the spread and development of mastitis. [23]

The prevention of mastitis, especially environmental is based on hygiene of the farm, and specifical parameters to discuss is the moisture, temperature and air flow. High humidity and especially pooling of manure and dirt gives high chances of environmental bacteria to grow. On the other hand, the prevention of overcrowding is crucial, and adequate clean and dry bedding regardless of housing systems. The manure handling is also of essence, especially in the laying areas where the udder is in contact with the environment. [24].

The udder is suspended from the median and lateral suspensory ligament originating from the pelvis, and each of the four quarters has its own parenchymal tissue functioning as a separate gland. The parenchymal tissue consists of alevoli, ducts and connective tissue. Milk production occurs in the alveoli where it drains into smaller ducts, larger ducts and gland cistern. The gland cistern empties into the ventrally located teat cistern. (Figure 1) [25, 26].

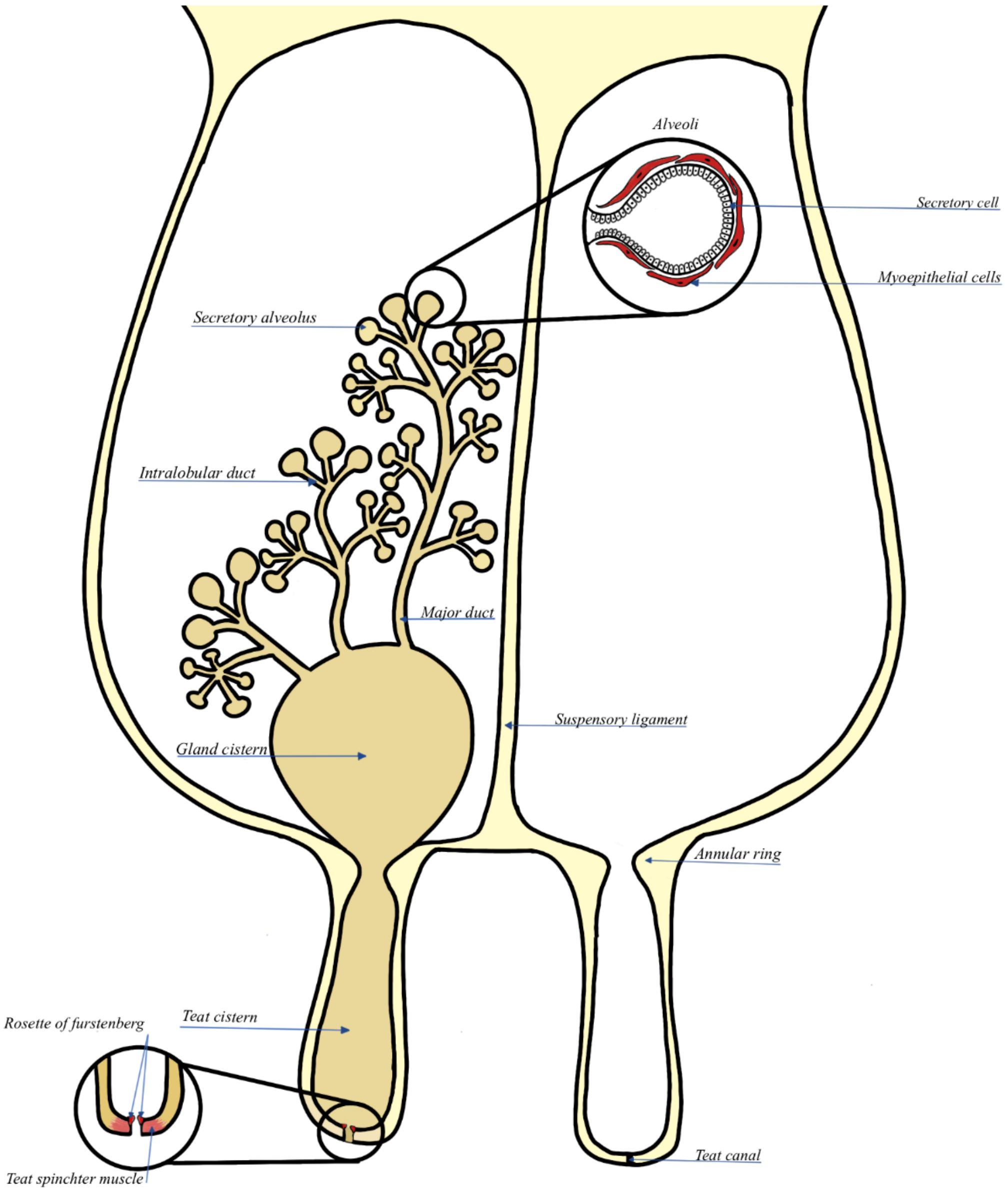

Figure 1: Anatomy of the udder, labelled [25, 27]

The rosette of Furstenberg at the teat end is the first line of defence against pathogens, and servers as a barrier preventing milk to be let out and infective against to enter. However the teat end stays open between 30 minutes and 2 hours after milking making the udder susceptible to intramammary infections [12]. At drying off a keratin plug is formed which acts as a barrier of first line physical defence during the whole dry period. However, the production of a fully developed keratin plug is variable, and literature suggests that several days are needed. In addition, high-yielding dairy cows have higher chances of not producing a functional keratin plug several weeks after drying off. [28, 29]. Literature suggest as high as 25% of teat ends remain open up to six week after drying off [30].

To compensate for the absence of a natural keratin plug, internal teat sealant products are available which can reduce the risk of developing intramammary infections by over 70%. Teat sealants function by minimizing bacterial invasion into the teat canal and cistern. Additionally, these products can be used in conjunction with intramammary antibiotic infusions if milk sampling results indicate a necessity for such treatment. [31, 32].

There are multiple tools to detect mastitis, and in conventional milking systems the farmer is key in the essence of forestripping which will help to identify clinical mastitis and other abnormalities. Other on-farm screening methods are also commonly used to investigate somatic cell count (SCC) such as the California Mastitis Test (CMT) which provides a qualitative result on the severity of the inflammation but does not identify the underlying cause. Increased SCC are correlated with the presence of mastitis [15]. Built-in most automatic milking systems (AMS) the electrical conductivity measures the Na^+ and Cl^- and these parameters increases in case of mastitis and is influenced by the udder health status of the cow [33]. Forestripping, SCC and electrical conductivity measurement can help the farmers to select the correct cow for required sampling and treatment. With laboratory testing of the specific pathogen causing mastitis, the decision of treatment, or not treating at all should be done in accordance with general guidelines.

2.2 Norwegian guidelines for treating mastitis

This is a summary of the Norwegian guidelines for treating mastitis found in the Norwegian Medical products agency. [34]

General Guidelines

The recommendations focus on infections of significant importance in cattle where antibacterial treatment is applicable. The goal is to limit the use of antibacterial agents, and treatment should only be used for animals that have undergone thorough diagnostics and where an effect from antibacterial treatment can be expected. The development of resistance is prevented through proper use, and critically important antibiotics for humans should be avoided. The European Medicines Agency (EMA) recommends the use of as narrow-spectrum agents as possible and local treatment where feasible. For instance, intramammary applications are considered to drive less resistance than systemic treatment in cases of mastitis [35]

It is crucial to emphasize disease prevention. When an infection manifests as a herd problem, antibacterial treatment should only be part of a comprehensive plan to reduce the incidence of infections. Environmental and individual preventive measures must be central to such a plan.

Additionally, biosecurity is critical, both when purchasing animals and through the use of a hygiene barrier for personnel entering the farm.

Udder

Diagnostics

It is recommended to take individual quarter samples for bacteriological examination and resistance sampling in connection with all mastitis treatments. Bacteriological diagnostics and resistance determination should be performed at a quality-assured laboratory. Knowledge of the agent and resistance in the individual herd should guide the choice of therapy. When treatment of clinical cases of mastitis is initiated before laboratory diagnosis is available, gram staining and microscopy of mastitis secretion smears can be performed to distinguish between gram-positive cocci/bacilli and gram-negative bacilli. The choice of treatment depends on clinical status, lactation stage, prognosis, and possibly knowledge of bacteriological diagnosis.

Bacteriological Findings

In Norway, most clinical mastitis cases are caused by gram-positive cocci sensitive to benzylpenicillin procaine. The most common bacteriological findings in glands with clinical mastitis in 2020 were: *Staphylococcus aureus* (28%), *Streptococcus dysgalactiae* (10%), *Trueperella pyogenes* (8%), *Streptococcus uberis* (7%), *coagulase-negative staphylococci* (CNS) (5%), and *Streptococcus agalactiae* (<1%). 20% of the isolates were coliform bacteria, and 0.7% of the isolates were penicillin-resistant *S. aureus* [36]. The main causative bacteria for subclinical mastitis are S. aureus, CNS, and streptococci [37]. Compared to isolates from clinical mastitis, the proportion of penicillin-resistant S. aureus isolates is somewhat higher. The occurrence of penicillin resistance varies between different CNS species [36]

Treatment Outcomes

Healing clinical mastitis after initial treatment with an intramuscular injection of benzylpenicillin procaine followed by benzylpenicillin procaine intramammary for four days has been shown to be around 75%. Healing depends on the bacteria and varies from around 30% for glands infected with *S. aureus* to 90% for infections caused by *Str. uberis* [38]

Severe and moderate clinical mastitis

Definition: mastitis with one or more classic signs of inflammation in the udder (swelling, pain, heath, redness). Visible signs of illness such as elevated temperature, decreased appetite, and depression may be present. The milk is usually altered [39]

Treatment

To ensure animal welfare, it is necessary to initiate treatment at an early stage, often before an exact bacteriological diagnosis is available. Unless microbiological results from previous cases of severe and moderate clinical mastitis suggest that the herd deviates significantly from the country otherwise, it should be assumed that the cause is a penicillin-sensitive bacterium. Benzylpenicillin procaine as an intramammary infusion is used as the first choice unless there is a high likelihood that a penicillin-resistant bacterium is present.

Penicillin sensitive bacteria

For severe clinical mastitis, an initial intramuscular injection of benzylpenicillin procaine is given in addition to intramammary applications. The MIC for relevant penicillin-sensitive bacteria in Norway suggests that a dosage of 20,000-30,000 IU per kg is appropriate. A sufficiently high dose should be administered. Subsequent treatment involves intramammary administration of benzylpenicillin procaine for an additional 3-4 days.

Preliminary results from a Norwegian therapy trial in 2020-2021 indicate that *S. aureus* responds poorly to only intramammary treatment, even in cases of moderate clinical mastitis without general distress. In herds with a good overview of the agents causing clinical mastitis, the initial intramuscular injection can be omitted if it is unlikely that *S. aureus* is the cause of the problem. Treatment can then be limited to benzylpenicillin procaine intramammary for 3-4 days.

After day 1, there is no demonstrated additional effect of supplementing intramammary treatment with further systemic treatment with benzylpenicillin procaine [40].For instance, in cases of teat trauma, it might be appropriate to replace intramammary treatment with systemic treatment.

If *Str. agalactiae* is detected, a systematic mapping and control of this bacterium in the herd should be initiated. If treatment is deemed necessary, benzylpenicillin procaine is used. *Str. agalactiae* generally responds well to treatment with intramammary agents.

T. pyogenes can cause mastitis in lactating cows and is then treated with benzylpenicillin procaine. In cases of summer mastitis in non-lactating animals, where *T. pyogenes* is most frequently detected, the mastitis is often chronic and incurable. The prognosis for recovery of the gland is quite poor meaning that treatment with antibiotics should only be considered for animal welfare reasons.

Penicillin-Resistant Staphylococci

If culturing shows that the cause is penicillin-resistant *S. aureus*, the choice of antibiotics is limited. Treatment of mastitis caused by penicillin-resistant *S. aureus* generally yields very poor results [38, 41]. Culling should be considered at an appropriate time in the lactation if a subsequent sample still shows penicillin-resistant *S. aureus*.

Attempted treatment may include intramammary agents with amoxicillin and clavulanic acid. Trimethoprim-sulphonamide can be used experimentally for systemic treatment, but the efficacy of such treatment is uncertain.

Gram-Negative Bacteria

If the cause of mastitis is gram-negative bacteria (most commonly *Escherichia coli*), treatment with antibacterial agents provides minimal or no additional effect beyond that handled by the udder's own defence [42, 43]. Therefore, as a rule, the use of antibacterial agents is not recommended for coliforms. The same applies to other gram-negative agents, such as *Klebsiella spp*. Supportive therapy such as fluid therapy, pain management, and milking out may be necessary to reduce the effects of endotoxins and prevent dehydration.

Supportive Treatment

NSAIDs should be considered for all animals with clinical mastitis. NSAIDs have shown an anti-endotoxic effect in some experimental studies if the treatment is administered at an early stage of the infection [44]. In animals experiencing pain and distress, mild sedation may be useful before examination. In cases of severe and moderate clinical mastitis, it has been recommended to frequently empty the affected gland. However, scientific evidence for the effectiveness of frequent milking and the use of oxytocin on the clinical and bacteriological recovery of the gland is limited. Nevertheless, this type of supportive treatment may be sensible from an animal welfare perspective. Moderate hypocalcaemia can occur due to endotoxin effect [45, 46]. Cautious intravenous infusion of calcium may be considered, but the dose should be lower than that recommended for milk fever [47]. Fluids should be administered to dehydrated animals.

Mild clinical mastitis

Definition: mastitis with visibly abnormal milk and/or chronic changes in the udder. Signs of severe and moderate mastitis such as swelling, pain, heath, or redness in the udder or visible signs of illness are absent in cows with mild clinical or chronic mastitis [39]

Treatment

In cases of mild clinical mastitis, it may be advisable to wait for the results of bacteriological culturing before initiating treatment. If mild clinical mastitis is a problem in the herd, the treatment should be part of an eradication plan.

In cases with evident chronic clinical symptoms (atrophy, fibrosis, reduced production) and high cell counts (above 600,000 cells/mL on average) for a longer period, there is no point in treating the gland with antibacterial agents either during lactation or in the dry period. The gland should be dried off or the cow culled. If there are no clear gland changes, treatment may be considered but samples for bacteriological examination should be taken before initiating antibacterial treatment. If *S. aureus* is detected, the prognosis is poor, and there is little point in treating during lactation. If *Str. dysgalactiae* is detected and the herd is actively working to control it, treatment during lactation may be appropriate – in this case, with benzylpenicillin procaine. If other bacteria, such as CNS are detected it is generally not appropriate to treat with antibacterial agents. If *Str. agalactiae* is detected an eradication plan should be initiated to control the bacteria on a herd level. [34]

Subclinical mastitis

Definition: inflammation of the mammary gland that is not visible and requires the use of diagnostic tests to be detected. The most common tests include examination and evaluation of the cell count, such as the California Mastitis Test (CMT) or similar [39].

Treatment

In cases of subclinical infection during lactation, it is doubtful whether any potential therapeutic effect justifies the costs associated with treatment and milk withdrawing period. If treatment is considered milk samples must be taken for microbiological examination. If *Str. dysgalactiae* is detected and the herd is actively working to control it, treatment with benzylpenicillin procaine may be appropriate. If *Str. agalactiae* is detected, systematic mapping and eradication of this bacteria should be initiated.

If many cows in a herd have subclinical mastitis the herd should be investigated to identify and possible correct the causative factors. If the bulk milk tank somatic cell count is increased to such degree it results in deduction in quality and several cows have high cell counts in one affected gland the removal of individual gland can be considered an immediate measure.

Treatment at Drying Off

Definition: a treatment based on milk sampling or cell count examinations, etc., initiated in connection with drying off [39].

Bacteriological Examination of Candidates for Dry Period Treatment

Bacteriological examination of milk samples should always be conducted before dry period treatment. Samples should be taken as close to drying off as practically possible. Candidates for bacteriological examination are identified by assessing cell counts in the last part of lactation.

Treatment

Treatment depends on the prognostic assessment and bacteriological findings. The prognosis is poor if chronic clinical changes are present, or if the cow's cell count has been over 500,000 to 600,000 per mL in several measurements before drying off.

If *S. aureus, Str. dysgalactiae, Str. agalactiae*, or *Str. uberis* is detected in one or more glands and the prognosis is considered good, treatment at drying off is appropriate.

A pure penicillin preparation intended for use at drying off is preferred if it becomes available in Norway. Currently available dry period preparations include benzathine penicillin combined with penetamat hydroiodide and framycetin sulphate or cloxacillin benzathine.

EMA's AMEG categorization of antibiotics suggests that framycetin should be used more cautiously than cloxacillin. Cloxacillin is a beta-lactamase-resistant penicillin (narrow-spectrum/group D), while framycetin is an aminoglycoside (broad-spectrum/group C) [48]. Systemic treatment is not necessary for dry period treatment.

Due to the relatively high frequency of new infections at drying off, it cannot be ruled out that new glands may become infected after the samples are taken [49]. This is especially true for *streptococci*. Therefore, it is recommended that all glands are treated simultaneously at drying off. An international literature review shows no increase in resistant bacteria with the use of dry period preparations [50].

Cows that have been treated during the dry period should be followed up in the next lactation, for example with a milk sample for bacteriological analysis about one week after calving. Cows that still have an infection and high cell count are advised to be culled at an appropriate time in lactation.

Penicillin-Resistant *S. aureus*

Since the result of treatment generally is poor for infections with penicillin-resistant *S. aureus* culling should be considered after bacteriological examination in the next lactation. First choice dry period preparation if you decide to treat contains cloxacillin benzathine.

Removing/Letting Go of a Lactating Gland

A lactating gland with chronic clinical changes and reduced performance should as a rule, be removed without the use of antibacterial agents. The same should be done with glands with persistently high cell counts. In the case of a severe, acute teat injury, the risk of mastitis is greatly increased, and it may be appropriate to combine removal with antibacterial treatment (systemic or local). Benzylpenicillin procaine is the first choice [51].

In addition to the recommendations from the Norwegian Medical products agency there are some additional recommendations from TINE concerning the dry cow therapy.

- All cows with clinical mastitis in the lactation should be sampled for bacteriological examination before drying off.
- All cows with geometrical mean somatic cell count above 100 000 cells/ml should be sampled before drying off.
- All cows infected with *Staphylococcus aureus*, *Streptococcus dysgalactiae* or *Streptococcus agalactia* should receive dry cow therapy.
- Sample all cows treated at drying off 6 days after parturition. Cows with persistent high somatic cell count should be culled.
- Check the milking equipment and milking routine regularly.
- Do body condition scoring and check energy balance of the food throughout the lactation period.
- Do not give mastitis milk as fodder for calves.

These general guidelines are also accompanied by the prevalence of mastitis in Norway, which can be summarised in Table 1 where the udder health is assessed as either good, average or bad which serve as an indicator for the farmer to determine their herd's health compared to national data.

	Good udder health	Average	Bad udder health
Mastitis per year (cow/year)	<0.06	0.15	>0.28

Table 1: mastitis per cow/year, udder health scoring.[34]

2.3 The Norwegian standard of farming

The most common breed in Norway is the Norwegian Red Cow, a dual-purpose breed producing both milk and meat for human consumption carefully selected genetically accustomed to the Norwegian demands for farming. In 2022 as high as 84.3% of the newborn heifers were NRC [52, 53].

For the last two decades the Norwegians farms have gone through large changes mainly due to a new regulation in 2004 changing the structure of dairy farming and animal welfare. The Norwegian Ministry of Agriculture and Food encouraging farmers to transit from tie-stall structure to free-roaming by prohibiting tie-stalls housing from 1. January 2034. In addition Norwegian cows have a mandatory period of 8 weeks on pasture in the summer months to ensure cows being allowed to express natural behaviour and free movement [54].

The average milk yield per cow for NRC is 7926 kg, with low cell counts on average 61 000 SCC/ml. Even though the average cell count is low, mastitis is responsible for 1/3 of all veterinary interventions in dairy herds, but the trends for the last 10 years shows a reduction of 20% in the use of antibiotics per cow [55, 56]

The Norwegian Red Cow (NRC) has an extensive and excellent breeding history of which The Norwegian Dairy Herd Recording system (NDHRS) started by local farmers in 1898 featuring an organised recording of milk yield. NDHRS is a system of data collection also used today certified by ICAR. This system is available for all dairy farmers in Norway and approximately 97% of dairy farmers participate in the herd recording system today. In this data system all treatments from veterinarians are recorded with diagnosis and drugs, inseminations, embryo transfers, pregnancy checks also including registration from the AI-technicians. Advisors register conformation traits on heifers and cows, laboratory register the milk quality and quantity, claw trimmers register claw health, and the slaughterhouse register data of the carcass. This all together gives a huge data collection on individual and herd level and can give both veterinarians, advisors, and farmers a better picture on herd status, quality of breeding values as well as ensuring better breeding goals such as better udder health, milk quantity, claw health, fertility and temper of the animals [56, 57]. To become a member of the NDHRS there are a few criteria needed to fulfil, such as milk sampling including measurement of the composition of milk, cell count, culturing and measurement of milk yield within a certain time frame during the year. [58].

The breeding goals for the NRC has a broad spectrum of important traits and is reflected as animals giving good economical yield for the farmer and still ensuring good animal welfare. Lately a larger focus on sustainability and climate has arisen and since the NRC is a dual-purpose breed this will give meat and milk for less food, less waste, less water usage and the emission per kilo produced meat and milk is lower than the specialized breeds. In addition this gives an excellent usage of Norwegian resources [59]. The two top priorities in the selection process of genetic potential as for 2023 is milk (23%) and udder (27%) which emphasise the interest in decreasing the occurrence of mastitis in the Norwegian herds and is continuously monitored through GENO [60]. GENO is the breeding association continuously monitoring NRC and distribute cattle genetics including semen and embryos to farms both internationally and domestically with their slogan “Breeding for a better life”. NDHRS and GENO operate independently from each other, but collaborate and share data to advance breeding goals [61].

From a research project with two different breeding lines of NRC, one line containing cows bred solely for high milk yield and the other containing healthy individuals selected based only on prevalence of mastitis. The sole breeding against mastitis yielded good results and after only five generations the genetic difference between the two groups were 10 percent points in clinical mastitis. On the other hand, the selected breeding also show a decrease in occurrence of ketosis and retained placenta which are commonly seen together with mastitis and other diseases. This shows that specific breeding not only for milk yield but against diseases are important to create sturdy cows with decreased susceptibility against diseases and illnesses [62, 63]. High yielding Holstein-Friesian cows does also appear to be more susceptible to mastitis and other illnesses than medium-yielding cattle, such as the Jersey. [64, 65].The estimated genetic relationship between milk output and mastitis in dairy cattle reinforce this hypothesis [66].

2.4 Antimicrobial resistance and surveillance systems

Antimicrobial resistance (AMR) represents a complicated global health challenge that extends beyond individual bacterial species, including a complex network of gene exchanges across the microbiomes of animals, humans, and the environment. This interconnection facilitates the transfer of antimicrobial-resistant genes among varied bacterial communities, thereby exacerbating the threat of AMR [67].

A study identified multiple examples of resistant genes in soil which exhibited 100% homology to those present in clinical isolates. The study presents strong proof for horizontal gene transfer occurring between soil bacteria and pathogens, however, does not clarify the specific direction of this transfer. [68]

Farm animals is on the other hand utilize a large quantity of antimicrobials, and the essential questions is the potential risks to human health and the extent of those risks. Surprisingly above 70% of global usage of antimicrobials is estimated to be used in livestock, and it is crucial to obtain a comprehensive and quantitative grasp of the interactions among various bacteria, numerous drugs, and multiple resistance factors across different host and environmental settings [69, 70].

As an example of the rapid spread of AMR from food producing animals to humans, colistin resistant genes were found in *E. coli* of pigs in 2013, and it took less than 10 years from first emerging until global circulation in humans. [71–73]. An examination of a methicillin resistant *S. aureus* lineage affiliated with disease in both humans and animals revealed an original methicillin sensitive strain in the human population, however, during circulation in animals a methicillin resistant determinant occurred leading to consecutive transfer of the resistant strain to humans. Subsequent studies demonstrated that transfer of resistant strains was more prevalent from animals to humans, although instances of human-to-animal transmission were also observed [74]. However, studies also contradict these findings and definitive conclusions regarding the directional nature of the transmission remain unclear due to methodological limitations in the research and more research are needed to clarify the intricate transmissions dynamics of resistant bacteria across human and food animal populations. The core issue is the identical or similar AMR bacteria does not convey information about the direction of the transfer which could be one way, both or neither, potentially originating from other sources [75]. Even though some is still unclear, it is important to keep in mind the threat of AMR both in humans and animals to be able to have functional treatment for all species independent of the source.

There is growing acknowledgement that extensive antimicrobial application in food animal production may facilitate the emergence of resistance to antimicrobials frequently employed in human medicine, primarily due to the shared use of prevalent antimicrobials in both food-producing animals and humans. The application of Highest Priority Critically Important Antibiotics (HP CIA) in food animal production is regarded with significant apprehension [76, 77]. The relationship between antibiotic usage and resistance in food animals has significant public health implications for numerous zoonotic pathogens. The human and animal ecosystems are interconnected concerning various zoonotic bacteria, such as *Escherichia coli, enterococci*, and *Staphylococcus aureus*. A substantial body of evidence already exists regarding the various pathways for potential transfer between species of antimicrobial-resistant genes and bacteria, including through food, direct interspecies interaction, and indirect environmental exposure. These One Health concerns have driven policy changes, especially within the EU [78, 79].

There are multiple institutions on different levels which combat the surveillance of AMR and antimicrobial use in general both for veterinary and human medicine. In 2015 the World Health Organization (WHO) created "Global Action Plan to tackle Antimicrobial Resistance (GAP-AMR) with the vision "*to ensure, for as long as possible, continuity of successful treatment and prevention of infectious diseases with effective and safe medicines that are quality-assured, used in a responsible way, and accessible to all who need them*" [80]. The same year a comprehensive and collaborative global effort to standardize AMR surveillance was created by WHO formally known as Global Antimicrobial Resistance and Use Surveillance System (GLASS). GLASS aims to foster national surveillance system, harmonize global standards, monitor global AMR trends, estimate the global burden of AMR, detect emerging resistance, generate data to assess the impact of intervention, and inform, research and develop new tools for the prevention, diagnosis, and treatment of infectious bacterial diseases. The scope of GLASS includes standardized data collection, analysis, interpretation and sharing by participating countries with focus on monitoring the status of national surveillance systems. This system is of essence not only to monitor antimicrobial resistance but also to monitor the antimicrobial consumption. [80, 81]

Health Emergency preparedness and Response Authority (HERA) was launched in 2021 by the European Commission to prepare the EU for future pandemics and to improve its response to health crisis and potential health crisis following the COVID-19 pandemic. In 2022 AMR was identified by HERA as "top three priority health threats"[82, 83]. The United Nations Environment Programme (UNEP) estimate AMR to be responsible direct and indirect for five million deaths globally in 2019, while numbers are estimated to rise to 10 million deaths by 2050 which in 2019 was the same proportion of human deaths caused by cancer. In addition to the detrimental effects on animal and human health AMR also has a large economic impact and could by the next decade drive 24 million extra people into extreme poverty. [84] These numbers emphasize the importance of reducing antimicrobial drugs worldwide to reduce AMR and promote both human and animal health.

The Norwegian Veterinary institute surveils the antimicrobial resistance in animals under the auspices of NORM-VET. The data collected represent the occurrence of antimicrobial resistance the usage of antibiotics in veterinary medicine in Norway. The purpose of NORM-VET is to describe the connection between antimicrobial usage and bacterial resistance, risk assessment and to observe the trend and tendency over an extended period. NORM-VET was already established in 2000 as a part of a government plan to act against AMR and continued the requirement put down from the EU Commission since 2014 (regulation 2013/652/EU later 2020/1729/EU).

Milk samples from cows in the Norwegian herds are tested by TINE Mastitis Laboratory in Molde and reported in the Norwegian Dairy Herd Recording system. The laboratory is responsible for antimicrobial resistance testing on selected isolate as a key role in the surveillance of antimicrobial resistance in dairy herds [85].

Norway, along with Denmark, England, the Netherlands, Sweden, and Switzerland, showed low frequencies of resistance to antimicrobials compared to countries like Belgium, France, Italy, Latvia, and Spain, where many isolates were resistant to multiple antimicrobials tested. The European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) project provides data on antimicrobial sales and prescribing patterns in European countries, showing large differences between countries in terms of antimicrobial usage [86, 87].

3. Objectives

The objective of this thesis is to evaluate the prevalence of mastitis in some Norwegian dairy herds and to understand on farm level which practices are used to prevent mastitis. Specifically, the farmers were interviewed about their knowledge of pathogens causing mastitis, mode of transmission as well as their practice containing the disease. We think that targeted preventative measures can effectively lower the prevalence of mastitis simultaneously reduce antibiotic use in dairy sector leading to improved herd health and reduced risk of AMR. This consequently can result in increased milk production and overall profitability for the dairy farmers. The importance of this topic is to cover the emerging crisis of AMR, and to study on-farm methods to decrease the use of antibiotics.

4. Materials and methods

In this study 5 randomized farms were investigated all using automatic milking system, and they all answered the following questionnaire.

Questionnaire to farmers

1. What is the prevalence of mastitis in your herd
2. Do you have a farm profile on which bacteria is most common in your herd?
3. What is the current practice of preventing mastitis?
4. When you get the laboratory report about a specific pathogen who interprets the results and do you as a farmer actually understand where the specific pathogen comes from – is it environmental or contagious?
5. If you do not understand the bacterial results who do you ask?
6. What measures to you put in place after a clinical mastitis from an epidemiological point of view to reduce the spread?
7. Do you use any homeopathic/non-prescription drug which does not contain antibiotics?
8. What methods do you use for dry cow therapy?

The farmers were interviewed separately and without knowledge of other farmers answers, in addition, the farmers are completely anonymous and selected based on their milking systems (AMS). Furthermore, only farms sending milk samples regularly were interview for the possibility to interpret the laboratory results and look at the dominant pathogen.

5. Results

<u>Farm 1</u>

Number of cows: 68

1. What is the prevalence of mastitis in your herd?

"0.13/year. It differs based on seasonality, in the summer it is more frequent than in winter"

2. Do you have a farm profile on which bacteria is most common in your herd?"

"*S. aureus* is the most common case of clinical mastitis in my herd which is the one I test. In the summer the most common is *T. pyogenes*".

3. What is the current practice of preventing mastitis?

"Just the regular, separating the cow, milking by hand and discarding the milk. I take care to send milk samples regularly before drying off, in addition I always take care if the computer tells me the SCC is high and try to investigate if I can find any mistakes with the robot, bedding etc.".

4. When you get the laboratory report about a specific pathogen who interprets the results and do you as a farmer actually understand where the specific pathogen comes from – is it environmental or contagious?

"I have not done so much research about the topic, but I prefer asking the veterinarian if there are any problem."

5. If you do not understand the bacterial results who do you ask?

"I usually ask the veterinarian and follow their recommendations without too many questions asked, I do not really have time for this".

6. What measure do you put in place after a clinical mastitis from an epidemiological point of view to reduce the spread?

"First is usually to call the veterinarian, if there are multiple red cows on the computer, I check the environment and put more shave wood bedding on the laying area and check if the robotic slatter cleaner is working. The cow is separated into the sick pens, and while getting IM infusions they are milked either by hand or manual electric milking machine to prevent it from entering the bulk tank. In some cases I have to milk out by hand, especially the ones caused by *T. pyogenes* which the robot usually is not able to milk"

7. Do you use any homeopathic/non-prescription drug which does not contain antibiotics?

"No not any"

8. What methods do you use for dry cow therapy?

"For problematic cows I take a milk sample and the veterinarian help me to choose what is correct for that particular cow. We treat with Orbenin (cloxacillin) if needed, or just regular Mastipen (benzyl-penicillin procaine). Most of the cows are not treated at all at drying off".

Farm 2

Number of cows 78

1. What is the prevalence of mastitis in your herd

“0.17/year, about once or month in theory but in practice there are more in the summer”.

2. Do you have a farm profile on which bacteria is most common in your herd?

“*S. aureus*”

3. What is the current practice of preventing mastitis?

“The robot takes care of the most of it but of course I followed results given by the robot and try to call the veterinarian in time. ”

4. When you get the laboratory report about a specific pathogen who interprets the results and do you as a farmer actually understand where the specific pathogen comes from – is it environmental or contagious?

“I know bacteria such as *E. coli* is from the environment and the big bad one *S. aureus* is contagious. We have had some support from Lely trying to find solutions for us to be sure the robot is not spreading it”.

5. If you do not understand the bacterial results who do you ask?

“The veterinarian or my advisor”

6. What measures to you put in place after a clinical mastitis from an epidemiological point of view to reduce the spread?

“Separate the cow into sick-pen, and follow her convalescence separately. It is usually not a big problem”.

7. Do you use any homeopathic/non-prescription drug which does not contain antibiotics?

“No”

8. What methods do you use for dry cow therapy?

“Some cows are treated at drying off with Mastipen (benzyl-penicillin procaine) but usually none.”

Farm 3

Number of cows: 80

1. What is the prevalence of mastitis in your herd

Not very common, sometimes during the summer months I have the *Trueperella pyogenes*. Prevalence given by the computer: 0.15

2. Do you have a farm profile on which bacteria is most common in your herd?

"*S. dysgalactiae*, and it mostly occur during the summer when they are out on their mandatory summer holiday"

3. What is the current practice of preventing mastitis?

My robot takes care of the most, but of course I follow the computer screen about cleaning the robot and shaving the udders and tails.

4. When you get the laboratory report about a specific pathogen who interprets the results and do you as a farmer actually understand where the specific pathogen comes from – is it environmental or contagious?

"No the more knowledgeable people probably knows"

5. If you do not understand the bacterial results who do you ask?

"My veterinarian is very helpful, and so are my farmer friends."

6. What measures to you put in place after a clinical mastitis from an epidemiological point of view to reduce the spread?

"Just the regular, separating the cow, milking by hand, and treating the sick cow with intramammary infusions given by the veterinarian"

7. Do you use any homeopathic/non-prescription drug which does not contain antibiotics?

No

8. What methods do you use for dry cow therapy?

"Currently none, I've had some treated with orbenin (cloxacillin) after the veterinarian recommended it.".

<u>Farm 4</u>

Number of cows 49

1. What is the prevalence of mastitis in your herd

I think I have some more problems with it than others, from NDRHS I can see that I am above national values in percentage. I have however not explored all my options on what to do since I am fairly new in this game and just took over this farm from my father. Prevalence given by the computer: 0.26"

2. Do you have a farm profile on which bacteria is most common in your herd?

"*S. uberis* is most common"

3. What is the current practice of preventing mastitis?

"I have not any concrete practice, I call the veterinarian which comes out and usually give the cow injections and I get the intra-mammary infusions to give the cow for 5 days".

4. When you get the laboratory report about a specific pathogen who interprets the results and do you as a farmer actually understand where the specific pathogen comes from – is it environmental or contagious?

"No but I want to learn this and is one of my priorities the second I get some more time around here".

5. If you do not understand the bacterial results who do you ask?

"The laboratory gives out a comment about the bacteria, but I ask the veterinarian".

6. What measures to you put in place after a clinical mastitis from an epidemiological point of view to reduce the spread?

"I have started to not give calves milk from cows with mastitis, as well as I am aware of the fact that I have a contagious mastitis bacterium on my farm. In addition the veterinarian told me it could be spread by manure and bedding, this makes hygiene more of a crucial aspect, and I separate the cow."

7. Do you use any homeopathic/non-prescription drug which does not contain antibiotics?

"No I am not aware of anything here unfortunately"

8. What methods do you use for dry cow therapy?

“We have tried treating the cows affected with *S. aureus* with drying off preparations as recommended by the veterinarian, but I am not sure if it has succeed yet, we will have to wait and see. Most of the cows are not treated at drying off”.

Farm 5

Number of cows: 89

1. What is the prevalence of mastitis in your herd

"I don't have a big problem with it, sometimes I get it but it gets less and less prevalent. Prevalence on computer: 0.14"

2. Do you have a farm profile on which bacteria is most common in your herd?

"Mostly *S. aureus*"

3. What is the current practice of preventing mastitis?

"I keep the maintenance schedule as given by the operator for the robot, I monitor the bulk somatic cell count closely and if there are any alarms or events I try to investigate my problems as fast as possible."

4. When you get the laboratory report about a specific pathogen who interprets the results and do you as a farmer actually understand where the specific pathogen comes from – is it environmental or contagious?

"No but I know there is a brochure existing about it"

5. If you do not understand the bacterial results who do you ask?

"I have good advisors in TINE, as well as my veterinarian"

6. What measures to you put in place after a clinical mastitis from an epidemiological point of view to reduce the spread?

"Not much, really. I separate the cow from the rest of the flock, and I do not let her go into the robot. Instead, I milk her by other means. I usually send a milk sample straight away and call the veterinarian after it has arrived if there are no severe signs of the cow being ill".

7. Do you use any homoeopathic/non-prescription drug which does not contain antibiotics?

"No"

8. What methods do you use for dry cow therapy?

“Some cows after milk samples receive antibiotics from the veterinarian as intramammary infusions at drying off, but not all. Problematic cows I usually send for culling and do not keep for another lactation.”

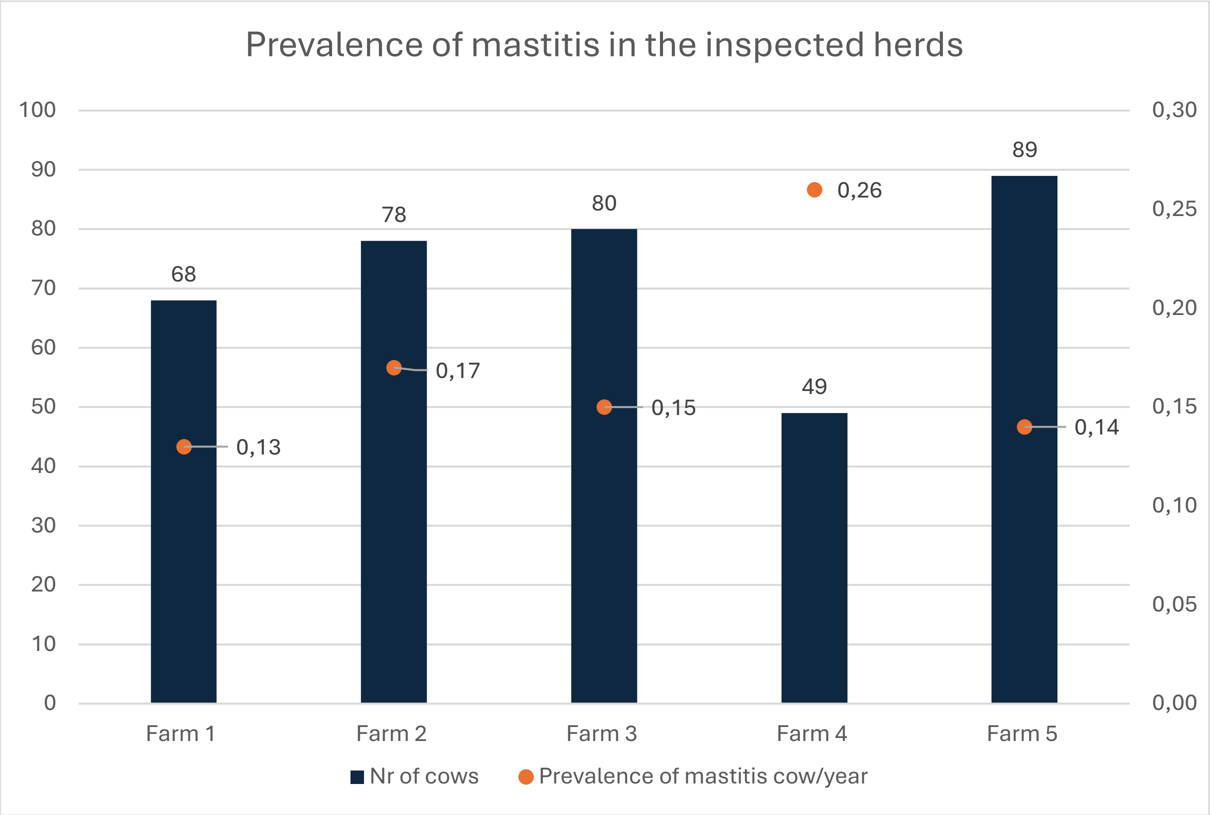

Figure 2: Number of cows and prevalence of mastitis in the inspected herds.

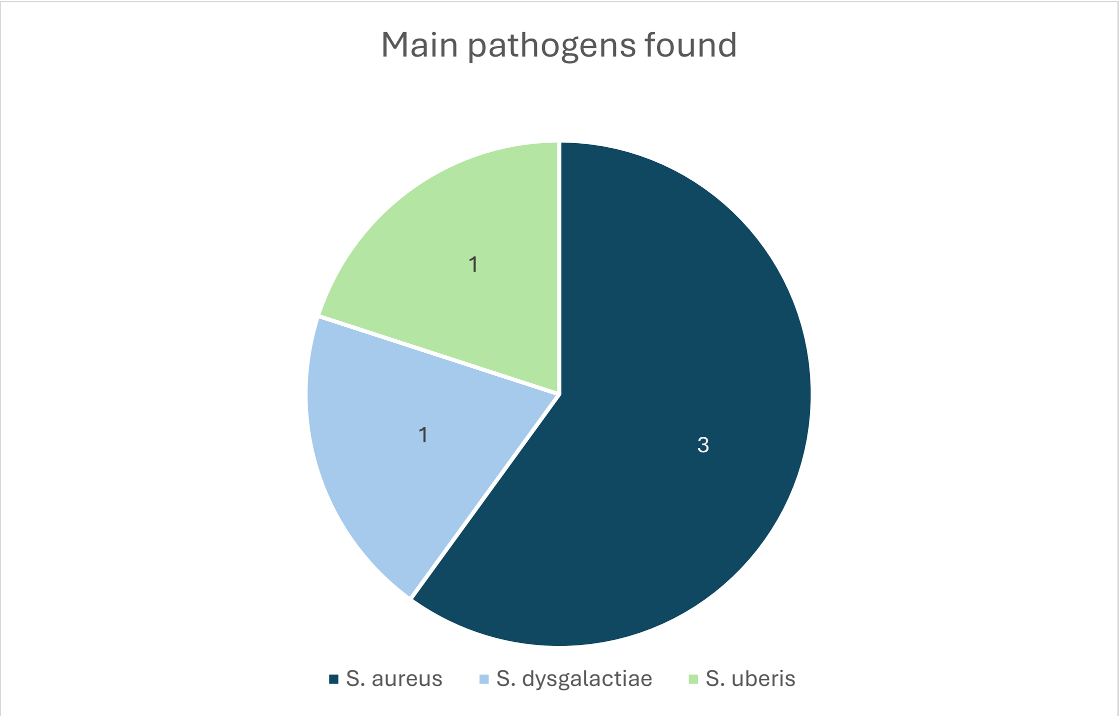

Figure 3: Diagram of the main pathogens found in the inspected farms based on milk sampling. S. auerus (3), S. dysgalactiae (1) and S. uberis (1).

Preventative measures

All five farms noted that they monitored the somatic cell count closely as preventative measures.

Measures put into action after a case of clinical mastitis

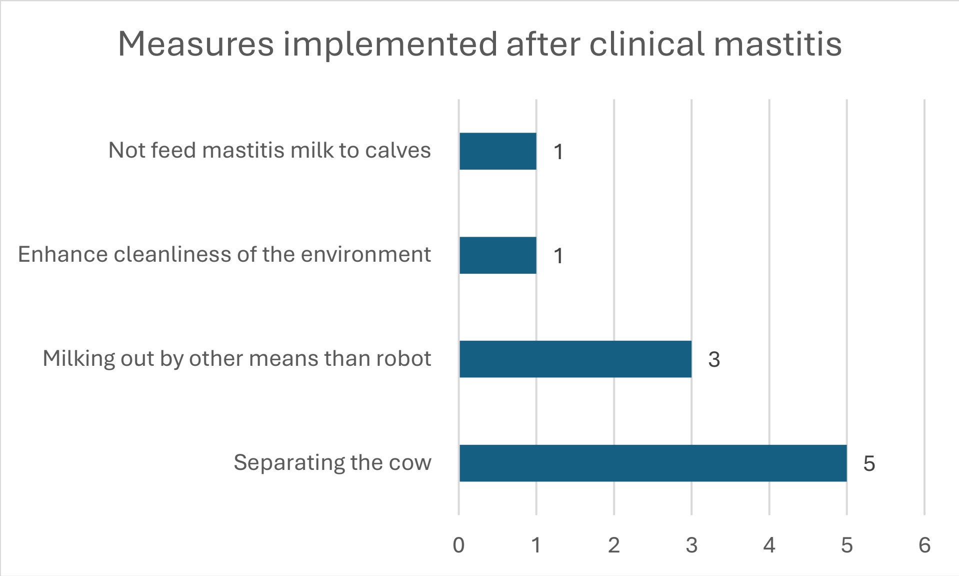

Figure 5: Measures implemented after clinical mastitis on the investigated farms. All farms were separating the sick cows into sick pens (5), three of the farms milked out by other means than robot (3), and one farmer focused on enhancing the environment of the cows by putting wood shavings on the laying area (1). One farmer does not feed the milk from cows with mastitis to calves.

Dry cow therapy

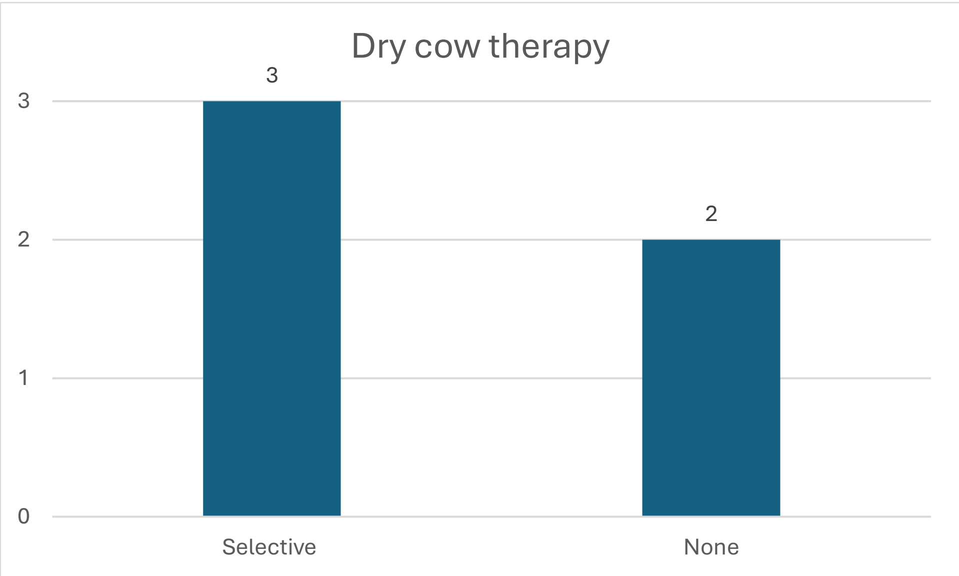

Figure 6: Diagram showing that three of the farms used dry cow therapy for drying off, while two farms used it inconsistently or not at all.

6. Discussion/Conclusion

The findings from this study of some Norwegian farms underscore important insight into the prevalence and management of mastitis. The results highlight *Staphylococcus aureus* as the most frequently identified pathogen. This pathogen was dominant on three of the five farms, aligning with the previous literature from the Norwegian mastitis laboratory, where they concluded *S. aureus* to be responsible for 28% from the study in 2020. Since this pathogen is associated with biofilm production and its contagious nature, it would be beneficial to investigate further the epidemiological control on these farms and, if necessary, launch eradication programs. The identification of *Streptococcus dysgalactiae* and *Streptococcus uberis* further broadens the pathogen diversity within these herds.

A noteworthy observation was that all farms investigated were within the category "average" of the udder health assessment (Table 1).

It is also worth mentioning that multiple farmers were mentioning *Trueperella pyogenes* as summer mastitis, which is also mentioned in the Norwegian guidelines for treating mastitis. Further research is needed to conclude if the increased incidence of this type of mastitis is correlated with the mandatory grazing period of Norwegian cows during the summer.

Farmers primarily rely on computer monitoring somatic cell count data, but it is not a preventative measure as most farmers mentioned, but rather a measure and tool to select the cows with mastitis.

The measures put in place after mastitis differ on the investigated farms. Still, as a rule, all of the farms separated the affected cow from the rest in the sick pens, reducing direct contact with healthy animals. Three of the farms milked the cows by other means than the robot in case of mastitis, which could be an important factor in the spread of contagious mastitis if the cleaning and maintenance of the robot is inadequate.

While some farmers knew the distinctions between contagious and environmental bacteria, others relied heavily on veterinarians and advisors to interpret the results. This highlights the probable need for increased farmer education focusing primarily on pathogens, epidemiology and biosecurity practices.

Three of the five farms relied on selective dry cow therapy, which is included as a recommendation in the Norwegian guidelines and aligns with the broader One Health objectives to minimise antimicrobial use. However, two of the farms did not do anything for dry cow therapy, which could be advantageous for the farmer in decreasing the chronic carriers of mastitis among the herd. Moreover, the usage of internal teat sealants or other homoeopathic drugs was absent on all examined farms. This suggests a potential for implementation, especially given their proven efficacy in reducing new intramammary infections.

Recommendations

The recommendations after this study include:

1. Enhance farmer education by closing knowledge gaps regarding pathogen transmission and biosecurity. This can help farmers make better decision and to create the best practices for their farm.
2. Utilise the possibility of using non-antibiotic interventions. Increasing awareness and use of internal teat sealants.
3. Enhancing hygiene protocols: farms with a higher prevalence of mastitis should prioritise finding the source of the infection, and keep in mind the maintenance and cleaning of milking equipment. It is also of essence to create a better environment, especially if the housing is not adequate.

Challenges and limitations of the study

The sample size of five farms limits the broader applicability of results to the wider Norwegian dairy industry, and the selection of farms that regularly send milk samples may also introduce bias. Furthermore, the reliance on self-reported data from farmers introduces the potential for recall bias, which may impact the accuracy of reported practices and pathogen prevalences.

Norwegian farmers benefit from strong veterinary support and national guidelines emphasising prudent antibiotic use. While these findings are valuable within Norway, caution should be exercised when generalizing to other countries.

This thesis supports a comprehensive, evidence-based approach to mastitis management combined with proactive monitoring, selective antibiotic use and enhanced farm hygiene. By following prudent-use guidelines and adopting preventative measures, dairy farmers can achieve better herd health, reduce antibiotic use and contribute to the global effort to combat AMR.

This study reinforces the importance of integrating scientific knowledge with on-farm practices. Possible further studies should explore a larger and more diverse farm sample to confirm the findings. It may also be beneficial to conduct a study comparing different countries to gain insight into the effectiveness or ineffectiveness of Norway's strict antibiotic policy compared to areas with less regulation.

Additionally, understanding genetic factors related to the susceptibility of mastitis may also facilitate more sturdy cows aimed at enhancing disease resistance, as has been shown with the lineages of NRCs and GENOs continuous breeding program.

Finally, more advanced studies on AMR trends in dairy herds will be essential to assess the long-term impact, with AMR posing a growing threat to public health. Continuous surveillance and research are crucial for developing effective, sustainable strategies that benefit both animal and human health.

7. Summary

Mastitis remains a central challenge in dairy cattle farming, impacting milk quality, quantity and overall herd health, driving antibiotic use. This thesis investigates the prevalence and management of mastitis in five Norwegian dairy herds, specifically focusing on reducing antibiotic use and combating AMR. Through structured interviews with farmers and milk samples analysis, the study identified *Staphylococcus aureus* in three of the five farms as the primary pathogens. *Streptococcus dysgalactiae* and *Streptococcus uberis* were also identified. We also evaluated current preventative measures and on-farm practices that were aligned with Norwegian Guidelines for prudent antibiotic use.

All farms relied on SCC monitoring via AMS for mastitis detection, but preventative measures were inconsistent. While most farms separated the cow with mastitis into sick pens, it is thought that following the guidelines closer would affect the udder health of the farm positively. Three of the farms utilised selective dry cow therapy in accordance with Norwegian guidelines, however, two of the farms did not implement any or little dry cow therapy, missing opportunities to address the chronic carriers of mastitis. Non-antibiotic-containing measures such as internal teat sealants were absent. This study highlights the importance of integrating scientific knowledge into on-farm practices and underscores the potential of targeted education to reduce the prevalence of mastitis and the risk of AMR.

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