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Mastitis and Milk Quality in Cows Held by Smallholder Farmers in Mzuzu Region in Northern Malawi

Mastitt og melkekvalitet hos melkekyr holdt av småskalabønder i Mzuzu-regionen i nordlige Malawi

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Preface

In an era of growing global awareness about sustainable agriculture and food security, the interaction between livestock health and milk production has become an imperative subject of exploration. As veterinary students with a genuine interest in cattle farming, we have chosen our topic "Mastitis and Milk Quality in Cows Held by Smallholder Farmers in Mzuzu Region in Northern Malawi". This resonates with our passion for bovine health and the challenges it can pose.

Our fascination for the relationship between dairy cattle, their environment and the farmers are rooted in our experiences gained from previous projects we have worked on in Africa, where we had the privilege of working with both wild and domesticated animals. These experiences ignited our interest to learn more about how routines and conditions in other parts of the world are compared to those we have here in Norway.

Through this research, we aim to shed light on the challenges faced by smallholder farmers in Malawi as they strive to ensure safe and effective milk production, with a special focus on the issue of mastitis. By doing so, we hope to provide valuable insights that can aid in the development of more effective and targeted strategies for improving dairy cattle health and milk production.

Summary

Title: Mastitis and Milk Quality in Cows Held by Smallholder Farmers in Mzuzu region in Northern Malawi

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Fieldwork for this study project was undertaken as part of the TRANSFORM initiative and FoodMa programme in the Mzuzu region of Northern Malawi. The aim of this study is to explore factors that can influence dairy cattle health and enhance milk production and quality in this region. A total of 40 dairy cows, owned by smallholder farmers, were examined, and milk samples were collected. For each cow, the California Mastitis Test (CMT) was performed, and a composite pooled milk sample was collected. The cleanliness of the cows was assessed through hygiene scoring. The milk samples from the cows were analyzed in the laboratory, and resistance testing was performed. The farmers in Malawi are organized into "bulking groups". In this study, three bulking groups were included, and milk samples were collected from each group. None of the cows exhibited signs of mastitis, but CMT revealed that 30% of the cows had subclinical mastitis. The bacteria identified in milk samples were mainly gram-positive cocci. The bulk milk samples were tested for the presence of penicillin and cephalosporin residues, but no such residues were found. Resistance to several types of antibiotics was detected. Even though the general appearance of the cows was good, we found that dairy milk production in Mzuzu have improvement potential.

Abbreviations

Term	Definition
UN	United Nations
USD	United States Dollar
SCC	Somatic Cell Count
WHO	World Health Organization
AMR	Antimicrobial resistance
AMU	Antimicrobial Use
LMIC	Low- and Middle-income Countries
SNF	Solid-Not-Fat
TS	Total solids
PPD-C	Purified Protein Derivates
FFA	Free Fatty Acids
NCA	Norwegian Church Aid
NMBU	Norges Miljø- og Biovitenskapelige Universitet (Norwegian University of Life Sciences)
LUANAR	Lilongwe University of Agriculture and Natural Resources
CMT	California Mastitis Test
CVL	Central Veterinary Laboratory
BCS	Body Condition Score
CFU/mL	Colony Forming Unit/mL

1 Introduction

1.1 TRANSFORM and FoodMa

This study was linked to the two major programmes, TRANSFORM and FoodMa. Both are in collaboration with NMBU (Norwegian University of Life Sciences) and LUANAR (Lilongwe University of Agriculture and Natural Resources). The TRANSFORM programme (Sustainable Food Systems for Rural Agriculture Transformation and Resilience Programme) is a four-year initiative (2021-2025) funded by the Royal Norwegian Embassy and managed by the consortium partners Norwegian Church Aid (NCA) and NMBU together with partnership of LUANAR (NCA, 2023).

The main objective of TRANSFORM is to strengthen local food systems in Malawi to improve food security, climate resilience, and income among agriculture-dependent rural households in Malawi. The program aims to assist 150,000 households across various districts in Malawi. Its overarching goal is to bolster local food systems and achieve four interconnected outcomes: 1) Increased productivity, production, diversification, and resilience to climate change at household and community levels; 2) Increased consumption of safe, nutritious, and diverse food; 3) Improved profitable market access and entrepreneurship; and 4) Improved research, policy and regulatory environment for agriculture transformation and climate resilience.

The main goal of the program “Sustainable food systems in Malawi” (FoodMa) is to contribute towards resilient and Sustainable Food Systems for better income and improved food and nutrition security in Malawi (LUANAR, 2020). The Programme objectives are presented in four Work Packages (WP) as follows:

- WP1: Strengthen food system governance and institutions;

- WP2: Enhance agricultural biodiversity, farming systems and seed security;
- WP3: Enhance climate smart agriculture through sustainable agriculture intensification; and
- WP4: Strengthen the capacity of LUANAR to respond to issues affecting the Malawi food system.

The present study was initiated by the LUANAR and NMBU partners in Transform and has been entirely dependent on the close collaboration with LUANAR facilitated by Dr. Liveness Banda and her two master`s students in animal science. We have had different areas of focus and shared results and observations. While we have concentrated on the veterinary aspects, including the general health of the animals, udder health, bacteriological findings in milk samples, antibiotic residues, and antibiotic resistance, the Malawian master's students have focused on surveying crucial factors that may influence milk quality and quantity through questionnaires.

1.2 Animal health, food safety and food security

Animal health, food safety, and food security are all interconnected terms that are part of the global food supply chain. Ensuring the health and well-being of livestock is crucial for maintaining both food safety and food security (Lewis, 2022).

Animal health plays an important role in food safety. Healthy animals are less likely to carry and transmit diseases to humans through the consumption of products like milk and meat. Veterinary care and disease management on the farms are essential to prevent contamination of the food with pathogens that may pose health risks to consumers (World Organization for Animal Health, 2023).

Animal health is also linked to food security. Livestock are valuable sources of protein and nutrients in many diets worldwide (UN Nutrition, 2021). With a growing world population

and many people already lacking adequate access to nutritious food, animal health is very important to ensure an adequate food supply. Healthy animals are more productive, leading to increased food production and a more stable food supply. Any outbreak of diseases among livestock can lead to reduced production, food shortages, and increased food prices, all of which can impact food security negatively (Capper and Williams, 2019).

1.3 Malawi

Malawi is a Sub-Saharan country, which borders Mozambique, Tanzania and Zambia. Malawi has an estimated population of 20 931 751 people (FN-sambandet, 2022). The capital and largest city is Lilongwe. Malawi is one of poorest and least developed countries in Africa (Johannesen, 2023). The United Nations (UN) estimates that 65,3% of the population lives in extreme poverty, which is defined as living below 1,9 USD a day (FN-sambandet, 2022).

1.4 Dairy production in Malawi

The economy in Malawi is mainly based on agriculture, and 64,2% of the total land area is cultivated land (FN-sambandet, 2023). More than 80% of the population lives in rural areas, and their income is largely dependent on agriculture (Food and Agriculture Organization of the United Nations, 2006). Dairy production in Malawi is situated around the three largest cities in the country; Lilongwe (Central region), Blantyre (Southern region) and Mzuzu (Northern region) (Tebug et al., 2011). There are some commercial farms, but dairy production is mainly dominated by smallholder farmers. Smallholder farmers is a term used more broadly to refer to rural farmers, primarily in developing countries, who rely mostly on family labor for farming and whose farms are their main source of income (Bhatti, 2016).

Smallholder farmers in Malawi are organized into milk bulking groups (Tebug et al., 2011). When the milk reaches the bulking center, the milk is weighed and tested (further described below). After the milk is weighed and tested, the milk is either sold to a commercial dairy or sold directly from the bulking group. In the Mzuzu region, the milk is sold directly from the bulking group to private buyers or venders. Venders are buying the milk from the bulking group and are selling it in the city. It is also likely that some of the farmers sell their milk directly to consumers.

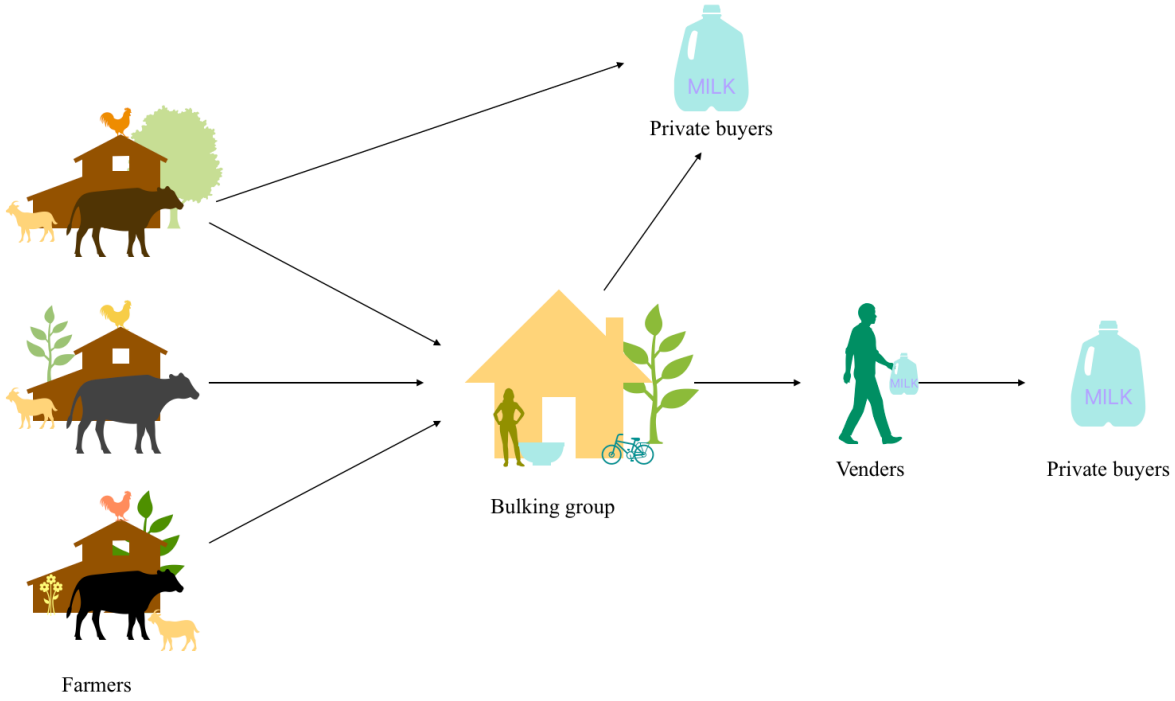

Figure 1. An illustration of the milk distribution in the Mzuzu region. The milk is transferred from the farm to the bulking group (or sometimes direct to private buyers). From the bulking group it can either be sold to private buyers, or through venders to private buyers.

1.5 Methods for testing the quality of the milk

1.5.1 Alcohol test

The alcohol test is based on coagulation of milk with low pH when ethanol is added. The alcohol test is performed with a milk testing gun. When milk is adulterated with soda or originates from a cow with severe mastitis or colostrum, the pH will decline, and the milk will coagulate (Dairy Knowledge Portal, 2023).

1.5.2 Density test

The density test is based on measuring the specific density of milk and is performed with a lactometer. If water is added to the milk, the specific gravity is lower than regular milk. Colostrum will have higher levels of density. Because milk has higher density than water, the lactometer will sink less in milk than in water (Draaiyer, 2009).

1.5.3 Soda test

This test is similar to the alcohol test, but also includes the addition of rosalic acid. The milk is adulterated with sodium carbonate or sodium bicarbonate if the milk changes color to pink/red following addition of rosalic acid (Centre for Science and Environment, 2023).



Pictures above. Left; An example of a milk testing gun. Middle; A lactometer used to measure the milk density. Right; Lusangazi Dairy, one of the collection points for the delivery of milk from farmers in the area. Pictures: Line Halbjørhus.

1.6 Mastitis and clinical signs

Mastitis is an inflammatory condition that affects the udder of the cow. It is a common health challenge in dairy production and is typically caused by bacterial pathogens. The condition can be classified into clinical and subclinical mastitis. Clinical mastitis is characterized by cardinal symptoms of inflammation in the udder, such as warmth, redness, swelling, pain and reduced function, and the cow often has fever and reduced general condition. The milk will also have an abnormal consistency, often containing clots. Subclinical mastitis on the other hand, lacks obvious clinical signs, but is detected through somatic cell count (SCC) analysis of the milk. Subclinical mastitis may be a precursor to clinical manifestation (Agriculture and Horticulture Development Board, 2023).

Both clinical and subclinical mastitis can negatively impact milk quality, both in terms of reduced milk yield and altered composition. In addition, the condition will result in poor animal health and welfare, making it crucial to diagnose and treat mastitis early to maintain healthy and profitable milk production (Gonçalves et al., 2020). The prevalence of mastitis in Malawi is scarcely documented, however a literature study from 2012 describes that as much as 39,5% of the farms in Malawi are suffering from losses due to mastitis (Baur et al., 2016), and reports that subclinical mastitis is much more common than clinical mastitis.

1.7 Antimicrobial use and resistance in Malawi

According to the World Health Organization (WHO), antimicrobial resistance (AMR) is one of the biggest threats to global health, food security and development today. It is well established that there is a strong association between the usage of antimicrobial agents and the occurrence of AMR (NORM/NORM-VET, 2020). In many low- and middle-income countries (LMIC) like Malawi, antimicrobial agents can be bought over the counter. This is an

important factor for increased antimicrobial use (AMU), which is the main driver behind the development of AMR (World Health Organization, 2020).

Surveillance and monitoring are acknowledged as critical components of the response to AMR. Many LMIC´s, like Malawi, do not have robust systems for collecting and analyzing data on AMU and AMR because of lack of resources and capacity (Interagency Coordination Group on Antimicrobial Resistance, 2018). AMU and AMR in livestock in Malawi are scarcely documented, but there have been some studies from medical hospitals. One study carried out between July 2006 and December 2007, concluded that there is resistance to almost all the antimicrobial agents that are empirically used in Malawi, and that antimicrobial agents that have not been widely introduced in Malawi show better laboratory performance (Makoka et al., 2012). It is not known whether the situation is similar for livestock.

A study performed in Blantyre stated that oxytetracycline was the most frequently administered antibiotic in livestock. The same trend is also seen in other parts of Africa, and worldwide. In Malawi, oxytetracycline is often dispensed in mixes with other antibiotics or together with vitamins (Mankhomwa et al., 2022).

There is little research available on AMU in Malawi, and how the smallholder farmers handle withdrawal periods for milk. In the study from Blantyre, they found that meat was often sold for human consumption without adherence to the withdrawal period for antimicrobial agents. This can increase the likelihood of humans becoming exposed to both resistant bacteria and antimicrobial residues through consumption of contaminated meat, hence jeopardizing food safety (Mankhomwa et al., 2022).

1.8 Lilongwe Dairy

Lilongwe Dairy is the largest milk processing company in Malawi, and is located in the Central region, in the capital Lilongwe. Lilongwe Dairy collects milk mostly from the Southern region, and on a smaller scale from the Central region. To date, Lilongwe Dairy does not buy milk from the Northern region (L. Banda, pers. communication, 2023). The farmers who deliver milk to Lilongwe Dairy are mainly smallholders that are organized into bulking groups. The farmers in the same bulking group deliver milk to the same cooling tank. When the cooling tank is full, milk transporting trucks from Lilongwe arrive and transport the milk to the dairy, and the processing can start. The trucks do not have any cooling system, so the milk is transported by night when the outside temperature is low. The night temperature varies by region and season but is approximately between 10-20°C (OpenAI, 2023).

Before the milk is further processed, several tests are performed on the dairy. These tests are the same as those performed on the milk from the cooling tank; temperature, density, soda-test and alcohol test. The dairy also examines the milk for fat, density, SNF (Sold-Not-Fat), TS (Total Solids), lactic acid, protein, lactose, casein, PPD-C (Purified Protein Derivates), FFA (Free Fatty Acids), glucose and urea. The composition of the milk decides what product the milk is further processed to.

Lilongwe dairy has no common routine for examining the milk for antibiotic residues. If they are planning to use it for making yoghurt or cheese, a small amount of the batch is added to a bacterial culture. If the milk thickens, the batch is approved for yoghurt and cheese making. If the batch does not thicken, it is likely that the milk contains antimicrobial agents, and the milk is used as either raw milk, or to make products that do not require a bacterial culture. The testing of the milk for antimicrobial residues is therefore not to ensure food safety or spread of AMR, but to avoid that the antimicrobials inhibit the starter culture when making yoghurt or cheese.

Lilongwe Dairy only utilizes 40% of their capacity and thus can process a lot more milk than they do today (Lilongwe dairy employees, pers. communication, 2023). Their limitation is the amount of milk they receive from the farmers. Another limitation is that they only collect milk from the Southern and the Central region of the country. If the smallholder farmers in the Northern region could provide milk of acceptable quality, it would be possible for the dairy to start collecting milk from the Northern region as well. Additionally, this could provide the Northern smallholder farmers with a more reliable income and consequently improve their living conditions.

2 Aims

2.1 Overarching aim

The overarching aim of this study was to investigate the factors that can influence dairy cattle health and enhance milk production and quality in the Mzuzu region in Northern Malawi, and thus increase the potential of delivering milk from this region to Lilongwe dairy. Specifically, we have focused on evaluating the occurrence of clinical and subclinical mastitis.

2.2 Objectives

The objectives are designed to guide our research efforts towards achieving our overarching goal.

- **Objective 1 - General health:** Assess the cow health by clinical examination and questionnaire information from the farmer.
- **Objective 2 - Udder health:** Evaluate the udder health by signs of inflammation and California Mastitis Test.
- **Objective 3 – Bacteriology:** Examine teat samples from the selected cows to identify the dominant bacterial species present in the milk.
- **Objective 4 – Antibiotic residues:** Investigate the presence of antibiotic residues in bulk milk.
- **Objective 5 - Antibiotic resistance:** Determine the prevalence of antibiotic resistance among the identified bacterial strains by using antibiotic disks.

3 Materials and methods

3.1 Ethical considerations

In this study all the ethical protocols were followed as assessed by the experts at the Lilongwe University of Agriculture and Natural Resources (LUANAR), Malawi (L. Banda, pers. communication, 2023).

For the survey questionnaire proper consent was taken before asking questions and participants were made aware about voluntary participation and maintained a commitment to "do no harm". Confidentiality and anonymity were taken care of to safeguard the privacy of the individuals involved.

The animal aspects have included ethical guidelines around humane handling, focus on protecting the animals from physical and psychological harm, and ensuring access to essential resources such as water, shelter, and food. A fundamental component of animal welfare, often summarized by the five freedoms, underscores the importance of allowing animals to fulfill their crucial behavioral and social needs, creating a framework that prioritizes their well-being (ASPCA, 2023). These are all important points emphasized in this research.

Balancing these considerations has been vital to conduct ethical and responsible research, promoting both the welfare of human and animal participants. This is first-hand information from the project leader of the overarching project in Malawi, where they are currently in the process of establishing an ethical committee to work on the ethical considerations.

3.2 The study population

The study population is lactating dairy cows belonging to smallholder farmers in the Mzuzu region in Northern Malawi. Dairy cows that had calved the last three days were excluded from the study. The farmers were selected by a local extension officer.
Farmers in the same geographic area are organized into bulking groups. Three different bulking groups were visited, and the study population was held by farmers associated with these bulking groups. The farmers deliver milk to a designated bulking group.

3.3 Questionnaire

3.3.1 Questionnaire farmers

The two master`s students in Animal Science at LUANAR prepared a questionnaire for the farmers. We have utilized some of these responses in our thesis to better form a comprehensive picture of the cows included in this study. The elements we have used is milk/day, days in milk, lactation number, age, and Body Condition Score.

3.3.2 Questionnaire bulking group

A questionnaire consisting of eight questions was prepared to get information about the milk processes at the bulking group (Appendix 1). The questions focused mainly on the quantity of milk received and how the quality of the milk is examined, the Malawian master`s students helped with the translation.

3.4 Sample collection

Consent from the farmers was obtained before the visits. After arriving at the farm and after a short briefing of the farmers, the data was collected. The two Malawian master`s students performed the questionnaire while we collected data from the cows. The farmer assisted in restraining the cow and provided water, soap, and a cloth to clean the udder. The person in direct contact with the cow performed the cleaning of the udder, California mastitis test, milk sampling and clinical examination, and the other one assisted and wrote down the information collected on a sheet (see section 3.4.1).

Each cow got their unique ID number. The first number in the ID of the cow was the farm number, and the second number was the cow number at the specific farm.

3.4.1 Data recording

An observation list was prepared in advance (Appendix 2). The same list was used for all cows and filled out at each farm visited. The list includes registration about housing, feed, water, hygiene-score, breed, clinical examination, udder examination and California mastitis test. The reference values we have used in the clinical examination are taken from the book "Klinisk diagnostikk hos produksjonsdyr" (Løken, 2013). These are also listed in Appendix 8.

3.4.2 Hygiene scoring

The hygiene score of the udder consists of two observations, the udder seen from behind and the udder seen from the side. The same method was used to assess the hygiene of the body, where one score was made on the lower hind legs and the other score upper rear part. Each cow got a score from one to four based on these two parameters. The form we used to evaluate the hygiene score is attached in Appendix 3. We chose to merge these two parameters to get an impression of the udder hygiene and the body hygiene. A total score

between two and four is considered good, and a total score between five and eight is considered poor.

3.4.3 California Mastitis Test

After the udder was cleaned and dried, the California Mastitis Test (CMT) was performed with CMT reagent (DeLaval, Norway) and a CMT paddle. Milk from each four quarters of the udder was mixed with CMT reagent on the CMT paddle. Each quarter got a score from one to five, depending on the change in viscosity of the mixture of CMT reagent and milk. Appendix 4 includes the scoring assessment. We chose to merge the CMT values from each teat of each cow, to make an average CMT score so that we could determine whether the cow had subclinical mastitis or not. A score of 2.5 or more was classified as subclinical mastitis. An average score less than 2.5 was classified as no mastitis. This assessment is based on TINE's CMT scale (appendix 4). A CMT score of 2 indicates a cell count between 150,000 and 550,000. Score 3 indicates a cell count between 400,000 and 1.5 million. If the cell count is above 200,000, TINE defines it as subclinical mastitis (Whist, 2017).

3.4.4 Milk sample collection from the farms

After the CMT was performed, the teats were sanitized using antibacterial wipes.
The milk samples are a pooled sample from all milk producing glands representing each cow in the project. The test tube was a 50 mL plastic tube (VWR, USA). The manual milking was performed using gloves to avoid transmission of infectious agents.
After collecting the milk sample, the test tube was marked with cow ID, date of collection and bulking group. Immediately after sampling, the test tube was placed in a cooling box with freezing elements (the exact temperature is unknown but estimated to be 10°C).
At the end of the day, the samples were transported to the veterinary laboratory in Mzuzu and stored at -20°C. After four days, the samples were moved to the Central Veterinary

Laboratory (CVL) in Lilongwe using a cooling box with freezing elements (the exact temperature is unknown but estimated to be approximately 4-10°C).

3.4.5 Milk sample collection from the bulking groups

A pooled sample was taken from the collection point in the bulking group. The milk was collected in 50 mL test tube using a clean mug (VWR, USA). The sample was marked with date, bulking group ID and approximately how many farmers had delivered milk at the time the sample was collected.

3.5 Laboratory work

The laboratory work we conducted in Lilongwe was performed at the Central Veterinary Laboratory (CVL). CVL is an important institution in Malawi, equivalent to Norway's Veterinary Institute. CVL plays a central role in monitoring animal health, diagnosing animal diseases, and conducting veterinary research in Malawi (GFAR, 2023).

3.5.1 Serial dilution of milk samples

Buffered peptone water was used for diluting the milk samples. Buffered Peptone Water powder (Thermo Fisher scientific, Waltham, USA.), 20 gram, was dissolved in one L of purified water. The solution was mixed under heating, and then autoclaved at 121°C for 15 minutes. The milk samples were thawed at room temperature before being diluted as shown in Figure 2, resulting in 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} dilutions. A new pipette was used after each transfer and the diluted samples were mixed by repetitive pipetting.

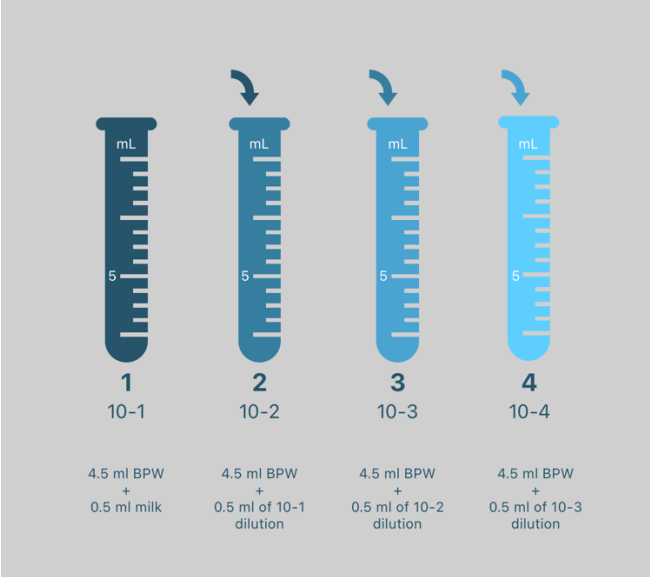

Figure 2. Serial dilution. The different dilutions were made as follows; The 10^{-1} dilution: 0.5 ml milk + 4.5 ml buffered peptone water. The 10^{-2} dilution: 0,5 ml of the 10^{-1} dilution + 4.5 ml buffered peptone water. The 10^{-3} dilution: 0.5 ml of the 10^{-2} dilution + 4.5 ml buffered peptone water. The 10^{-4} dilution: 0.5 ml of the 10^{-3} dilution + 4.5 ml buffered peptone water. 0.5 ml of the mixed dilution was taken out before further use. BPW: Buffered Peptone Water.

3.5.2 Blood agar and Baird-parker agar

After making the dilutions, 0.1 mL of the 10^{-2} dilution was transferred to a blood agar plate (Thermo Fisher scientific, Waltham, USA.), and a Baird-parker plate (Thermo Fisher scientific, Waltham, USA.). A disposable plate spreader (Thermo Fisher scientific, Waltham, USA) was used to spread the sample on the plate. A new plate spreader was used on each sample. After spreading the sample on the plate, the plate was incubated at 37°C for 48 hours. This procedure was performed on all samples, except samples 14.1-14.6 and 16.1 and 26.2, where the 10^{-1} dilution was used instead of the 10^{-2} dilution.

The colony morphology on the blood agar and the Baird-parker agar was studied after 24 and 48 hours. The colony size, number of colonies, color and hemolysis was described.

3.5.3 Gram staining procedure

A colony was picked from the blood agar and transferred to a glass slide with a loop. A drop of sterile water was also added on the glass slide and mixed with the bacterial colony. A gas

burner was used to burn the loop between every bacterial colony. The bacterial smear was dried and fixated by heating.

Gram staining was performed with 0.5% crystal violet, Lugol's iodine, acetone and safranin. See Appendix 5 for a complete description.

3.5.4 Microscopy

A microscope (Leica Microsystems CMS GmbH, Wetzlar, Germany) was used to determine whether the bacteria was gram-positive or gram-negative cocci or rods, and, if possible, Staphylococci or Streptococci.

3.5.5 Bacterial identification

For the bacterial identification, a VITEK machine was used. VITEK® MS (bioMérieux, Marcy l'Étoile, France) is an automated microbial identification system that utilizes Matrix Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) technology. This advanced system provides quick and accurate identification of microorganisms (Biomerieux, 2023).

3.5.6 Petrifilm

Milk from sample number 1.1-8.2 was incubated on petri-film. One mL of the 10^{-1} dilution was transferred to petri-film. The lid on a small petri dish was used to spread the sample on the petri film prior to incubation at 42°C for 24 hours.

3.5.7 Total bacterial count

The plate count agar was made from powder (Thermo fisher scientific, Waltham, USA). 17.5 grams of powder was mixed with 1 L of distilled water, the solution was heated until the powder was dissolved. Then the solution was autoclaved at 121°C for 15 minutes. The plate count agar was then stored at 60°C, so it did not stiffen. To count the total bacterial number in

the milk samples, two dilutions were used for each milk sample. The dilutions used were 10^{-2} and 10^{-4}, and 1 L of each dilution was spread onto an empty petri dish. After the samples had been added to the petri dishes, the plate count agar was taken out of the water bath (60°C) and poured onto the dishes. The petri dish was filled half full of agar. The petri dish was whirled, until the plate count agar and the sample were mixed properly. After the agar had stiffened, the petri dishes were incubated at 37°C for 72 hours.

3.5.8 Antibiotic residues, SNAP test

The IDEXX SNAP Beta-Lactam ST test (IDEXX, Westbrook, USA) was used on the bulk milk, to examine it for the presence of antibiotic residues. The SNAP test is sensitive to penicillin and cephalosporines in milk. The test kit includes a pipette, a test tube, and the snap test. The milk was thawed at room temperature and the sample tube was turned two to three times by hand, until the milk appeared homogeneous. The milk was then pipetted into the test tube and turned two to three times by hand. There is a tablet in the test tube that should be dissolved in the milk. After the tablet had dissolved, the milk was transferred to the snap test. The result is based on the three circles on the test. If the penicillin or the cephalosporine circle is lighter than the control, the milk is positive for penicillin or cephalosporine. If the penicillin or the cephalosporine circle is darker than the control the milk is negative.

3.5.9 Antibacterial susceptibility test

The petri dishes with characteristic colonies for *Staphylococcus aureus* both on blood agar and Baird-parker agar were selected for antibacterial susceptibility testing. Mueller-Hinton agar plates (Thermo fisher scientific, Waltham, USA) were used for testing for AMR. The most dominant colony was picked with a sterile swab and put into a test tube with 0.45% sodium chloride. The solution was mixed with a vortex mixer, and the optical density was measured using a VITEK Densichek device (bioMérieux, Marcy l'Étoile, France). After a

density between 0.5 and 0.6 was obtained, the solution was smeared onto the Mueller-Hinton agar and six different types of antibiotic disks were added: ciprofloxacin, trimethoprim sulfamethoxazole, penicillin, ampicillin, gentamicin, and tetracycline. After the disks with antibiotics were added, the dishes were incubated for 24 hours at 37°C.
The zone diameter was measured in millimeters and interpreted according to EUCAST.

3.6 Data treatment and statistical analysis

We created an Excel database containing individual identification numbers and corresponding measurements at the individual cow level. We utilized box plots to assess group differences, including an examination of the potential relationship between hygiene scores and subclinical mastitis. To illustrate data distribution and measures of central tendency, we employed descriptive statistics. Theoretically, we could have conducted further statistical analyses to explore differences among the three bulking groups. However, due to a highly skewed distribution of farms within each group (with one group having 30/40 cows, while the other two had only 5 cows), and significant within-group variation, we refrained from doing such analyses.

4 Results

4.1 On farm observations

4.1.1 Breed, housing, feed, and water

The breeds were mainly Friesian crosses (32/40), but also Friesian-Jersey crosses, some pure Jersey, Jersey crosses and Holstein crosses (Appendix 6).

All the cows in the study were stalled in a free stall outside. All except one had the possibility to be under a roof.

All the cows had free access to grass. Out of the 40 cows, 14 of them (35%) had free access to water. From them, 12 (86%) had clear water, while two cows had water with high turbidity. The turbidity was observed visually, with no further testing.

Picture. This is one of the cows examined in the study showing the typical breed and housing. Picture: Line Halbjørhus.

4.1.2 Hygiene score udder

In 62% of the cows, the udder hygiene score was one or two, and thus considered good. In 38% of the cows, the score was three or four and thus considered poor (figure 3).

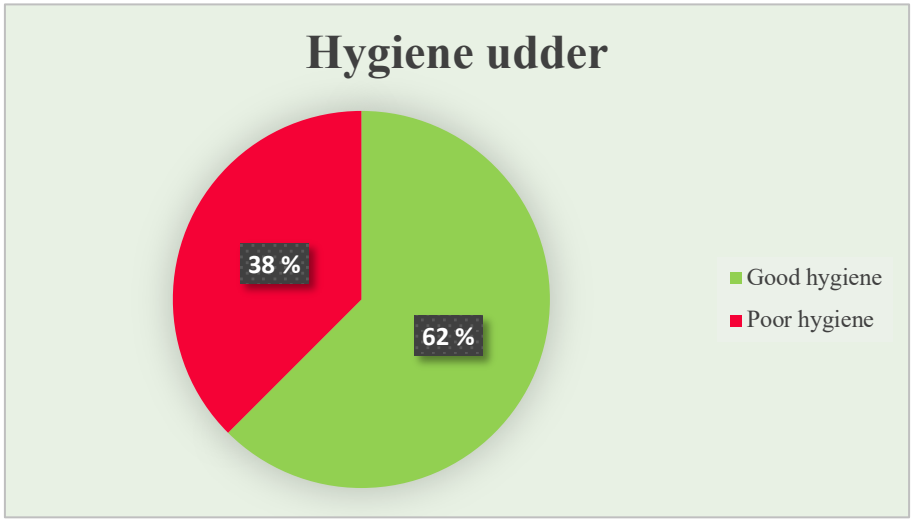

Figure 3. The udder hygiene was considered good in 62% (25/40) of the cows, and poor in 38% (15/40) of the cows.

4.1.3 Hygiene scores legs

In 35% of the cows, the hygiene score of the legs was one or two, and thus considered good. In 65% of the cows, the score was three or four and thus considered poor (figure 4).

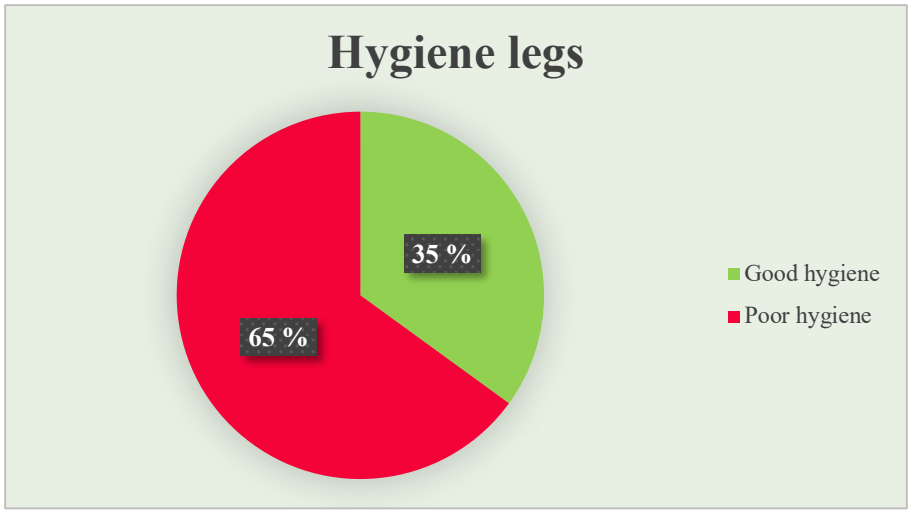

Figure 4. The hygiene was considered good in 35% (14/40) of the cows and poor in 65% (26/40) of the cows.

The cows that had poor hygiene on the udder were mostly the same that also had poor hygiene on the hind legs, but some of the cows with dirty hind legs still had clean udders.

4.2 Questionnaire LUANAR students

The average milk yield per cow per day was reported to be 8.18 liters, and the average BCS was 2.3. These are sourced from the questionnaire that the Malawian master´s students used on the farm, and the most relevant data for our study is compiled in table 1.

Table 1. This table shows milk yield/day in liters, days in milk, lactation number, age and BCS of the cows in this study. These data are extracted from the questionnaire of the LUANAR students that they will use in their unpublished master's thesis.

	Min	**Max**	**Mean**	**Median**	**Standard deviation**
Milk/day (liters)	1.00	20.00	8.18	7.00	3.79
Days in milk	7.00	300.00	162.80	180.00	75.76
Lactation number	1.00	7.00	2.20	1.50	1.60
Age (years)	3.00	13.00	5.00	5.00	2.03
BCS	2.00	2.75	2.30	2.50	0.23

4.3 Bulking group questionnaire

The results from the bulking group questionnaire can be found in Appendix 1.

4.4 General health

All the cows presented as bright and alert. One cow had a body temperature which indicates low grade fever. Three out of 40 cows were considered dehydrated based on reduced skin turgor. The heart rate, respiratory rate and temperature of the cows are listed in table 2. Some

deviations were found in the respective parameters, but the most significant deviations were observed in heart rate, where 18 out of 40 cows had heart rates above the reference values.

Table 2. This table shows the minimum and maximum values registered on heart rate, respiratory rate, and temperature. It also shows the mean and median values.

	Min	Max	Mean	Median	Standard deviation
Heart rate (beats/min)	52.0	132.0	82.0	80.0	18.19
Respiratory rate (breaths/min)	16.0	40.0	25.3	24.0	7.47
Temperature (°C)	37.8	39.4	38.5	38.5	0.41

4.5 Udder health

4.5.1 Cardinal signs and CMT-scores

Based on the five cardinal signs of infection: redness, warmth, swelling, pain, and loss of function, none of the cows showed any signs of infection in the udder.

The California mastitis test was performed on all 40 cows. The individual results are presented in table 3. It may appear that farms with more cows have a higher CMT score, but this has not been statistically verified.

Table 3. CMT-results.

Cow ID	CMT score	Cow ID	CMT score	Cow ID	CMT score	Cow ID	CMT score
1.1	1-1-1-2	**8.2**	Colostrum	**14.4**	Dry-5-4-5	**20.1**	2-2-2-2
2.1	1-1-1-1	**9.1**	1-2-2-2	**14.5**	1-2-1-1	**21.1**	1-1-2-1
2.2	2-1-1-1	**10.1**	1-1-1-1	**14.6**	3-4-2-5	**22.1**	3-5-4-4
3.1	1-1-1-2	**11.1**	2-1-1-1	**15.1**	3-3-2-3	**23.1**	1-3-1-3
4.1	2-1-2-1	**12.1**	1-1-2-1	**15.2**	3-5-3-5	**23.2**	1-1-1-2
5.1	1-2-1-3	**13.1**	3-2-2-2	**15.3**	3-2-2-3	**24.1**	1-1-1-1
5.2	1-2-1-1	**13.2**	1-1-1-1	**16.1**	4-4-3-3	**25.1**	3-2-3-2
6.1	1-1-1-1	**14.1**	3-3-2-1	**17.1**	2-2-1-1	**25.2**	1-1-1-1
7.1	2-1-1-1	**14.2**	1-1-2-2	**18.1**	1-1-1-1	**26.1**	5-5-3-4
8.1	1-1-1-2	**14.3**	1-1-2-3	**19.1**	4-4-4-3	**26.2**	2-3-1-1

4.5.2 Distribution of mastitis

None of the cows in this study had clinical mastitis. Out of 40 cows, 12 of them had an average CMT value of 2.5 or more, and thus were considered to have subclinical mastitis. Among the remaining 28 cows, we found neither subclinical nor clinical mastitis.

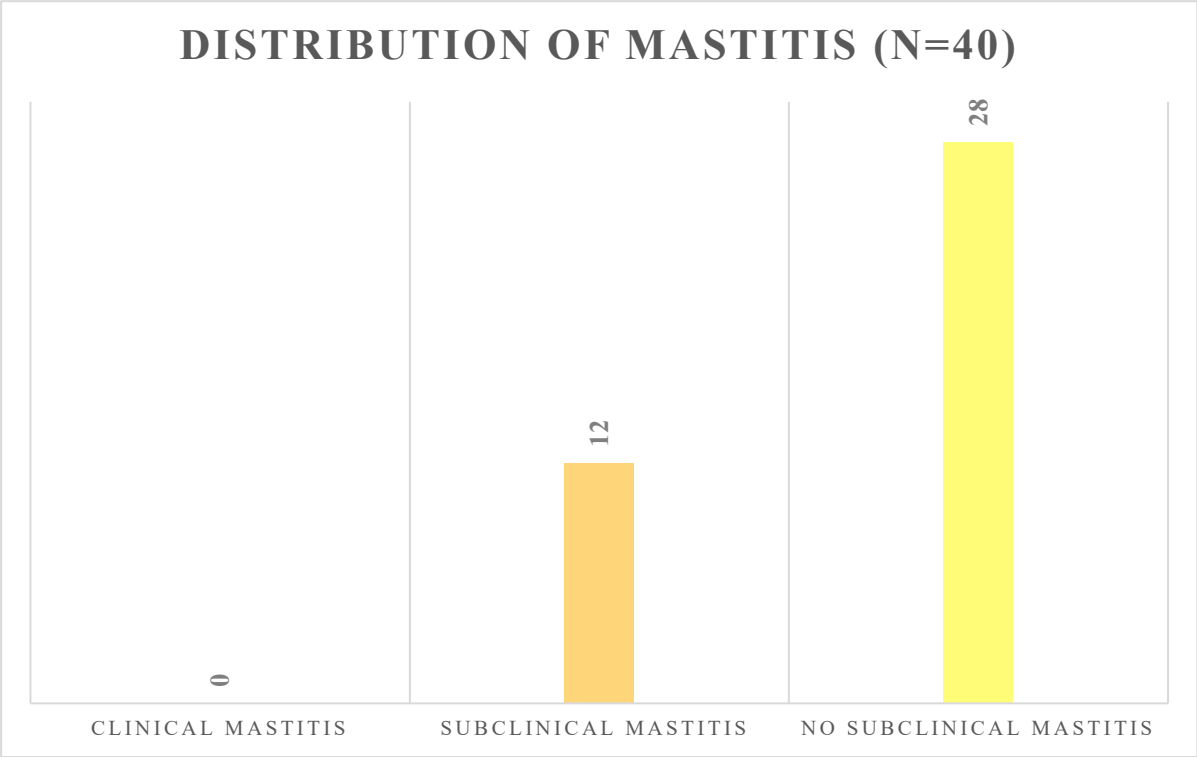

Figure 5. None of the cows in the study had clinical mastitis. 12 cows had subclinical mastitis and 28 cows had no subclinical mastitis.

4.6 Bacteriology

4.6.1 Cultivation and total bacterial count

Out of 40 blood agar plates, 34 showed bacterial growth. A total of 20 plates contained colonies of different morphologies. The number of colonies on the plates with growth varied greatly. The one with the least growth had 1,000 colony-forming units per mL (CFU/mL), and the one with the most growth had 400,000 CFU/mL.

A total of 17 Baird Parker agar plates contained colonies with morphologies characteristic for Staphylococci. The results can be found in Appendix 7.

Eleven samples were cultivated on petrifilm for detecting *E.coli*. Only sample nr 8.2 showed bacterial growth on petrifilm with colony morphology characteristic for *E. coli*. The petrifilm contained only two colonies which correspond to 20 CFU/ml in the original milk sample.

All petri dishes with plate count agar for total bacterial count were unreadable and thus unsuccessful.

4.6.2 Antibiotic residues in bulk milk

The snap tests we used on the three bulking groups were negative. Therefore, no presence of penicillin and cephalosporin residues were found in the samples.

4.6.3 Bacteria typing

The petri dishes with bacterial growth on blood agar (34/40) were gram stained. Out of the 34 dishes, 33 contained gram-positive cocci and one contained gram-positive rods. The distribution is shown in figure 6.

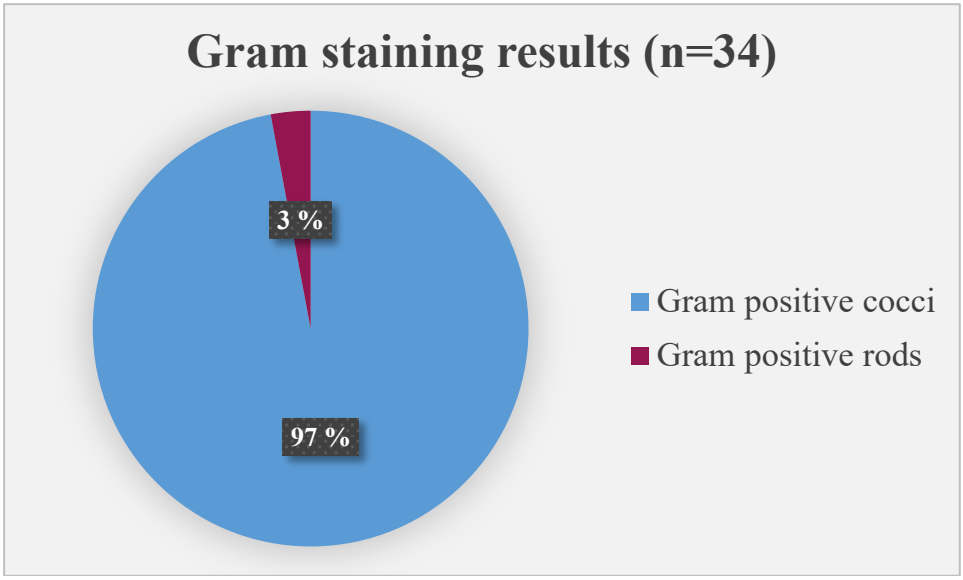

Figure 6. Results from Gram staining of the milk samples.

The most common bacteria detected from the VITEK machine were *Staphylococcus epidermidis* (six samples) and *Staphylococcus aureus* (five samples). In 14 of the milk samples, the bacteria could not be detected by the VITEK machine.

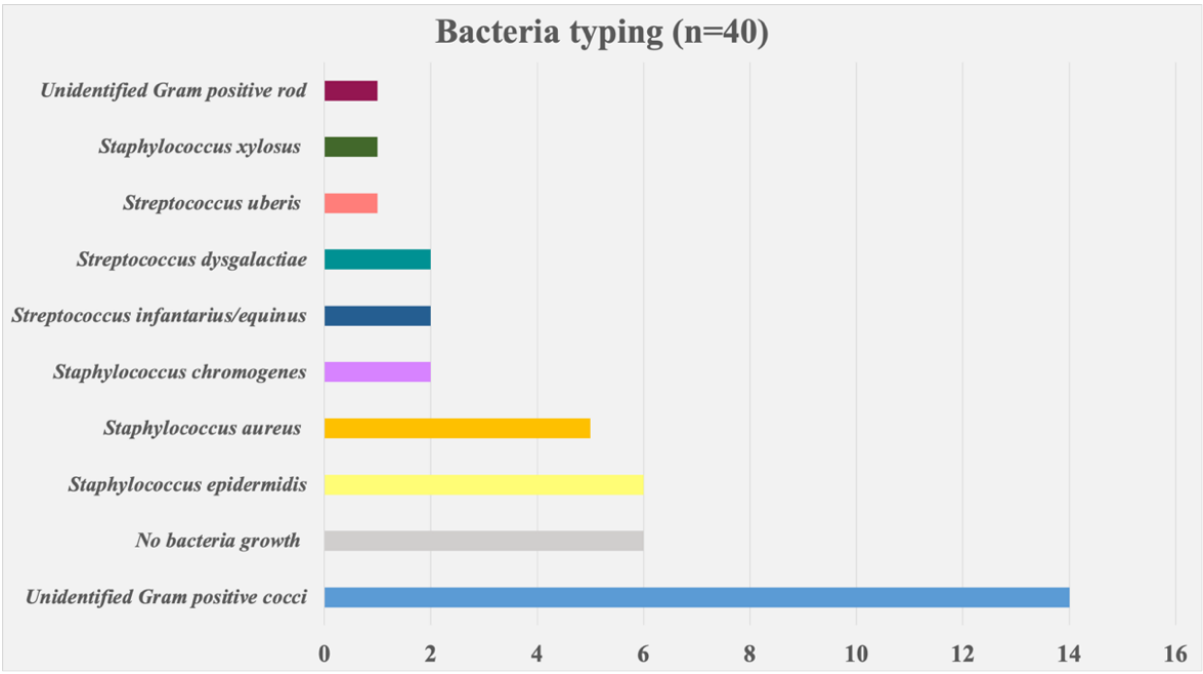

Figure 7. Distribution of the species and types in the milk samples. The largest proportion of the samples contained either unidentified Gram-positive cocci, Staphylococcus epidermidis, Staphylococcus aureus or no bacterial growth.

4.6.4 Bacteria detected in cows with subclinical mastitis

Among the 12 cows diagnosed with subclinical mastitis, three samples contained unidentified gram-positive cocci. *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus dysgalactiae* were detected in two milk samples each. *Staphylococcus chromogenes* and *Streptococcus uberis* were identified in one sample each. In one of the milk samples from a cow with subclinical mastitis, no bacteria growth was detected. The percentage distribution of bacteria detected in cows with subclinical mastitis is presented in figure 8.

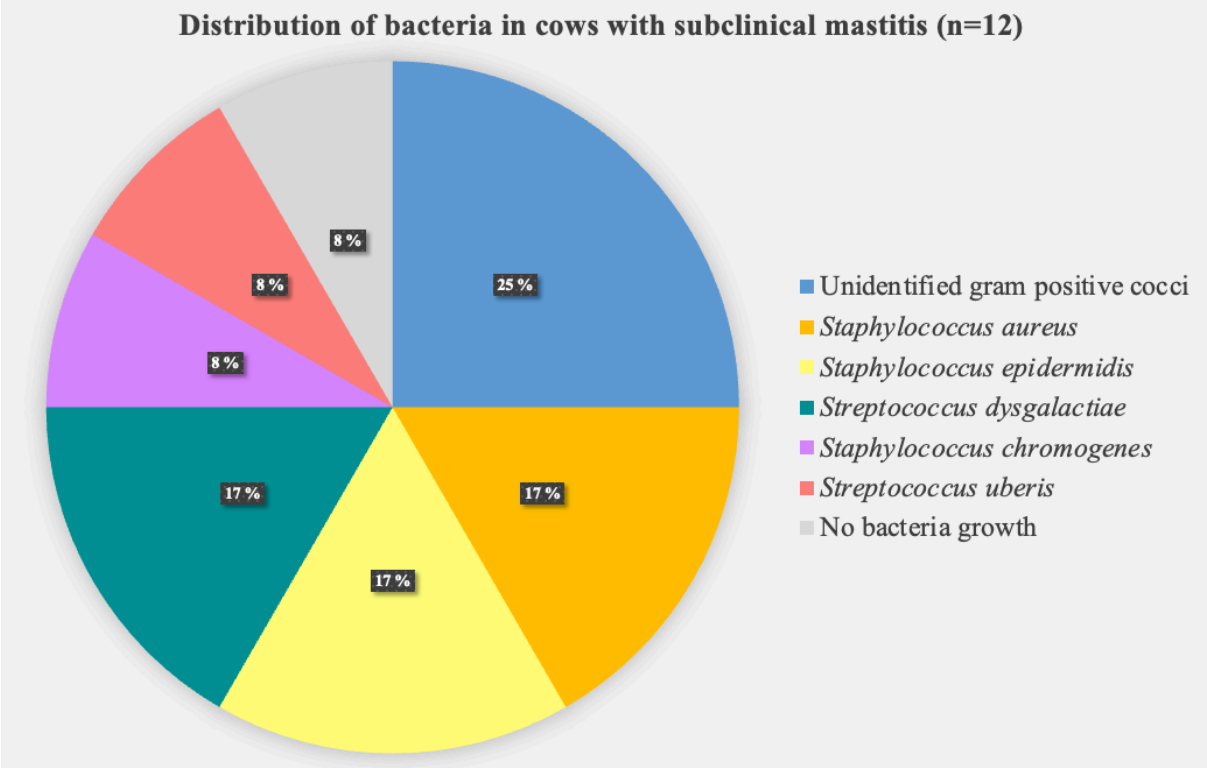

Figure 8. Bacterial species detected in the cows diagnosed with subclinical mastitis.

4.6.5 Bacteria detected in cows with no subclinical mastitis

In most of the milk samples from cows with no subclinical mastitis, unidentified gram-positive cocci were detected. In five of the samples, no bacterial growth was detected. *Staphylococcus epidermidis* was detected in four samples, *Staphylococcus aureus* was detected in three milk samples. The percentage distribution of bacteria detected in cows with no subclinical mastitis is presented in figure 9.

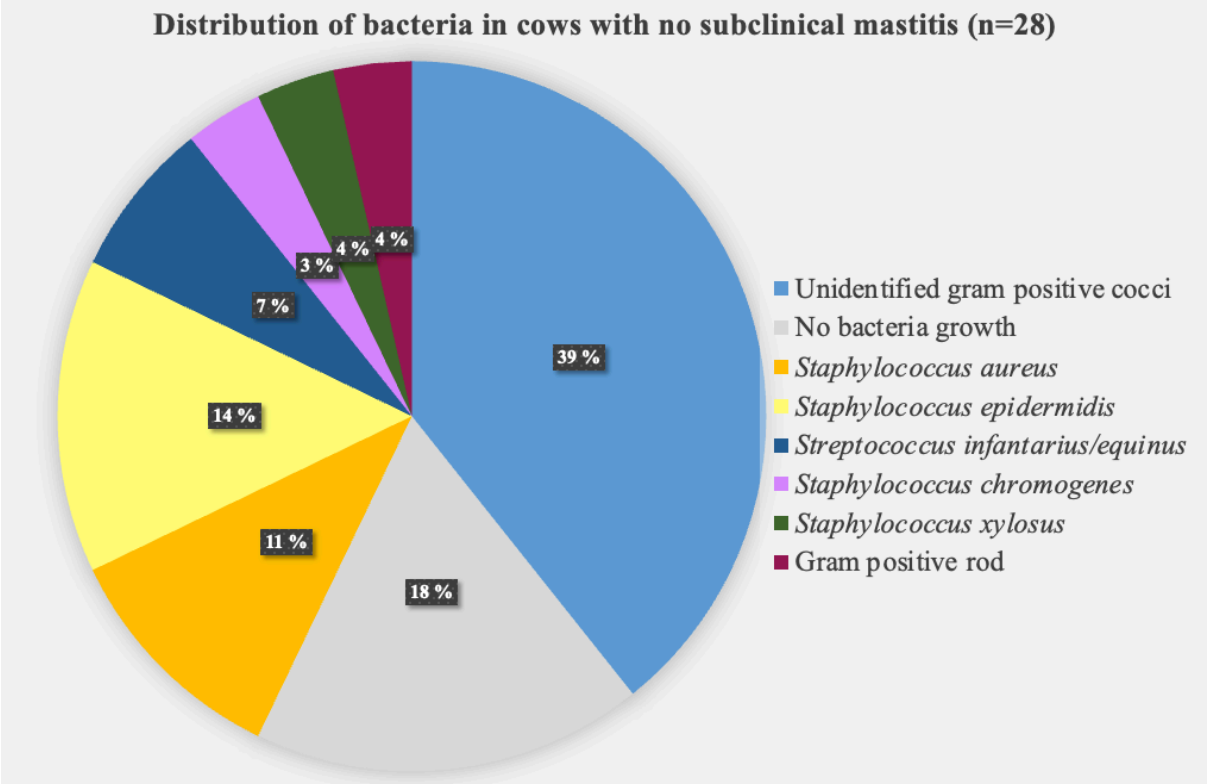

Figure 9. Bacteria detected in cows with no subclinical mastitis.

4.7 Antibiotic resistance

All three samples of *Staphylococcus aureus* were resistant to penicillin and sensitive to trimethoprim-sulfamethoxazole. One of them is also resistant to tetracycline. One sample of *Staphylococcus epidermidis* was resistant to penicillin, trimethoprim-sulfamethoxazole, and tetracycline. The other sample of *Staphylococcus epidermidis* was sensitive to penicillin, trimethoprim-sulfamethoxazole, and tetracycline, but is resistant to gentamicin. One of the samples with *Streptococcus dysgalactiae* was resistant to penicillin and intermediate resistant to trimethoprim-sulfamethoxazole. The other sample of *Streptococcus dysgalactiae* was sensitive to penicillin, tetracycline, and gentamicin but resistant to trimethoprim-sulfamethoxazole. The diameter of inhibition zones and whether they are classified as sensitive or resistant are shown in table 4.

Table 4. The diameter of the inhibition zones measured on the 11 agar plates we tested and whether they are classified as sensitive or resistant. AM: Amoxicillin, P: Penicillin, CIP: Ciprofloxacin, SXT: Sulfamethoxazole-Trimethoprim, TE: Tetracycline, GM: Gentamicin.

Cow ID	Bacteria	AM	P	CIP	SXT	TE	GM
5.1	*Staphylococcus aureus*	29 mm	20 mm Resistance	27 mm	26 mm Sensitive	9 mm Resistance	24 mm Sensitive
14.1	*Gram positive cocci*	11 mm	19 mm	25 mm -	22 mm	29 mm	29 mm
14.2	*Staphylococcus epidermidis*	19 mm Sensitive	13 mm Resistance	29 mm -	8 mm Resistance	10 mm Resistance	29 mm Sensitive
14.3	*Staphylococcus xylosus*	29 mm	29 mm	27 mm -	19 mm	28 mm	22 mm
14.4	*Staphylococcus aureus*	9 mm Resistance	8 mm Resistance	26 mm -	18 mm Sensitive	26 mm Sensitive	20 mm Sensitive
14.6	*Gram positive cocci*	10 mm	8 mm	26 mm -	23 mm	22 mm	21 mm
15.2	*Streptococcus dysgalactiae ssp dysgalactiae/equisimilis*	26 mm	20 mm Sensitive	33 mm -	14 mm Resistance	27 mm Sensitive	28 mm Sensitive
16.1	*Streptococcus dysgalactiae ssp dysgalactiae/equisimilis*	18 mm	16 mm Resistance	22 mm -	16 mm Intermediate	23 mm Sensitive	19 mm
19.1	*Staphylococcus aureus*	10 mm	16 mm Resistance	29 mm -	22 mm Sensitive	26 mm Sensitive	23 mm Sensitive
23.2	*Staphylococcus epidermidis*	29 mm Sensitive	30 mm Sensitive	23 mm -	18 mm Sensitive	23 mm Sensitive	18 mm Resistance
26.2	*Gram positive cocci*	38 mm	37 mm	26 mm -	9 mm	23 mm	18 mm

5 Discussion

The overarching aim of this study was to explore the factors that could improve dairy-cattle health and enhance milk production and quality in Mzuzu region in Northern Malawi, and thus increase the potential for delivering milk of good quality from this region to Lilongwe dairy.

Previous studies from Malawi have concluded that dairy cow health is a challenge among smallholder farmers (Banda, 2012). However, we found the overall dairy cow health in this study to be quite good. The cows presented as bright and alert, with good general appearance. Nevertheless, the majority of the cows (30/40) had deviations in some of the health parameters, such as high heart- and respiratory rates. Additionally, 14 cows had lower body temperatures than the reference value, one cow had low-grade fever and three cows had reduced hydration status. We suggest that the deviations in respiratory and heart rate are due to stress due to restraining and handling by new people, and possibly because of heat stress. It is widely acknowledged that increased heart rate and respiratory rate are physiological responses to stress (Chu et al., 2022). Physiological responses to heat stress include increased sweating rate, respiratory rate, breaths per minute, panting score and body temperature (Lees et al., 2019). The cow breeds in the study were mainly Holstein, Friesian and Jersey crossbreds. It is widely known that Bos indicus breeds like Zebu, have greater heat tolerance compared to Bos taurus breeds like Holstein, Friesian and Jersey (Lees et al., 2019) (Kunzler, 2022). It is not unlikely that the cows in the study have some interference of Malawian Zebu genes, and thus have better heat tolerance. Studies have also shown that some Bos taurus genotypes are tropically adapted, and thus have better heat tolerance (Lees et al., 2019). We can thus only suggest that the deviations in heart rate and respiratory rate are due to stress related to restraining and handling, and possibly because of heat stress rather than disease.

Three cows had clinical signs of reduced hydration status. As much as 62.5% of the cows in our study did not have free access to water but were given water during milking. It is well documented that restricted water intake leads to reduced milk yield and has negative effects on animal health and welfare (Kononoff, 2017). Considering the hot weather, and the fact that the majority of the cows did not have free access to water, we were surprised that not more than three cows showed clinical signs of dehydration. Studies have shown that some Bos taurus genotypes are considered tropically adapted, and thus able to cope with hot weather. (Lees et al., 2019). Although, it is likely that the well-being, health and milk yield of the cows could increase by providing them with free access to water. Further work should focus on training of farmers and facilitation of access to clean drinking water for livestock in this region of Malawi.

The mean BCS of the cows in the study were 2.3 (ranging between 2.0 and 2.75). The mean duration of milking was 162.8 days (ranging from seven to 300 days). The BCS of a dairy cow is an assessment of the proportion of body fat that it possesses, and it is an important factor in dairy cattle management. Assessing BCS is important due to its relationship to milk yield, reproductive traits, health, and disease (Roche, 2009). In the Norwegian dairy cattle breed Norwegian Red, a BCS between 3.25 and 3.75 is recommended at calving, and the BCS-loss should not be more than 0.5-0.75 the first two months post-partum. After three months post-partum, the BCS should slowly increase until drying, and reach 3.25-3.75 again before calving (Stevenson, 2022). Thus, the BCS of the cows in this study are lower than the Norwegian recommendations. Although, it is doubtful whether the Norwegian recommendations also can apply to Malawian conditions, due to the large differences in the dairy cow breeds. The dairy cows were of different breeds, and mostly crossbreeds of exotic breeds like Holstein Friesian and Jersey. Genetic tests would be necessary to determine the exact genetic origin. The ideal BCS of Holstein cattle at calving are 3.0-3.25, and above 2.5 in

early lactation and thus a bit lower than the Norwegian recommendations. (Mishra, 2016). It is conceivable that the different cow breeds in the study store fat in different ways, and that this could have influenced the BCS. Most of the cows in the study had free access to feedstuff and were fed maize concentrate during milking. The cows were fed what seemed to be low quality grass, or available vegetation or leftovers from harvest. [illegible]observation fits well with a previous study conducted in Malawi where farmers in the Northern region was asked to rank the constraint to milk production, and possible causes. The farmers ranked feed shortage the third constraint. Possible causes for this were limited knowledge on the use of locally available feedstuffs, lack of pasture seeds, less land for pasture improvement and poor pastures, especially during the dry season (Tebug ET AL., 2011). It is well documented that inadequate feeding causes reduced BCS and thus reduced milk yield (Roche, 2009). The climate in Malawi is tropical, with rainy season from October to April, and dry season from June to September (FN-sambandet, 2022). The present study was performed just after the rainy season, the landscape was lush and green, and the access to feedstuff was adequate. It is reasonable to assume that the BCS of the cows varies a lot from the rainy season to the dry season, due to the availability of sufficient feedstuff. The suboptimal BCS of the cows shows that the good impression of the general health may not be so good after all.

None of the cows in the study had clinical mastitis, but 30% of the cows had subclinical mastitis. Cows with subclinical mastitis have a high somatic cell count (SCC), which will negatively affect milk quality. Milk with high SCC has a lower lactose and casein content, and a higher content of minerals. Such milk will also have a higher pH than milk with a low SCC. Altogether, such alterations in high-SCC milk will make it less suitable for consumption and cheese processing, and thus make the milk less attractive for dairies (Bobbo et al., 2016). The average milk yield of the cows in the study was 8.175 liters per day. The milk yield will be affected by where the cow is in the lactation period. However, the milk yield of the

Malawian cows is a big contrast to those delivering milk to the Norwegian dairy industry, where cows with milk yield of 15 liters or less a day is considered dried (Whist and Brodshaug, 2016). There may be many factors that influence the milk yield. Genetics, management practices, suboptimal feeding, water availability, climate conditions and housing infrastructure are some factors that could result in low milk yield (Baur et al., 2016). Studies have shown that a high SCC can correlate negatively with milk yield (Schreiner, 2003). With the relatively high occurrence of subclinical mastitis in the present study, it is not unlikely that this can be one of the factors that affects the milk yield negatively.

A study from the US concluded that there is a connection between the cleanness of cows and the prevalence of subclinical mastitis (Schreiner, 2003). In the present study, we did not find any connection between cleanness of the cows and subclinical mastitis. The udder hygiene was considered good in 62% of the cows. This is not very surprising since the udder is cleaned with water and soap twice a day before milking. The cows were a lot dirtier on the legs, and only 35% were considered clean. We suspect that the cleanness of the cows varies a lot with the seasons. Most of the cows were stalled in enclosures with mud floors and a roof made of wood and grass. Without proper draining, the mud floors can become very slurry, especially in the rainy season. The present study was performed after the rainy season and the ground was relatively dry, slurry enclosures were not unusual. We observed seven (17,5%) very dirty enclosures where the cows had mud far up on their legs. In the other enclosures, the hygiene was adequate. Accumulation of slurry is detrimental to the animals and provide a medium for pathogens to grow as well as high risk of milk contamination (Banda, 2012).

One observation we made during the field work was the connection between high CMT scores and the presence of more than one cow at the farm. This is just an observation and has not been proven by any statistics. This discovery may indicate that contagious bacteria are causing subclinical mastitis, rather than environmental bacteria. Amongst the 12 cows with

subclinical mastitis, unidentified gram-positive cocci (three samples), *Staphylococcus aureus* (two samples), *Staphylococcus epidermidis* (two samples), *Streptococcus dysgalactiae* (two samples), *Streptococcus uberis* (one sample) and *Staphylococcus chromogenes* (one sample) was identified. Amongst these bacteria *Staphylococcus aureus*, *Streptococcus dysgalactiae* and *Streptococcus uberis* are considered contagious mastitis bacteria. Both *Staphylococcus epidermidis* and *Staphylococcus chromogens* are considered environmental bacteria but can act as contagious mastitis bacteria if the conditions are right. *Staphylococcus epidermidis* has its main reservoir on human skin, and infection from humans during milking can therefore not be ruled out (TINE medlem, 2022).

Previous studies have shown that *Staphylococcus aureus* and *Streptococcus agalactiae* are the most common bacteria isolated from subclinical mastitis amongst dairy cows in Malawi (Klastrup, 1977). In this study, the two most common bacterial species detected were *Staphylococcus epidermidis* (15%) and *Staphylococcus aureus* (12,5%). *Streptococcus agalactia* was not isolated from any of the milk samples. *Staphylococcus aureus* is an important bacterial pathogen that contaminates food of animal origin. The bacteria produce heat stable toxin, which induces food poisoning in humans. It is well documented that prolonged storage of milk at room temperature can lead to further proliferation of *Staphylococcus aureus*, and thus the production of toxins (Borena, 2023). Studies show that there are few smallholder farmers in Malawi that have access to electricity, the milk is therefore stored over night when the temperature is low (*Reuben et al., 2021)*. Due to the long distance from the farms to the bulking group, the milk is delivered to the bulking group only in the morning. Therefore, the evening milk is stored, and delivered together with the morning milk the day after. One surprising observation was that the bulking groups had milk tanks with the possibility of cooling capabilities but did not use them due to expensive electricity. The milk is therefore not cooled along the value chain from the farmer to the consumer. This

creates a favorable environment for *Staphylococcus aureus* to proliferate and to produce toxins.

Since the milk is not sold through a commercial dairy, it is not pasteurized before consumption unless the buyers do it themselves. Pasteurization is a milk-processing procedure that heats the milk to a minimum of 72 degrees for at least 15 seconds, or at equivalent combinations of temperature and time. Most microorganisms in raw milk are killed during pasteurization (Mattilsynet, 2023). In one study from the Mzuzu Agricultural division in the Northern region, 34% out of 140 farmers responded that they consumed unpasteurized milk (Tebug, 2014). When visiting the bulking groups, we got the impression that the consumers boiled the milk before consumption. Consuming raw milk increases the risk of attracting a variety of infectious diseases. Examples of pathogenic agents that can be transmitted by unpasteurized milk are *Staphylococcus aureus, Escherichia coli*, *Salmonella* spp., *Bacillus cereus, Listeria monocytogenes, Campylobacter, Brucella abortus* and *Mycobacterium bovis*. These pathogens have in common that they are a threat to public health and can be greatly reduced with pasteurization. If the milk was delivered to a commercial dairy, like Lilongwe dairy, the milk would be routinely pasteurized and thus safer to drink. Even though pasteurization is an effective way of reducing the risk of pathogenic bacteria in the milk, it is not effective to reduce heat stabile *Staphylococcus aureus* toxins and *Bacillus cereus* spores. Therefore, adequate hygienic standards of milking and storage of milk is crucial to avoid milk borne disease, even though the milk is boiled or pasteurized.

In the present study, no residues of penicillin or cephalosporine were found in any of the bulk milk samples. It is worth noting that tetracyclines are reported to be the most used antibiotics in Malawi (World Health Organization, 2020). Therefore, it is not surprising that penicillin and cephalosporine residues were not present in the milk. The information collected from the farmers indicated to practice withdrawal periods after use of antibiotics. Notably, neither the

bulking groups nor the Lilongwe dairy test the incoming milk for the presence of antibiotic residues. Dairy milk consumers in Malawi have therefore no assurance that the milk is free from antibiotics. This jeopardizes food safety, public health and increases the risk for development of AMR (Sachi, 2019). If the bulking groups and the dairies were provided with snap tests for antibiotic residues, and stopped delivery of contaminated milk, the farmers could be less tempted to use antibiotics preventively. This is one possible way of reducing AMU in the dairy industry in Malawi.

All three of the *Staphylococcus aureus* strains detected in the milk samples were resistant to penicillin. This finding does not correspond to the non-detection of penicillin residues as stated above. However, there is a widespread worldwide prevalence of penicillin resistant *Staphylococcus aureus* (Monaco, 2017). In an Ethiopian study conducted between 2020 and 2021 samples from cow milk, udder swabs and milkers hand swabs were collected, and antibiotic susceptibility testing was performed. In that study, high levels of penicillin resistance were detected, with 94% of the samples being resistant to penicillin (Tibebu, 2021). Even though we only resistance tested three milk samples with Staphylococcus aureus, it is not unlikely that the situation is similar in Malawi. One of the samples with *Staphylococcus aureus*, and one of the samples with *Staphylococcus epidermidis* was resistant to tetracycline. The relatively small number of samples resistant to tetracycline is surprising considering the reporting of being the most frequently used antibiotic in the country (World Health Organization, 2020). The total number of samples tested for AMR in this study is quite low, and in some of the samples we did not reveal the specific bacteria species. More research is required to document the antibiotic resistance situation amongst dairy cattle in Malawi.

Methodological considerations

Performing field and laboratory studies in Malawi have some challenges with regards to availability of laboratory facilities and equipment, electricity, and long distances between

sampling sites and the laboratory with reduced ability to keep a stable cooling chain. During the laboratory work, we could have benefited from another strategy for the selection of colonies for Maldi-tof analyses and bacteria identification prior to the antibiotic susceptibility testing.

Despite these challenges this study has documented important information about the cow's health, milk quality and the presence of AMR bacteria in milk from Mzuzu. Even though 40 cows are not representable for all cows in Mzuzu, it is likely that the findings herein reflect the current status in cows held by smallholder farmers in Mzuzu.

6 Conclusion

In this project, several subjects have been examined, and various methods, both in the field and in the laboratory, have been used. The general appearance of the cows in this study was considered good. The presence of subclinical mastitis was measured using CMT, and the cleanness of cows was scored from one to four. No pattern was found between the cleanness of the cows, and the presence of subclinical mastitis. We suspect that our results are highly impacted by the season, and it is not unlikely that our results would have been different if the data collection had been performed in the rainy season.

Milk samples were collected from each cow in the project. In the laboratory, the milk samples were cultivated, and by using the Maldi-tof method, the bacteria present in the samples were identified. The bacteria detected were mainly gram-positive cocci. No penicillin or cephalosporin residues were found in the bulk milk. However, antibiotic resistant bacteria were present in the udders of dairy cows in Mzuzu warranting further research.

Four things are imperative to ensure food safety and reducing the risk of transmission of milk borne pathogens: a low burden of zoonotic diseases in dairy cattle, sufficient hygienic measures, maintenance of the cold chain from the farm to the consumer, and pasteurization of the milk. If this is not in place, it can also affect food security through the spoilage of milk destined for human consumption (Krosness, 2023). By working with this thesis, we found that these prerequisites are not fulfilled by the dairy production in Mzuzu. It is likely that there is a high burden of zoonotic diseases in the dairy cattle. In this study we discovered the presence of *Staphylococcus aureus* in milk. Through our fieldwork, we got the impression that the farmers were aware of the importance of good hygiene while milking. A factor of great concern is the absence of a cold chain. Importantly, the bulking groups should be advised to

use the milk tanks they have access to. Since the farmers don't have access to electricity, it is challenging to keep the milk cold. One solution can be to encourage them to deliver milk twice a day, instead of only in the morning. This will reduce the time the milk is stored at room temperature. One of the challenges with delivering the milk to the bulking group twice a day is the long distance from the farms to the bulking groups. It can be hard to convince the farmers to walk or cycle this far twice a day. Finally, the factor of greatest concern is the lack of pasteurization of the milk. In a future perspective, we hope that these factors can be addressed. If these factors are fulfilled, and the farmers are provided with guidance on management of dairy cattle, we believe that the milk quality and quantity will increase to an acceptable level for Lilongwe dairy to start collecting milk from the Northern region as well.

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Sammendrag

Tittel: Mastitt og melkekvalitet hos melkekyr holdt av småskalabønder i Mzuzu-regionen i nordlige Malawi.

Forfattere: Line Halbjørhus og Synne Selboskar

Veiledere: Lars Olav Eik (Institutt for internasjonale miljø- og utviklingsstudier, Noragric), Marina Elisabeth Aspholm (Institutt for parakliniske fag), Veslemøy Sunniva Oma (Institutt for produksjonsdyrmedisin) og Mette Helen Bjørge Müller (Institutt for produksjonsdyrmedisin)

Som en del av TRANSFORM prosjektet og FoodMa programmet, har det i arbeidet med denne fordypningsoppgaven blitt gjort et feltarbeid i Mzuzu regionen i nordlige Malawi. Målet med studien var å undersøke helsen til melkekyrne, og identifisere faktorer som kan øke melkeproduksjonen og melkekvaliteten i denne regionen. Totalt 40 melkekyr, holdt av småskalabønder, ble undersøkt og tatt melkeprøver av. For hver enkelt ku, ble det også utført CMT og det ble tatt en samlet speneprøve fra juret. Kyrnes renhet ble også vurdert i form av hygienescoring. Bøndene i Malawi er organisert i såkalte "bulking groups", tre slike ble besøkt, og det ble tatt melkeprøver fra samlemelken der. Ingen av kyrne viste tegn til mastitt, men CMT avdekket at 30% av kyrne hadde subklinisk mastitt.

Speneprøvene fra kyrne ble dyrket og resistenstestet på laboratoriet i Malawi. Bakteriene som ble påvist var hovedsakelig gram positive kokker. Det ble påvist resistens mot flere typer antibiotika. Tankmelken ble testet for penicillin og cephalosporin rester, men det ble ikke funnet i noen av prøvene. Selv om vi oppfattet den generelle helsen til melkekyrne som god, fant vi ut at melkeproduksjonen i Mzuzu har forbedringspotensialer når det gjelder melkekvalitet og melkeytelse på kyrne.

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Appendices

Appendix 1. Questionnaire and Answers, Bulking Group

Questionnaire:

1. How many members does this bulking group have?
2. How much milk do you deliver each day?
3. How many times a day do you receive milk from the farmers?
4. What kind of tests are performed on the milk at the bulking center?
5. Does it happen that milk is rejected?
6. What is the reason for the rejection?
7. What is the price?
8. How is the milk distributed afterwards?

Table 5. Here are the answers from the three bulking groups. ** some farmers are in a program where they are given a cow. If she has a female calf, they must give this calf to a neighbor. That is why some of the bulking groups have a lot of members, but they don't deliver any milk. They are in the program.

	Bulking group 1 (Lusangazi)	**Bulking group 2 (Mndundudu)**	**Bulking group 3 (Kapacha)**
Members	200 (66 delivering right now **)	60 members. 13 is delivering right now **	120 members. 16 delivers right now **
Liters of milk received a day	500 L (capacity 3600 liters, two tanks)	Maximum 200 L per day.	80 L. (Have a tank with capacity of 1500 L. Not in use)
How many times a day do you receive milk?	Once a day. In the morning.	Once a day. In the morning.	Once a day. In the morning.
Test performed on the milk at the bulking group	Alcohol test and density test	Alcohol test and density test.	Alcohol test and density test.
Do you sometimes reject the milk	Yes, sometimes.	Yes, sometimes.	Yes, sometimes.
Reasons for rejection	Sour or adulterated milk. Penalty 1000 MK.	Adding water. Adulterated milk.	Adulteration or sourness.
Price	The farmers get paid 250 MK/L. The bulking group 400 MK/l	The farmer gets 250 MK/L. The bulking group get 400 MK/l	The farmer gets 300MK/L. The bulking group get 350 MK/l.
Distribution of the milk afterwards	Private buyers or venders.	Private buyers or venders.	Private buyers or venders.

Appendix 2. Observation list used on the farms.

Housing

	Type of housing			Inside or outside		
Cow	Tie stall	Free stall	Other types of housing, if so – how?	Outside	Inside	Combination
1						
2						
3						
4						
5						

Feeding and water

	Yes	No	Comments
Do the cows have access to feed?			
What kind of feed do they get?			
Do the cows have access to water?			
Is the water clean?			
Other relevant observations			

Hygiene score (1-4)

Cow	Back of the udder	Side of the udder	Rear part of the cow	Hind legs of the cow
1				
2				
3				
4				
5				

Clinical examination

Cow	Breed (Zebu/Holstein/ other)	Overall impression	Degree of hydration	Heart rate	Resp. rate	Temp.	Other
1							
2							
3							
4							
5							

Examination of the udder

Cow	Teat condition score (1-4)	Cardinal signs of inflammation	CMT
1		☐ Calor = heat ☐ Dolor = pain ☐ Rubor = redness ☐ Tumor = swelling ☐ Functio laesa = loss of function	Teat 1: Teat 2: Teat 3: Teat 4:
2		☐ Calor = heat ☐ Dolor = pain ☐ Rubor = redness ☐ Tumor = swelling ☐ Functio laesa = loss of function	Teat 1: Teat 2: Teat 3: Teat 4:
3		☐ Calor = heat ☐ Dolor = pain ☐ Rubor = redness ☐ Tumor = swelling ☐ Functio laesa = loss of function	Teat 1: Teat 2: Teat 3: Teat 4:
4		☐ Calor = heat ☐ Dolor = pain ☐ Rubor = redness ☐ Tumor = swelling ☐ Functio laesa = loss of function	Teat 1: Teat 2: Teat 3: Teat 4:
5		☐ Calor = heat ☐ Dolor = pain ☐ Rubor = redness ☐ Tumor = swelling ☐ Functio laesa = loss of function	Teat 1: Teat 2: Teat 3: Teat 4:

Appendix 3. Hygiene score, TINE Rådgiving

(Whist and Sølverød, 2017)

Appendix 4. CMT-value description

(Whist and Sølverød, 2017)

CMT-value	Description	Estimated cells/ml milk
1	The solution appears just like milk.	Under 200 000
2	Small irregularities that will disappear after a short while.	Between 150 000 and 550 000
3	The solution makes a gel. The gel will not stick together	400 000 – 1,5 million
4	The solution makes a gel-lump that will stick together.	800 000 – 5 million
5	Strong gel formation. When you pour the solution of the board the solution will fall out as one lump of gel.	Over 5 million

Appendix 5. Gram staining procedure

1. Apply 0,5% crystal violet for 1 minute and wash with tap water.
2. Replace with lugo´s iodine for 1 minute then wash off with tap water.
3. Decolorize with acetone for 1-2 seconds.
4. Quickly wash with tap water for a few seconds.
5. Counter stain with Safranin for 1 minute
6. Wash off with tap water.

Lugo´s iodine: iodine 1 gm, Potassium iodide 2 gm, distilled water 100 mL.

Appendix 6. Breed

Table 6. Distribution of breeds among the cows in the study obtained from unpublished master's thesis.

Breed	Number of cows
Friesian cross	32
Friesian/Jersey	3
Jersey	2
Friesian/Holstein	1
Jersey cross	1
Holstein cross	1

Appendix 7. Bacterial growth

Table 7. Characteristic growth on Baird Parker agar: Black and shiny with a white boarder, surrounded by an opaque zone. NG: No growth.

Cow nr.	Cow ID	Bacterial growth on Blood agar	Colonies of different morphologies	Characteristic growth on Baird Parker agar	CFU/ml
1	1.1	+	-	-	2 000
2	2.1	-	NG	-	-
3	2.2	+	+	-	3 000
4	3.1	+	+	+	6 000
5	4.1	+	-	-	1 000
6	5.1	+	-	+	60 000
7	5.2	-	NG	-	-
8	6.1	-	NG	-	-
9	7.1	+	+	-	16 000
10	8.1	+	+	-	7 000
11	8.2	+	+	-	2 000
12	9.1	+	+	-	6 000
13	10.1	+	-	-	400 000
14	11.1	+	-	-	30 000
15	12.1	+	+	-	138 000
16	13.1	-	NG	-	-
17	13.2	-	NG	-	-
18	14.1	+	+	+	400 000
19	14.2	+	-	+	140 000
20	14.3	+	+	+	150 000
21	14.4	+	-	+	240 000
22	14.5	-	NG	-	-
23	14.6	+	-	+	400 000
24	15.1	+	+	+	60 000
25	15.2	+	+	+	36 000
26	15.3	+	+	-	5 000
27	16.1	+	+	+	40 000
28	17.1	+	+	-	6000
29	18.1	+	-	+	27 000
30	19.1	+	-	+	150 000
31	20.1	+	+	-	60 000
32	21.1	+	+	-	60 000
33	22.1	+	+	-	21 000
34	23.1	+	+	+	30 000
35	23.2	+	-	+	6 000
36	24.1	+	+	+	200 000
37	25.1	+	-	-	22 000
38	25.2	+	-	-	150 000
39	26.1	+	+	+	50 000
40	26.2	+	-	+	50 000

Appendix 8. Overall results presented in Excel

Tables below. Here are excerpts from the Excel sheet with the overall results at the individual level. This includes the results from the observation list, clinical examination, udder examination, bacteria typing, and number of microbial cells (CFU/ml) found in each cow. Red and yellow font indicates values above the reference value (red is the most severe). Blue font indicates values below the reference values.

EXPLANATIONS
Cow ID: "Farm nr"."Cow nr on the farm".
FC: Friesion cross
FJC: Friesion/Jesey cross
JC: Jersey cross
J: Jersey
HC: Holstein cross
FHC: Friesian/holstein cross
FSO: Free stall outside
FSOwR: Free stall outside with roof
FAF: Free access to food (grass/plants)
NFAF: Not free access to food
Hygiene score: 1-4. Back udder-Side udder-Rear part-Hind legs
Degree of hydration: Not dehydrated (ND), mildly dehydrated (MD), badly dehydrated (BD)
Heart rate, reference: 60-80/min
Respiratory rate, reference: 10-30/min
Temperature, reference: 38,3-39,3 degrees Celcius
Teat condition score: 1-4
Cardinal signs udder: Heat (calor), pain (dolor), redness (rubor), and swelling (tumor), lack of function (functio laesa)
CMT: HF=1 VF=2 HB=3 VB=4
Bacteria: Type of bacteria found in the teat sample
SE= Staphylococcus epidermidis
G+C= Gram + cocci
NG: No growth
SA: Staphylococcus aureus
SC: Staphylococcus chromogenes
G+R: Gram + rods
SX: Staphylococcus xylosus
SD: Streptococcus dysgalactiae
SI: Streptococcus infantarius
SU: Streptococcus uberis
CFU: Colony-forming unit
MD: Mildly dehydrated

Bulking gruoup 1 - Lusangazi Dairy

Cow nr.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Cow ID	**1.1.**	**2.1.**	**2.2.**	**3.1.**	**4.1.**	**5.1.**	**5.2.**	**6.1.**	**7.1.**	**8.1.**	**8.2.**	**9.1.**	**10.1.**	**11.1.**	**12.1.**
Breed	FC	FC	FC	FC	FC	FC	FC	FC	FC	FC	FC	FC	FC	FC	FC
Housing	FSOwR	FSOwR	FSOwR	FSOwR	FSOwR	FSOwR	FSOwR	FSOwR	FSOwR	FSOwR	FSOwR	FSOwR	FSOwR	FSOwR	FSOwR
Free access to food	FAF	FAF	FAF	FAF	FAF	FAF	FAF	FAF	FAF	FAF	FAF	FAF	NFAF	FAF	FAF
Free access to water	No	No	No	No	No	No	No	Yes	No	No	No	No	No	No	No
Hygiene score	1-1-1-1	2-1-3-2	1-1-2-2	2-1-3-4	1-1-1-3	3-3-4-4	3-3-4-4	3-2-3-4	3-3-3-3	2-2-4-4	3-4-4-4	2-1-2-4	4-4-4-4	2-2-3-3	3-3-4-4
Degree of hydration	ND	ND	ND	ND	ND	ND	ND	ND	ND	MD	ND	ND	ND	ND	ND
Heart rate	76	84	76	68	92	60	60	88	112	80	104	80	72	112	68
Resp. rate	24	24	20	20	20	24	20	20	20	32	40	36	32	40	40
Temerature	37,8	38,3	38,0	38,5	37,8	38,2	38,8	38,9	38,5	38,4	39,4	38,7	38,2	38,1	39,2
Teat condition score	1,5	1,5	1	1	2	2	2	1	1	1	1	2	2	1	1
Cardinal signs udder	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CMT	1-1-1-2	1-1-1-1	2-1-1-1	1-1-1-2	2-1-2-1	1-2-1-3	1-2-1-1	1-1-1-1	2-1-1-1	1-1-1-2	Colostrum	1-2-2-2	1-1-1-1	2-1-1-1	1-1-2-1
Bacteria ID	SE	NG	G+C	G+C	SE	SA	NG	NG	G+C	SC	G+C	G+C	G+R	G+C	G+C
CFU/ml	2 000	NG	3 000	6 000	1 000	60 000	NG	NG	16 000	7 000	2 000	6 000	400 000	30 000	138 000

16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
13.1.	**13.2.**	**14.1.**	**14.2.**	**14.3.**	**14.4.**	**14.5.**	**14.6.**	**15.1.**	**15.2.**	**15.3.**	**16.1.**	**17.1.**	**18.1.**	**19.1.**
FC	FC	FC	FJC	J	FJC	J	FJC	FC	JC	FC	FC	FC	HC	FC
FSOwR	FSOwR	FSOwR	FSOwR	FSOwR	FSOwR	FSOwR	FSOwR	FSOwR	FSOwR	FSOwR	FSOwR	FSOwR	FSOwR	FSOwR
FAF	FAF	FAF	FAF	FAF	FAF	FAF	FAF	FAF	FAF	FAF	FAF	FAF	FAF	FAF
No	No	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	No	Yes	No	No
2-1-2-3	3-2-2-3	1-1-2-2	1-1-2-1	1-1-2-2	1-1-2-2	1-2-3-2	1-2-2-2	1-1-2-2	2-2-1-2	1-1-2-2	2-2-4-4	2-1-2-3	3-2-4-4	2-2-4-4
ND	ND	ND	ND	ND	ND	ND	ND	ND	MD	MD	ND	ND	ND	ND
96	88	72	60	76	60	120	84	132	64	120	80	52	100	80
20	16	20	24	24	16	20	28	32	28	24	20	40	24	24
38,0	38,4	38,0	38,3	38,1	38,1	37,9	38,4	38,8	38,8	38,3	38,7	38,6	39,3	38,8
2	1	1	2	2	1	1	2	1	1	1	1	1	1	1
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3-2-2-3	1-1-1-1	3-3-2-1	1-1-2-2	1-1-2-3	Dry-5-4-5	1-2-1-1	3-4-2-5	3-3-2-3	3-5-3-5	3-2-2-3	4-4-3-3	2-2-1-1	1-1-1-1	4-4-4-3
NG	NG	G+C	SE	SX	SA	NG	G+C	SE	SD	G+C	SD	SI	SI	SA
NG	NG	400 000	140 000	150 000	240 000	NG	400 000	60 000	36 000	5 000	40 000	6 000	27 000	150 000

Bulking group 2 - Mndundundu

Cow nr.	36	37	38	39	40
Cow ID	**24.1.**	**25.1.**	**25.2.**	**26.1.**	**26.2.**
Breed	FC	FC	FC	FC	FC
Housing	FSOwR	FSOwR	FSOwR	FSOwR	FSOwR
Free access to food	FAF	FAF	FAF	FAF	FAF
Free access to water	No	Yes	Yes	No	No
Hygiene score	1-1-2-1	1-2-3-3	1-1-2-1	3-3-4-4	3-3-4-4
Degree of hydration	ND	ND	ND	ND	ND
Heart rate	96	72	88	92	68
Resp. rate	28	20	20	24	16
Temerature	38,6	38,6	38,8	38,1	38,0
Teat condition score	1	2	2	2	1
Cardinal signs udder	-	-	-	-	-
CMT	1-1-1-1	3-2-3-2	1-1-1-1	5-5-3-4	2-3-1-1
Bacteria ID	SA	SE	SA	G+C	G+C
CFU/ml	60 000	60 000	21 000	30 000	6 000

Bulking group 3 - Kapacha

Cow nr.	31	32	33	34	35
Cow ID	**20.1.**	**21.1.**	**22.1.**	**23.1.**	**23.2.**
Breed	FC	FC	FHC	FC	FC
Housing	FSOwR	FSO	FSOwR	FSOwR	FSOwR
Free access to food	FAF	FAF	FAF	FAF	FAF
Free access to water	Yes	No	Yes	Yes	Yes
Hygiene score	3-2-3-4	2-2-3-3	1-2-2-2	3-3-4-4	4-3-4-4
Degree of hydration	ND	ND	ND	ND	ND
Heart rate	80	88	76	84	76
Resp. rate	28	20	28	36	20
Temerature	38,0	38,8	38,5	39,0	38,9
Teat condition score	1	2	1	1	2
Cardinal signs udder	-	-	-	-	-
CMT	2-2-2-2	1-1-2-1	3-5-4-4	1-3-1-3	1-1-1-2
Bacteria ID	G+C	SC	SU	SE	G+C
CFU/ml	200 000	22 000	150 000	50 000	50 000

Appendix 9. Bacteria typing

Table 8. These are the results from bacteria typing with use of the VITEK machine.

Cow ID	Bacteria detected	Cow ID	Bacteria detected	Cow ID	Bacteria detected
1.1	Staphylococcus epidermidis	12.1	Gram positive cocci	18.1	Streptococcus infantarius ssp infantarius / Streptococcus equinus
2.1	No bacteria growth	13.1	No bacteria growth	19.1	Staphylococcus aureus
2.2	Gram positive cocci	13.2	No bacteria growth	20.1	Gram positive cocci
3.1	Gram positive cocci	14.1	Gram positive cocci	21.1	Staphylococcus chromogenes
4.1	Staphylococcus epidermidis	14.2	Staphylococcus epidermidis	22.1	Streptococcus uberis
5.1	Staphylococcus aureus	14.3	Staphylococcus xylosus	23.1	Staphylococcus epidermidis
5.2	No bacteria growth	14.4	Staphylococcus aureus	23.2	Gram positive cocci
6.1	No bacteria growth	14.5	No bacteria growth	24.1	Staphylococcus aureus
7.1	Gram positive cocci	14.6	Gram positive cocci	25.1	Staphylococcus epidermidis
8.1	Staphylococcus chromogenes	15.1	Staphylococcus epidermidis	25.2	Staphylococcus aureus
8.2	Gram positive cocci & E-coli	15.2	Streptococcus dysgalactiae	26.1	Gram positive cocci
9.1	Gram positive cocci	15.3	Gram positive cocci	26.2	Gram positive cocci
10.1	Gram positive rod	16.1	Streptococcus dysgalactiae		
11.1	Gram positive cocci	17.1	Streptococcus infantarius ssp infantarius / Streptococcus equinus		

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