OCCURRENCE OF SUBCLINICAL MASTITIS AND EFFECTIVENESS OF DRY COW THERAPY IN SMALLHOLDER DAIRY FARMS IN KIAMBU COUNTY, KENYA

A Thesis Submitted in Partial Fulfillment of Requirements for **Master of Veterinary Medicine (M. Vet. Med),** (Department of Clinical Studies, University of Nairobi)

Ronald Kiprono Sang (BVM)

University of Nairobi

Faculty of Veterinary Medicine

Department of Clinical Studies

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

Dr. Ronald Kiprono Sang, BVM

Signature……………………………. Date 7/06/2021

This thesis has been submitted for examination with our approval as university supervisors.

Prof. George K. Gitau (BVM, MSc & PhD)

Professor, Department of Clinical Studies, Faculty of Veterinary Medicine, University of Nairobi.

Signature……………………………. Date 07-06-2021

Prof.John Vanleeuwen (DVM, MSc & PhD)

Professor, Atlantic Veterinary College, University of Prince Edward Island

Signature……………………………. Date 07-06-2021

DEDICATION

I dedicate this thesis to my parents for their great support during my study. My fiancée, siblings, aunt Gladys and grandmother thank you for your prayers.

ACKNOWLEDGEMENT

First I would acknowledge Our Creator for the gift of life and health he gave me during the study period.

I am greatly indebted to Prof. George Gitau for his unwavering support and mentorship during my master’s studies. Without his assistance I would not have managed to carry out my master’s project. My sincere gratitude to Prof. John Vanleeuwen, University of Prince Edward Island for his support through the research grant and his supervisory role. I am also grateful to Kabete Dairy Cooperative and the farmers who allowed me to work with them and their animals. Thanks to Dr. Peter Kimeli for introducing me to these farmers. I am grateful to the members of the Department of Clinical Studies for the moral support and encouragement throughout my studies. My gratitude also goes to the laboratory technologists who assisted me during my work and in particular, Mr. Paul Gitau, Mr. Nickson Simani, Ms. Hilda Nakhweya and Ms. Jane Kamau.

To my comrades in the struggle for further education, thank you for being team players and I wish all of you all the best. Special mention to Dr. Serem Essau for your guidance when I reported for master’s studies.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATION

CMT- California Mastitis test

DCT- Dry cow therapy

ITS- Internal teat sealant

USD – United States Dollar

ABSTRACT

Bovine mastitis is a very important production disease in cattle dairy herds in Kenya. The objectives of this thesis were to determine: 1) the types of bacterial infections in dairy cows using the California Mastitis Test (CMT) and culture at dry-off; 2) the effectiveness of different dry cow therapy options for treating existing mastitis infections; and 3) factors associated with *Staphylococcus aureus* infections at dry-off and post-calving in smallholder dairy farms in Kiambu County, Kenya.

The study targeted cows at the point of dry-off, and farms with such cows were recruited through cooperative societies and the help of artificial insemination service providers. On twenty farms, 32 cows with CMT-positive quarters at dry-off were recruited into the study, including 75% Friesian, 19% Ayrshire and 6% Jersey, totaling 121 quarters. From all the CMT-positive quarters, milk samples were aseptically collected for bacterial culture. Additionally, the positive quarters were randomly allocated to receive either dry cow therapy (DCT) plus internal teat sealant (ITS) or ITS alone, and farm- and animal-level factors were captured through a questionnaire. The project was undertaken between the months of September 2019 to March 2020.

The mean herd size of the farms was 11 cattle, with the composition of 4.2, 1.3, 2.9 and 2.3 milking cows, dry cows, heifers and calves respectively. Mean daily milk production for the dairy farms was 55 liters. On milking practices, 75% of the farms use hand-milking only, of which 47% and 53% squeeze and pull the teats, respectively, while 15% use machine milking and 10% use both machine and hand milking methods. All farms use a cloth for udder cleaning, with 90% using one cloth for all the cows in the farm. Additionally, 80% of the farmers were drying their cows gradually, but only 40% were sometimes using dry cow therapy.

Subclinical *S. aureus* mastitis was among the most common type of infection at dry-off (54.6% of CMT-positive quarters and 81.3% of CMT-positive cows were infected with *S. aureus*). Dry cow therapy significantly reduced the proportion of quarters infected with *S. aureus* from 67.9% at dry-off to 44.0% post-calving (35% reduction), but did not significantly reduce other infections, although proportions of other isolates were low. The final multivariable logistic regression model found there were 0.35 times lower odds of *S. aureus* infection post-calving than at dry-off. The odds of *S. aureus* infection were 0.14 times lower when milk production was over 2.5 kg/day than when it was under 2.5 kg/day. There were 0.29 times lower odds of *S. aureus* infection when udders had scant dirtiness versus when they were clean. However, compared to "clean" lower leg cleanliness, scant and moderate dirtiness had 8.7 and 4.5 times higher odds of *S. aureus* infection, respectively. Finally, compared to no bedding, mattresses and sawdust/grass/leaves/crop waste had 0.14 and 0.06 times lower odds of *S. aureus* infection, respectively.

This study points out the importance of subclinical mastitis at dry-off in smallholder dairy farms, and therefore provides information on how dry cow therapy can be used to address the problem. Preventive measures against subclinical mastitis (e.g. hygiene in the cow cubicle and use of post-milking teat dip) and strategic screening of milking cows with CMT, especially at dry-off so that cows with subclinical mastitis at dry-off can be treated with DCT, can go a long way in the control of the disease, and thus help reduce the cost associated with it.

CHAPTER ONE: INTRODUCTION

1.1: Introduction

Mastitis continues to be a major constraint in the dairy industry world-wide and is associated with economic losses and changes in the udder (Tremblay *et al*., 2013; Gomes *et al.*, 2016; Kashif *et al*., 2016). The disease is mostly caused by bacterial organisms, especially *Streptococcus*, *Staphylococcus* and coliforms (Gomes *et al*., 2016), and there are over 150 bacterial species that have been isolated in bovine mastitis (Shaheen *et al*., 2016). Fungi often cause mastitis following excessive use of antibiotics in the udder, leading to favorable conditions for fungal growth (Shaheen *et al*., 2016).

There are different classifications of bovine mastitis that have been described in the literature. Classifications can be based on the primary source of organisms: 1) contagious, for example *Staphylococcus aureus* and *Streptococcus agalactiae*; 2) environmental, such as *Escherichia coli* and *Klebsiella* organisms; or 3) opportunistic, such as *Staphylococcus epidermidis* and *Staphylococcus simulans* organisms (Shaheen *et al*., 2016).

Mastitis can also be classified based on the presentation of infection: clinical or subclinical. Clinical mastitis is characterized by physical changes in milk such as discoloration and clots. Subclinical mastitis is characterized by increased somatic cell counts >200,000 cells and presence of leucocytes, lower milk pH and higher ion concentration, without any visible clinical sign of udder or milk changes (Reshi *et al*., 2015; Kibebew, 2017).

In Kenyan dairy farms, subclinical mastitis is widely distributed at both the quarter and animal levels, and the major organisms isolated from two studies were *S. aureus, S. agalactiae*, *Corynebacterium bovis, Klebsiella* and *coagulase negative Staphylococcus* (Gitau *et al*., 2014;

Mureithi & Njuguna, 2016). A study in the former Nakuru and Mukurwei-ini districts registered a cow-level prevalence of subclinical mastitis of 47% and 53% on the first visit of the study, and 60% and 57% on the second visit, respectively, while prevalence of clinical mastitis was low at <1% (Gitau *et al*., 2014). Ondiek *et al.,* (2013) reported *S. aureus* at 58.8%, *Streptococcus* species at 11.8%, mixed infections of *E. coli*, *Streptococcus* and *Staphylococcus* at 20.5% and *E. coli* at 8.9% at the cow level. The incidence rate of subclinical mastitis in early lactating cows in smallholder dairy farms in Mukurwe-ini, Kenya, was 0.30 cases per cow-month, with the most isolated organisms being *S. aureus* and *S. agalactiae* (Richards *et al*., 2019).

New intramammary infections can occur up to 7 times faster during the dry period than during lactation, when milking is no longer flushing bacteria out, and in the United Kingdom, 60% of new intramammary infections during the dry period are caused by *E. coli* and *S. uberis* organisms (Green *et al*., 2002). Pantoja *et al.,* (2009) estimated that 10-17% of cow quarters develop new intramammary infections during the dry period in the United States of America, which are caused by environmental bacteria.

Currently, in Kenya, there are no data on the types of subclinical mastitis at dry-off. Furthermore, the effectiveness of the different dry cow therapy methods in the Kenyan smallholder dairy farm context has yet to be determined, despite the substantial losses that occur for dry cow mastitis in the industry.

1.2 Objectives of the study

1.2.1 General objective

The general objective of the study was to determine the occurrence of subclinical mastitis at drying off and the effectiveness of different options of dry cow therapy in smallholder dairy farms in Kiambu County, Kenya.

1.2.2 Specific objectives

1. To determine the types of quarter- and cow-level udder infections in dairy cows at dry-off among smallholder dairy farms in Kiambu County, Kenya.
2. To determine the effectiveness of different dry cow therapy options (dry cow therapy and/or internal teat sealant) on existing infections in smallholder dairy farms in Kiambu County, Kenya.
3. To determine factors associated with *S. aureus* infections at dry-off and post-calving in smallholder dairy farms in Kiambu County, Kenya.

1.3 Research hypotheses

1. The types of quarter- and cow-level udder infections in dairy cows at dry-off are unknown among smallholder dairy farms in Kiambu County, Kenya.
2. Dry cow therapy is not effective in treatment of existing udder infections in smallholder dairy farms in Kiambu County, Kenya.
3. There are no factors associated with S. aureus infections at dry-off and post-calving in smallholder dairy farms in Kiambu County, Kenya.

1.4 Research problem

Mastitis is an economically important production disease in the dairy industry in Kenya and the world. It causes losses through loss of milk production, cost of treatment, cost of control, culling of cows and loss of income that would have been generated from sale of milk. It has been shown that out of the production diseases, mastitis is a big challenge in most farms and hence leads to losses in production and overall performance of the dairy industry (Katsande *et al*., 2013). The milk production losses associated with mastitis make it difficult for farmers to meet the demand for milk as the human population continues to grow. Subclinical mastitis contributes to most of these losses since it is difficult to detect; hence, it causes prolonged reduction in milk production along with the cost of treatment (Mdegela *et al*., 2009; Ayano *et al*., 2013).

In Kenya's dairy industry, DCT has not been adopted widely, thus farmers continue to face the challenge of retaining mastitis through the dry period, and develop new cases of mastitis detected at calving.

1.5 Justification

The dry period in dairy herds is very important since it is the time to achieve higher cure rates for existing cases of mastitis, and to prevent new cases of mastitis developing during the dry period that extend into the next lactation (Bradley *et al*., 2011). With the concerns of antimicrobial resistance increasing in the world, the use of blanket dry cow therapy (DCT) is being discouraged; hence, dairy producers are being sensitized to use selective DCT (Cameron *et al*., 2015). Selective DCT involves testing of all the quarters for intramammary infections at dry-off, and only the positive quarters receive the DCT.

Use of DCT intramammary antibiotics and internal teat sealants (ITS) have been shown to be effective in the treatment and prevention of intramammary infections (Cameron *et al*., 2015). Bradley *et al.* (2010) recorded a cure rate of 90% of mastitis caused by various bacteria including: *S. uberis*, *E. coli*, coagulase-positive *Staphylococci* and *S. agalactiae* following dry cow treatment with cephalonium. It was also noted that the combination of antibiotic DCT and ITS is only beneficial in cows that record high somatic cell count at dry-off compared to those with low somatic cell count (Halasa *et al.* 2009a and b).

CHAPTER TWO: LITERATURE REVIEW

2.1 Definition of mastitis

Mastitis refers to the inflammation of the mammary gland marked by chemical and physical changes in milk and pathological changes in the glandular tissue in response to injury from infectious agents, their toxins, trauma and/or chemical agents (Kibebew, 2017).

2.2 Etiology of mastitis

Mastitis has multifactorial causes, ranging from chemicals, injury and infectious agents such as bacteria, viruses and fungi (Majeed, 2016). Pathogens that cause the disease vary depending on climate, management practices, place and the species of the animal (Lakshmi, 2016). Worldwide incidence risks of bacterial species of mastitis have been reported as 3.7% for *S. dysgalactiae*, 1.5% for coagulase negative *Staphylococcus*, 1.59% for coagulase positive *Staphylococcus*, 0.83% for *S. uberis*, 1.04% for *Enterococcus*, 1.59% for *Pseudomonas* and 1.56% for *E. coli* (Parker *et al*., 2007). Listeria and Lactobacilli bacteria have also been reported to cause bovine mastitis, with their origin being traced to human infections (Shaheen *et al*., 2016). Up to 17 species of *Mycoplasma* bacteria have been isolated in bovine mastitis and also *Nocardia* species (Shaheen *et al*., 2016).

Continuous use of antibiotics coupled with strict control of bacterial mastitis has led to incidences of mycotic mastitis caused by *Aspergillus fumigatus*, *Candida albicans* and *Cryptococcus neoformans* (Shaheen *et al*., 2016).

2.3 Classification of mastitis

Mastitis can be classified based on the presentation of infection: clinical or subclinical. Clinical mastitis is characterized by physical changes in milk such as discoloration and clots. It is further classified into three categories, namely peracute, acute and sub-acute mastitis. Peracute clinical mastitis is characterized by change in composition of milk, decrease in milk production, and changes in udder and systemic signs of fever, shivering, depression and loss of appetite (Lundberg, 2015). Acute clinical mastitis has similar clinical signs to the peracute type but systemic signs are less pronounced, whereas in sub-acute clinical mastitis, there is minimal udder change and no systemic signs (Kibebew, 2017). The visible signs of udder inflammation observed include heat, pain, redness and swelling, and their persistence leads to damage of udder tissues and replaced with connective tissue (Ganguly *et al*., 2018). Subclinical mastitis is characterized by increased somatic cell counts >200,000 cells and presence of leucocytes, lower milk pH and higher ion concentration, without any visible clinical sign of udder or milk changes (Kibebew, 2017; Reshi *et al*., 2015).

There are also three broad categories of mastitis based on the type of causative agent of the disease: contagious, environmental and opportunistic mastitis. Some cases of clinical or subclinical mastitis can be chronic in nature if they do not self-cure or are left undetected and untreated, and chronic subclinical mastitis cases can show periodic alteration in milk composition and occasional clinical flare-ups of the disease (Kibebew, 2017).

Contagious mastitis is caused by bacteria that live on the skin of the teat and in the udder and can be spread from one animal to the other during milking (Ganguly *et al*., 2018). This form of mastitis is further classified as: 1) clinical, which shows obvious signs of inflammation; and 2) subclinical. Additionally, clinical mastitis is subdivided into three types; peracute, acute and sub-acute. Peracute clinical mastitis is characterized by obvious signs of inflammation, reduced milk production, changes in milk composition and systemic involvement marked by shivering, fever and loss of appetite. In acute clinical mastitis, systemic signs are less pronounced compared to peracute, but udder and milk changes are marked. Sub-acute clinical mastitis has little change in the mammary gland and no systemic involvement (Ganguly *et al*., 2018). Examples of contagious mastitis pathogens include *Staphylococcus aureus* and *Streptococcus agalactiae* (Shaheen *et al*., 2016).

Environmental mastitis is caused by organisms that do not live in the udder or teat of the animal but are found within the environment in feces, feeds and beddings (Ganguly *et al*., 2018). Environmental mastitis can also be clinical, subclinical, or chronic, with clinical cases also potentially being peracute, acute or sub-acute. Self-curing is not uncommon with this type of mastitis. Examples of environmental mastitis pathogens include *Escherichia coli* and *Klebsiella* organisms. Some pathogens are described as both environmental and contagious, such as *Streptococcus uberis and dysgalactiae* because they can be contracted both from other cows and from the environment (Shaheen *et al*., 2016).

Opportunistic mastitis pathogens are typically resident skin colonizers on the teat, rarely causing clinical mastitis. Opportunistic mastitis is usually subclinical, rarely causes clinical signs, and can be chronic, although self-curing is possible. Examples of opportunistic mastitis pathogens include bacteria in the coagulase-negative *Staphylococcus* group, such as *Staphylococcus epidermidis* and *Staphylococcus simulans* organisms (Shaheen *et al*., 2016).

2.4 Prevalence and incidence of mastitis

Prevalence and incidence of mastitis vary from place to place, as management and risk factors vary from place to place, as shown by the literature. In Germany, *Staphylococcus aureus* is the most widely isolated bacteria (Kadlec *et al.* (2019). In Poland, prevalence of subclinical mastitis was found to be 36.7% in examined cows and 15.7% in examined quarters, with coagulase negative *Staphylococci*, *S. agalactiae, S. aureus* and fungi being the most commonly isolated organisms (Sztachanska *et al*., 2016). In Canada, the most isolated organisms were *Staphylococcus aureus* with 21.7%, *Escherichia coli* at 17.6%, *Streptococcus uberis* at 13.3% and Coagulase-negative *Staphylococcus* at 10.7% (Riekerink *et al*., 2008).

Management in developing countries can be quite different from developed countries, leading to different mastitis occurrence. In China, it has been reported that incidence of mastitis is 3.3% per month in a thirty-month study, with 45% of cases caused by the gram-positive bacteria, and 33% by gram-negative bacteria (Gao *et al*., 2017). Dieser *et al.*, (2014) reported the most isolated organism in Argentinian dairy farms as coagulase-negative *Staphylococci* at 52.1%, *S. aureus* at 21.3%, *Corynebacterium* species at 5.2%, and *S. agalactiae* and *S. dysgalactiae* at 4.4% each. Katsande *et al.*, (2013) documented prevalence of 27.6% for coagulase-negative *Staphylococci*, 25.2% for *E. coli*, 16.3% for *S. aureus*, 15.5% for *Klebsiella* and 1.6% for *Streptococcus* species in Zimbabwe. In Tanzania, 74% of prevalent bacterial mastitis infections were coagulase-negative *Staphylococcus* and 20.4% were *S. aureus* (Mdegela *et al*., 2009).

In Kenyan dairy farms, subclinical mastitis is widely distributed at both the quarter and animal levels, and the major organisms isolated from two prevalence studies were *S. aureus*, *S. agalactiae*, *Corynebacterium bovis*, *Klebsiella* and *coagulase negative Staphylococcus* (Gitau *et al*., 2014; Mureithi & Njuguna, 2016). A study in the former Nakuru and Mukurwe-ini districts registered a cow-level prevalence of subclinical mastitis of 47% and 53% on the first visit of the study, and 60% and 57% on the second visit, respectively, while prevalence of clinical mastitis was low at <1% (Gitau *et al*., 2014). Ondiek *et al.*, (2013) reported *S. aureus* at 58.8%, *Streptococcus* species at 11.8%, mixed infections of *E. coli*, *Streptococcus* and *Staphylococcus* at 20.5% and *E. coli* at 8.9% as cow-level prevalence. The incidence rate of subclinical mastitis in early lactating cows in smallholder dairy farms in Mukurwe-ini, Kenya, was 0.30 cases per cow-month, with the most isolated organisms being *S. aureus* and *S. agalactiae* (Richards *et al*., 2019).

2.5 Risk factors of mastitis

There are multiple predisposing risk factors of mastitis in cattle and these have been categorized into animal and farm level factors. Animal- and quarter-level factors that have been shown to be significantly associated with prevalence of mastitis in Ethiopia are breed, age, parity, stage of lactation, milk yield, udder conformation and teat morphology (Abebe *et al*., 2016). Leelahapongsathan *et al.,* (2014) noted that quarter-level factors, such as teat position, distance from udder to the floor and previous infections, are important in the occurrence of mastitis in Thailand dairy herds. Prevalence of *S. aureus* mastitis was also lower in cows in first parity and in those that had a short milking time (Leelahapongsathan *et al*., 2014). Shape of the teat, tone of the teat sphincter and weakness of the suspensory ligament of the udder are genetically inherited and are important in the development of mastitis (Lakew *et al*., 2019).

Farm-level factors such as improper milking procedures, inadequate housing and poorly maintained milking machines also play a role in the occurrence of mastitis (Reshi *et al*., 2015). Milking machines that lack enough vacuum, poor udder cleaning prior to milking, and lack of dry cow therapy treatment are other risk factors of occurrence of mastitis (Oliveira *et al*., 2015). Herd size can also contribute to the occurrence of mastitis in a farm, as farmers can introduce new mastitis pathogens when they purchase cattle (Lakew *et al*., 2019).

In Kenya, a risk factor study showed that cows with dirty udders had high prevalence of mastitis compared to those with clean udders (Mureithi & Njuguna, 2016). Gitau *et al.*, (2014) attributed the high prevalence of *S. aureus* mastitis to failure to use gloves and different wash cloth for each cow, causing the spread of the organism between cows on a farm.

2.6 Diagnosis of mastitis

There are different diagnostic methods for mastitis available to detect mastitis. On-farm, physical examination of the udder, California mastitis test, and the strip cup test are widely used qualitative ways to identify abnormalities in the udder or milk quality. On farms with advanced technology, the electrical conductivity test and somatic cell count provide farmers with quantitative evidence of abnormal milk quality. These tests are also frequently used by milk processors and laboratories to quantify milk abnormalities, Molecular and biosensor tests are becoming common in some laboratories. Other tests that are less common (and therefore not presented here) include: Wisconsin mastitis test, Modified Whiteside test, pH determination test, Chloride test, Methylene blue reduction test, Milk antitrypsin assay and N-acetyl-β-D-glucosaminidase test.

2.6.1 Somatic cell count of milk

Somatic cell count can be expressed as bulk milk and individual cow somatic cell count (Lakshmi, 2016). In normal milk, leucocytes and other glandular cells should be less than 200,000cells/ml and should comprise of 66-88% macrophages, 10-27% lymphocytes, 1-11% neutrophils and 0-7% epithelial cells, but in instances of mastitis, neutrophils dominate up to 80% (Ganguly *et al*., 2018). This test gives an early warning to the presence of an infection when count changes (Deb *et al*., 2013). In presence of mastitis, the number of somatic cells can be in excess of 5,000,000 (Ganguly et al., 2018).

2.6.2 California mastitis test

This is a popular test since it is cheap, easy to carry out, rapid and most importantly, reliable as it gives an accurate extent of the disease (Lakshmi, 2016). The test utilizes the reaction of somatic cell DNA with the test reagent to form a gel. It is a cow-side test for subclinical mastitis where quarters with scores of 2 and 3 are considered positive, while those with scores of 0 and trace are negative, and a score of 1 is sometimes considered equivocal (Deb *et al*., 2013). There is a direct correlation between score of the test and the somatic cell count such that score 0 has between 0 and 200,000, trace has between 150,000 and 500,000, score 1 has between 400,000 and 1,500,000, score 2 has between 800,000 to 5,000,000 and score 3 has over 5,000,000 somatic cells (Ganguly *et al*., 2018).

2.6.3 Strip cup test

This is widely used in milking parlors for testing for clinical mastitis before machine milking commences. Few strips of milk from each quarter is milked into the cup and examined for flakes, clots, wateriness or blood which are indicative of mastitis (Ganguly *et al*., 2018).

2.6.4 Electrical conductivity test

Electrical conductivity in milk from a mastitic cow is high compared to normal milk because of the increased sodium, potassium, calcium, magnesium and chloride ions (Duarte *et al*., 2015). This test is however a screening test since electrical conductance can vary from animal to animal and therefore further tests should be carried out in positive animals (Duarte *et al*., 2015; Lakshmi, 2016). This test can be done using a portable electrical conductivity meter and units given in milk seimens/cm (Ms/cm) (Ganguly *et al*., 2018) and is commonly used in farms that use milking robots (Duarte *et al*., 2015). Electrical conductivity in normal milk ranges from 5.5-6.5 mS/cm while that of milk from mastitic quarters can be as high as 9 mS/cm (Norberg *et al*., 2004).

2.6.5 In-vitro culture

In-vitro culture is the gold standard for diagnosis of mastitis, where milk samples are collected aseptically and submitted to the laboratory for bacterial, fungal or viral culture in specific media for isolation and biochemical tests (Deb *et al*., 2013). Bacterial cultures can be carried out at the quarter level, cow level or herd level (bulk milk), and is helpful in management of transmission by identifying the pathogen type and therefore likely source of infection (Lakshmi, 2016). Fungal isolation is possible in normal bacterial culture, although it requires patience for the time to grow. Viral isolation is difficult, especially in chronic cases with mixed infection (Deb *et al*., 2013).

2.6.6 Molecular tests

Polymerase chain reaction has been widely used in recent years in detection of mastitis, despite being cumbersome and costly (Lakshmi, 2016). However, this method has the advantage of high sensitivity and specificity, and is helpful in the diagnosis of more than one causative organism (Martins *et al*., 2019). Multiplex Polymerase Chain Reaction (PCR) diagnosis of pathogens causing mastitis is helpful, especially in cases involving fastidious organisms which sometimes are hard to diagnose (Deb *et al*., 2013). This method is also helpful in understanding virulence, vaccine development and antimicrobial resistance of organisms (Gurjar *et al*., 2012).

2.6.7 Biosensors

Biosensors are able to recognize biological molecules and send them to a transducer that measures the strength of the signal. This has been aided by advances that have been realized in nanotechnology; nanostructures and metal nanoparticles have improved transduction and amplification of signals (Martins *et al*., 2019). Nanotechnology detection of pathogens reduces the time taken to detect and collect samples, and make a diagnosis of the disease (Duarte *et al*., 2015).

2.7 Control of mastitis

Control of mastitis should be a multi-pronged approach. Preventive efforts are essential and should be coupled with swift diagnosis and treatment of mastitis cases in order to reduce the reservoir of pathogens within a herd as a source for new infections. Culling to reduce the reservoir of pathogens should also be considered where treatment is undesirable or unsuccessful. These control methods are discussed in the following sub-sections.

2.7.1 Environmental management

Sanitation and hygiene play a key role in the control of mastitis since the major sources of infection are infected quarters, milker's hands, teat cups, milking machines, dirty udders, washing clothes and flies (Kibebew, 2017). Proper disposal of slurry, disinfection of premises, proper cleaning of the udder, milker's hands and milking machines are important as they serve to reduce the exposure of teat ends to agents of mastitis (Shaheen *et al*., 2016). There are also different methods that have been used in the control of the disease such as dry cow therapy, lactation therapy, gene therapy, and mastitis autogenous vaccine.

2.7.2 Vaccination

Autogenous vaccine is used to minimize new intramammary infections, reduce severity of new intramammary infection, or eliminate chronic intramammary infection (Shaheen *et al*., 2016). However, there is limited evidence on their efficacy. Vaccines have been widely tried against *S. aureus* and *S. agalactiae*, but their efficacy studies have shown that they are not reliable (Ismail, 2017). There is a vaccine against *E. coli* that has been shown to reduce the severity of coliform mastitis, but it does not reduce the incidence of infections (Klaas and Zadoks, 2018).

2.7.3 Treatment of mastitis during lactation

Mastitis treatment is generally determined by bacteriological culture and sensitivity, clinical manifestations, prognosis and regulations on the use of drugs in a particular country (Lundberg, 2015). Currently, there are two approaches to treatment of bovine mastitis that are being practiced, namely, antimicrobial treatment and alternative treatment. Antimicrobial treatment is the most widely used and its success is affected by the volume of milk in the udder, accumulation of debris resulting from the disease process, and the impermeability of the blood-udder barrier to certain antimicrobials (Shaheen *et al*., 2016). In the United States, intramammary antimicrobials are frequently used in the management of mastitis, and most are B-lactams such as ceftiofur and penicillin (Oliveira & Ruegg, 2014).

In the alternative approach, the use of herbal derivatives has been utilized, especially with the advent of antimicrobial resistance and residues in milk. Gel made from *Cedrus deodara*, *Curcuma longa*, and *Ocimum sanctum,* among others, has been used in the management and prophylaxis of subclinical mastitis (Shaheen *et al*., 2016). However, the efficacy of these products is unclear. Some environmental pathogens (e.g. coliforms) are known to self-cure in some cows, especially with frequent stripping of the udder, thereby not requiring antimicrobial treatment unless there are systemic signs of infection (Kromker and Leimbach, 2017).

2.7.4 Dry cow therapy

Dry cow therapy (DCT) refers to the use of intramammary antibiotics at dry-off and is a widely used method for mastitis control (Neelam *et al*., 2017). Dry cow therapy is key in the treatment of mastitis that has not responded to conventional treatment during lactation, such as cases of *S. aureus*. Treatment of *S. aureus* during lactation often has low cure rates, and thus use of DCT is advised, especially if the case is chronic (Petersson-wolfe *et al.*, 2010). There are two reasons why DCT is more effective in treating mastitis than antibiotic treatment during lactation: 1) the DCT intramammary tube has a higher concentration of antibiotic than a lactation intramammary tube; and 2) at dry-off, the antibiotic is no longer removed with the subsequent milking. These two reasons ensure high concentration of antibiotics released into the udder tissues at the start of the dry period. The slow metabolism of the DCT during the early dry period also prevents new intramammary infections when the keratin plug is being formed in the teat sphincter

(Green *et al*., 2002). Therefore, DCT is advantageous compared to lactation therapy since high doses of antibiotics can be used without milk withdrawal, there are also high cure rates, there is prolonged activity of the drug, and there is minimal chance of residues in milk (Waldner, 2007).

There are two major approaches that have been used in the practice of DCT in dairy industry; blanket and selective DCT. Blanket DCT involves treatment of all quarters at dry-off without necessarily taking into consideration the status of intramammary infection, while selective DCT is treatment of quarters that are positive for intramammary infections.

Blanket DCT has been the most preferred DCT method of dairy farmers in North America and most parts of the world for decades, but with the campaign against antimicrobial resistance increasing, this has changed (Dufour *et al*., 2012; van der Wagt, 2017). This method is highly effective at clearing existing infections at dry-off and preventing new infections in the early dry-off period, Blanket dry cow therapy is an expensive approach in the management of intramammary infections because every quarter of every cow is treated at dry-off, but can be helpful on farms with increased prevalence of clinical mastitis and reduced antimicrobial resistance (Scherpenzeel *et al*., 2018). Conversely, selective dry cow therapy is more economical when incidence of clinical mastitis and bulk somatic cell count are low (Scherpenzeel *et al*., 2018). Therefore, the appropriate approach for a farm depends on the risks of mastitis and the restrictions in use of antibiotics (van der Wagt, 2017).

Apart from use of the antibiotics, there are also other approaches to the prevention of new infections during the dry period, such as the use of teat sealants. Naturally, within two weeks after dry-off, the keratin plug forms in the teat sphincter to act as a barrier to prevent the entry of harmful organisms (Crispie *et al*., 2004). However, the formation of the plug may fail in some cows and the integrity of the plug decreases as the dry period advances; therefore, the cow becomes prone to intramammary infections (Mutze *et al*., 2012). Teat sealants act as this natural barrier and are applied at the dry-off.

There are two types of teat sealants that are being used in the dairy industry; internal and external teat sealants. Crispie *et al.* (2004) found that external teat sealants are not as effective as internal teat sealants, but offer some protection as compared to unsealed teats. Teat sealants can be used in combination with antibiotic DCT, and the continued worry of antimicrobial resistance has led to its increased usage of teat sealants (Crispie *et al*., 2004). Newton *et al* (2008) reported that incidences of mastitis in cows treated with antibiotic DCT and internal teat sealant is lower as compared to those treated with antibiotic alone or with no treatment at all.

Internal teat sealants when used alone or together with antibiotic DCT have been shown to reduce mastitis post-calving by up to 25% (Rabiee & Lean, 2013) and by 75% (Wanjala, *et al*., 2020) as compared with untreated cows. Mutze *et al.* (2012) and Wanjala, *et al*. (2020) noted that the combination of internal teat sealants and long acting dry cow antimicrobials significantly reduced clinical mastitis caused by environmental pathogens.

2.7.5 Other management options

Culling of the mastitic cows is the only certain way of eliminating infected cows as a source of infection in the farm, especially when treating of cases is unsuccessful, and record-keeping is sub-optimal (Hillerton & Booth, 2018). Breeding for resistant cows against mastitis is also being adopted, and with advances in molecular genetics and marker-assisted genetic selection, this option is growing in viability and popularity (Deb *et al*., 2013). Addressing aspects of nutrition which play a role in the occurrence of mastitis, such as deficiencies of vitamin E and selenium, also helps in the control of the disease (Ganguly *et al*., 2018).

2.8 Economic losses due to mastitis

Mastitis is considered the greatest constraint to dairy farming in many parts of the world, with losses incurred as a result of: 1) discarded milk following antibiotic treatment, 2) veterinary services, 3) premature culling of the cow, 4) loss of production, 5) deaths from peracute cases, and replacement costs (Majeed, 2016). Staphylococcal mastitis is the major contributor of the economic losses in most parts of the world, while in Europe, Streptococcal mastitis is the main concern (Shaheen *et al*., 2016).

In Canada, Aghamohammadi *et al.*, (2018) reported losses as 495 USD per cow per year and 48% contributed by subclinical mastitis, 34% by clinical mastitis, and 15% being the cost of preventive measures undertaken to control the disease, such as teat disinfectants and milking gloves. In India, losses were estimated to be between 289.1 to 1178.3 USD per lactation per animal (Rathod *et al*., 2017). Romero *et al.* (2018) estimated the cost of subclinical mastitis in Colombian dairy farms to be over 800USD per farm with small (10-25 milking cows) and medium-sized (26-100 milking cows) farms mostly affected. With these high costs, validated control measures are likely to be financially beneficial, not to mention good for animal welfare.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study area

The study was carried out in Kikuyu Sub-County of Kiambu County in the Central part of Kenya (Figure 3.1). The County covers a total area estimated at 2543.5 km^2 (1˚ 10’ 29’’N and 36˚ 49’ 49’’E), and borders Nairobi and Kajiado to the South, Machakos to the East, Murang’a to the North and Northeast, Nyandarua to the Northwest and Nakuru to the West. The County experiences a cool and warm climate with night-time temperatures ranging between 12^{0}C and 18.7^{0}C, and day-time temperatures ranging between 12.2^{0}C and 26.7^{0}C. Annual rainfall averages 1000mm, which makes the County suitable for agriculture. Crop production and livestock-keeping are the major economic activities in the area due to the ready market access in nearby Nairobi city. Dairy farming contributes immensely to the economy of the Kiambu area, and this is evident by the presence of milk-processing plants such as Brookside, Palmside, Githunguri, and Ndumberi Dairies, among others.

Kiambu County was conveniently selected for the study for various reasons: 1) there was another ongoing project among the smallholder dairy farms, 2) there is intensive smallholder dairy farming in the county, 3) it was financially feasible given the limited research funds, and 4) it had close proximity to the Department of Clinical Studies Laboratory, allowing reliable milk culture procedures without compromising the quality of the milk samples collected.

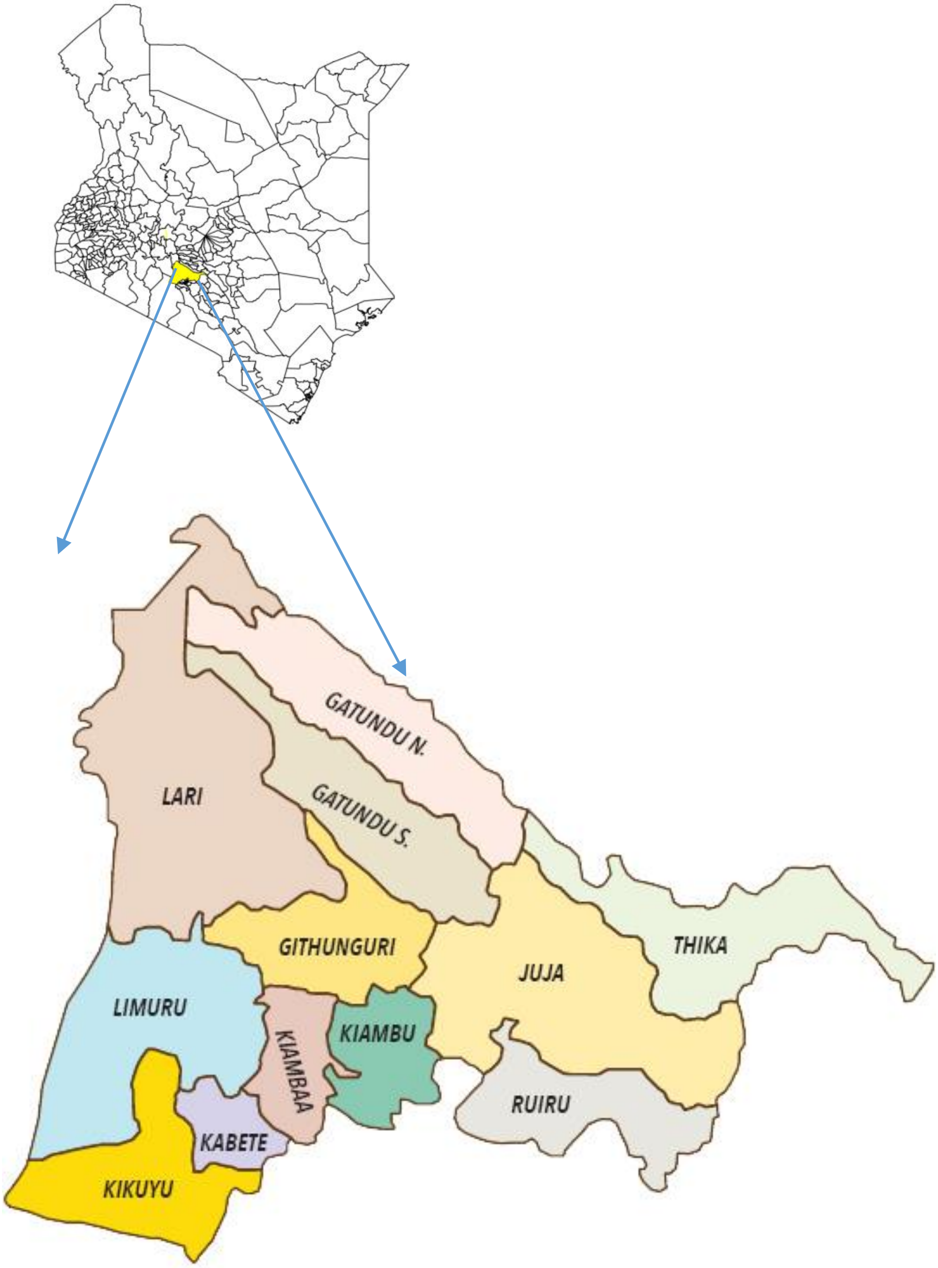


Figure 3.1: Geographical location of Kikuyu in Kiambu County, Kenya

3.2 Study design and selection of study farms

This study was a randomized controlled trial which targeted cows to be dried off. Inclusion criteria for eligible farmers for the study included: 1) farmers who had a cow that was being dried off; and 2) farmers who were actively delivering milk to the Kabete and Muguga Dairy

Cooperative Societies. Therefore, farms were conveniently selected based on the availability of a cow at the point of drying off. Inclusion criteria for eligible cows for the study included cows that tested positive for CMT on at least one quarter at dry off on the eligible farms.

The sample size for the treatment part of the study (the primary goal of the study) was 100 positive quarters in total and was estimated using a DCT cure rate of 70% in the treatment group and 40% in the control group, as reported in the Netherlands (Vanhoudt *et al*., 2018). This sample size was computed based on a power of 0.8 and significance level of 0.05.

3.3 Data and sample collection

Between July and October 2019, farms were initially visited on the days during which drying off of cows was happening, and one week after calving of recruited cows, with some farms visited a third time two weeks after calving to confirm post-calving culture-negative status. The sampling unit was the individual quarter of the cows which were subjected to California Mastitis Test at dry-off. The mastitis-positive quarters were those with a score of 2 and 3, while those considered negative were those with a score ≤ 1. Quarters that were CMT-positive were enrolled in the study while the CMT-negative were ignored if the farmer was not practicing dry cow therapy while in instances where they practiced it was recommended to them to apply.

Questionnaires were administered during the farm visits to capture information on farm- animal- and quarter- level factors. Farm-level factors of interest included farmer demographics, cow housing management, milking practices, and reported past diseases, such as mastitis, and their control, among others. Animal level factors were breed of cow, age, parity, current level of production, and month of lactation. Quarter-level factors included current and past mastitis status, current and past milk leakage status, and current teat-end status, among others.

From all the CMT-positive quarters, milk samples were collected aseptically for culture at the bacteriology laboratory of the Department of Clinical Studies, University of Nairobi. The CMT-positive quarters were randomly allocated to receive either DCT (Multimast®; Neomycin sulphate 100mg, penethamite hydriodide 100 mg, procaine benzyl penicillin 400mg per 4.5 g syringe) and ITS (ORBESEAL®; 65% bismuth subnitrate) as treatment or ITS only as control. The randomization was achieved by randomly allocating a number to each quarter so that quarter number 1 received the treatment while quarter number two received the control.

All milk samples were kept on ice until they were submitted to the laboratory for culture later in the day, and not frozen in order to increase the chances of culturing coliform organisms. The study follow up and sampling was concluded in March 2020.

3.4 Bacterial culture and identification of micro-organisms

Using blood agar and MacConkey, 10 microliters of milk was streaked on each plate and incubated at 37^0C for 48 hours, with the remaining milk being frozen at -20^0C. Growth was observed, and in samples that showed no growth, the frozen milk samples were cultured to check for presence of *Staphylococcus aureus*. The organism is normally found in leucocytes hence their lysis due to freezing releases them. Colonies were described and gram-staining and biochemical tests were used to characterize the organism, such as the coagulase test. This coagulase test was used to differentiate *S. aureus* from coagulase negative *Staphylococcus* such as *S. epidermidis* and *S. saprophyticus*. The results of both CMT and culture were used to assess the effectiveness of treatment.

3.5 Data entry and analysis

Data collected using the questionnaires, along with the results from the laboratory, were entered into Microsoft Excel (Microsoft Inc., Sacramento, California, USA) where they were cross-checked for accuracy and coded, before being imported to Stata 15.1 (StataCorp LLC, College station, Texas, USA) for analyses. Proportions (presented as percentages) were determined for categorical variables, such as breed, and ranges, means and standard deviations were determined for continuous variables, such as age. To determine if the intervention of DCT and ITS was better than ITS only at dry-off, the proportions of infected quarters at dry-off were compared to the proportions of infected quarters post-calving, for all types of infections, and by bacteria. This comparison was done by testing to see if there was a significant interaction between time of sampling and treatment group when stratifying the data and using infection as the outcome of interest.

Univariable analysis were carried out using multilevel mixed-effects logistic regression in order to determine unconditional associations between the farm-, animal- and quarter-level predictor data and presence of *Staphylococcus aureus* as the outcome since it was cultured in a high proportion of positive samples, and was an objective measure in the study. Univariable associations with $p \leq 0.15$ were eligible for multivariable analysis.

Initially, a multivariable mixed logistic regression analysis was fitted for all the eligible predictors associated with *S. aureus*. The final model was developed using backward stepwise elimination, leaving those variables which had a p-value ≤ 0.05. Correlations between predictor variables were identified using pair-wise correlation, and where two or more variables were highly correlated (correlation coefficient>0.5), statistical significance and biological plausibility were used to identify which variable would be removed during the modeling process. Explanatory variables were considered confounders if their removal from the multivariable model modified the coefficients of other significant variables by 30% or more. Interactions between significant explanatory variables in final model were also tested and the significant interaction terms (p-value

≤0.05) were included in the final models (Dohoo *et al*., 2009). The area under the curve (AUC) of the receiver operating curve (ROC) was used to evaluate the overall model performance. Area under the curve ranging of 0.7 to 1 is considered acceptable (Mandrekar, 2010).

CHAPTER FOUR: RESULTS

The study involved 20 smallholder dairy farms, out of which a total of 32 cows were recruited, giving a total of 121 CMT-positive quarters (3 quarters were non-functional while four were CMT-negative). During the first visit (dry-off), all the 121 quarters were sampled. During the second visit, only 97 samples were collected because three cows were lost during the study, two through disposal following Downer cow syndrome and one through sale. Another 12 samples from three other cows could not be processed in time following disruption of normal laboratory functions due to Covid-19, leading to 26 cows cultured post-calving.

4.1 Farm-level characteristics

The mean age of the participating farmers was 51 years and mean number of years in the dairy business was 15 years (Table 4.1). The mean number of milking cows, dry cows, heifers, calves and bulls are 4, 1, 3and 2, respectively, with the mean herd size being 11 animals (Table 4.1). Mean average milk production for the dairy farms was 55 liters, with dairy contributing to an estimated mean percentage income of 38% (Table 4.1).

The mean number of cow cubicles in the farms was 8 (Table 4.2). The results showed that 70% of the farmers interviewed were male, while 30% were female. Of all the farmers interviewed, 50% had attained tertiary education, with 15% and 35% having attained primary and secondary education, respectively. Additionally, 95% were married and 5% were single, with 60% of the farms being under the management of husbands, followed by wives (25%), children (10%) and employees (5%) (Table 4.2).

The results showed the most common disease reported by the farmers in the farms was mastitis (75%), followed distantly by metabolic diseases (20%) and pneumonia (15%), and diarrhea and East Coast Fever at 10% each (Table 4.3). Foot conditions, skin diseases, aflatoxicosis, foot-and-mouth disease and lumpy skin disease were not common in the smallholder dairy farms.

Table 4.1 Descriptive statistics of continuous variables from 20 smallholder farms in Kiambu County, Kenya, in September 2019 - March 2020

Variable	Mean	Range	Standard deviation
Primary farmer age (years)	51.0	27 – 75	13.9
Number of adults in household	3.9	1-12	2.3
Number of years in dairy farming	15.2	6-40	9.0
Farm acreage (acres)	0.67	0.125-3	0.77
Number of dry cows	1.3	0-4	1.1
Number of milking cows	4.2	1-13	3.1
Number of heifers	2.9	0-6	2.0
Number of calves	2.3	0-11	2.6
Herd size	10.9	3-34	6.9
Average milk production per day (kg)	54.7	1-200	53.1
Percentage income from dairy	37.5	5-100	30.2
Number of cubicles in animal house	8.4	2-15	4.1

Table 4.2 Descriptive statistics of categorical variables of the farm demographics from 32 cows on 20 smallholder farms in Kiambu County, Kenya, in September 2019 - March 2020

Variable	Category	Frequency	Proportion (%)
Gender of primary farmer	Male	14	70
	Female	6	30
Education of primary farmer	Primary	3	15
	Secondary	7	35
	Tertiary	10	50
Marital status of primary farmer	Single	1	5
	Married	19	95
Person in charge of farm management	Children	2	10
	Wife	5	25
	Husband	12	60
	Employee	1	5
Dairy cooperative society	None	1	5
	Kikuyu	5	25
	Limuru	2	10
	Muguga	6	30
	Kabete	6	30
Breed of Cow	Friesian	24	75
	Ayrshire	6	19
	Jersey	2	6

Table 4.3 Descriptive statistics of the common diseases reported by the farmers in 20 farm smallholder dairy farms in Kiambu County, Kenya, in September 2019 - March 2020

Variable	Category	Frequency	Proportion (%)
Mastitis	No	5	25
	Yes	15	75
Diarrhea	No	18	90
	Yes	2	10
Lumpy skin disease	No	19	95
	Yes	1	5
Helminthiasis	No	19	95
	Yes	1	5
Aflatoxicosis	No	19	95
	Yes	1	5
Skin diseases	No	19	95
	Yes	1	5
Foot and mouth disease	No	18	90
	Yes	2	10
Infectious diseases	No	19	95
	Yes	1	5
East Coast fever	No	18	90
	Yes	2	10
Metabolic diseases	No	16	80
	Yes	4	20
Pneumonia	No	17	85
	Yes	3	15
Foot conditions	No	18	90
	Yes	2	2

Most of the dairy units had an intact roof (95%), while 70% of the cow cubicle floors were concrete (Table 4.4). Walk alley floors were mostly concrete (70%) and the frequency of cleaning the floors was mostly daily (55%). The frequency of adding new bedding in the cow cubicle was primarily weekly (60%). Cows lying backwards in the cubicles was reported in 20% of the farms. Additionally, 50% of cows were lying on the walk alley. The cleaning frequency of the walk alley was 55% for once daily, 40% for twice daily and 5% for thrice daily (Table 4.4).

Most farms (75%) used hand-milking and of these, only 47% squeezed (instead of pulled) the teats during milking, while 15% used machine milking and 10% used both methods (Table 4.5). Additionally, 100% of the farms used a cloth for udder cleaning, with 90% using one cloth for all the cows in the farm. The study showed that 70% of the farmers reported that clots in milk was a sign of mastitis in cows, while 45% reported swollen udder and 25% reported pain during milking. Most (85%) of the farmers said that mastitis lasted for one week following treatment, with 90% said that mastitis resulted in decreased milk production. Additionally, 80% of the farmers dried off their cows gradually over 14 days, with 40% reporting that they sometimes used DCT (Table 4.5).

Table 4.4 Descriptive statistics of the dairy unit from 20 smallholder dairy farms in Kiambu County, Kenya, in September 2019 - March 2020

Variable	Category	Frequency	Proportion (%)
Status of the roof	Intact	19	95
	Leaking holes	1	5
Type of floor in cubicle	Concrete	14	70
	Dirt	5	25
	Soil	1	5
Type of bedding in cubicle	None	7	35
	Mattress	8	40
	Sawdust	2	10
	Grass/leaves	2	10
	Crop waste	1	5
Frequency of adding beddings	Weekly	3	60
	Twice weekly	2	40
Level of manure in udder area	Clean	2	10
	Scant	8	40
	moderate	5	25
	Excessive	5	25
Cows lying backwards in cubicle	No	16	80
	Yes	4	20
Cleaning frequency of cow cubicle	Once daily	9	45
	Twice daily	9	45
	Weekly	1	5
	Every 3 months	1	5
Type of floor in walk alley	Concrete	14	70

	Stones	6	30
Cows lying in the walk alley	No	10	50
	Yes	10	50
Cleaning frequency of the walk alley	Once daily	11	55
	Twice daily	8	40
	Thrice daily	1	5

Table 4.5 Descriptive statistics on milking practices from 20 smallholder dairy farms in Kiambu County, Kenya, in September 2019 - March 2020

Variable	Category	Frequency	Proportion (%)
Milking method	Hand	15	75
	Machine	3	15
	Both	2	10
Hand milking method (n=17 farms)	Squeeze	8	47
	Pull	9	53
Feeding method	Zero-grazing	19	95
	Open-grazing	0	0
	Both	1	5
Pre-milking udder cleaning	No	0	0
	Yes	20	100
Temperature of water used for udder cleaning	Warm	19	95
	Cold	1	5
Addition of disinfectant into water for udder cleaning	No	17	85
	Yes	3	15
Udder drying before milking	No	2	10
	Yes	18	90
Type of material used for udder drying	Cloth	18	90
	None	2	10
Use of separate cloth per cow	No	18	90
	Yes	2	10
Washing hands before milking	No	0	0
	Yes	20	100
Cloth washing between milking	No	1	5
	Yes	19	95
Cloth drying between milking	No	1	5

	Yes	19	95
Use of teat dip post-milking	No	17	85
	Yes	3	15
Use of milking jelly pre-milking	No	4	20
	Yes	16	80
Feeding immediately after milking	No	5	25
	Yes	15	75
Number of milking per day	2x	16	80
	3x	4	20
History of mastitis in the last one year	No	3	15
	Yes	17	85
Management of mastitis in the last year (n=17 farms)	Treated	16	94
	Sampled and treated	1	6
Person who treats mastitis	Self	2	10
	AHA	17	85
	Vet	1	5
Swollen udder as sign of mastitis	No	11	55
	Yes	9	45
Abnormal milk as sign of mastitis	No	16	80
	Yes	4	20
Clots in milk as sign of mastitis	No	6	30
	Yes	14	70
Reduced milk as sign of mastitis	No	17	85
	Yes	3	15
Udder fibrosis as sign of mastitis	No	17	85
	Yes	3	15
Fever as sign of mastitis	No	19	95
	Yes	1	5
Pain as sign of mastitis	No	15	75

	Yes	5	25
Impact of mastitis on milk production	Decreased	18	90
	Constant	1	5
	Increased	1	5
Duration of mastitis	Two days	1	5
	One week	17	85
	Above one week	2	10
Method of drying cows	Abrupt	4	20
	Gradual	16	80
Use of dry cow therapy	No	12	60
	Yes	8	40
Blanket dry cow therapy	No	14	70
	Yes	6	30
Use of teat sealants at dry-off	No	20	100
	Yes	0	0
Use of teat sealants and dry cow therapy	No	20	100
	Yes	0	0

4.2 Animal-level characteristics

There were 44% of cows being milked twice daily, and 28% each being milked once and thrice daily, with 56% having no history of mastitis (Table 4.6). Additionally, 62.5% and 37.5% of the study cows had intermediate and high udder depth, respectively.

In terms of udder balance, 59.4% of the cows had the same level between front and rear udders, while 21.9% and 18.8% had deep rear and front balance, respectively (Table 4.6). The results further showed that 46.9% had intermediate udder attachment, 40.6% and 12.5% had strong and loose udder attachment, respectively.

Lower leg cleanliness scores showed that 13.2% of the cows had clean legs, 28.9% were slightly dirty, 51.2% were moderately dirty and 6.6% were excessively dirty (Table 4.6). The cleanliness score of the upper flank was 12.4%, 34.7%, 39.7% and 13.2% for clean, slight, moderate and excessive dirtiness, respectively. Additionally, the udder cleanliness scores were 25.6%, 49.6%, 18.2% and 6.6% for clean, slight, moderate and excessive dirtiness, respectively (Table 4.6).

Table 4.6 Descriptive statistics of categorical data of animals level factors from 20 smallholder dairy farms in Kiambu County, September 2019 - March 2020 (n = 32 cows)

Variable	Category	Frequency	Proportion (%)
Milking frequency	1	9	28
	2	14	44
	3	9	28
Mastitis history	No	18	56
	Yes	14	44
Udder depth	Intermediate	20	62.5
	High	12	37.5
Udder attachment	Loose	4	12.5
	Intermediate	15	46.9
	Strong	13	40.6
Udder balance	Deep rear	7	21.9
	Same level	19	59.4
	Deep front	6	18.8
Lower leg cleanliness score	Clean	16	13.2

	Slightly dirty	35	28.9
	Moderately dirty	62	51.2
	Excessively dirty	8	6.6
Upper leg and flank cleanliness	Clean	15	12.4
	Slightly dirty	42	34.7
	Moderately dirty	48	39.7
	Excessively dirty	16	13.2
Udder cleanliness	Clean	31	25.6
	Slightly dirty	60	49.6
	Moderately dirty	22	18.2
	Excessively dirty	8	6.6

Milking frequency categories:

1= once daily; 2= twice daily; 3= thrice daily

4.3 Quarter-level characteristics

Farmers reported that 55% of the quarters that had mastitis occurred in early lactation, 40% in mid-lactation and 5% in late lactation (Table 4.7). Additionally, 78.6% of quarters that had mastitis treatment at early lactation, 14.3% during mid-lactation and 7.1% during late lactation. Only 8.3% of the teats were leaking, and from examinations of teat ends for eversion, it was noted that 70% of teat ends had a smooth ring, 19% had a rough ring following callous formation, and 11% did not have a ring. Finally, 81.8% of the teats had deep front placement, while 18.2% had teat placement at the same level (Table 4.7).

Table 4.7 Descriptive statistics of categorical variables at the quarter level from 32 cows on 20 smallholder dairy farms in Kiambu County, from September 2019 - March 2020

Variable	**Category**	**Frequency**	**Proportion (%)**
Time of mastitis in current lactation (n=20)	Early lactation	11	55
	Mid lactation	8	40
	Late lactation	1	5
Time of last mastitis treatment (n=14)	Early lactation	11	78.6
	Mid lactation	2	14.3
	Late lactation	1	7.1
Teat leakage (n=121)	No	111	91.7
	Yes	10	8.3
Teat end score (n=121)	No ring	13	10.7
	Smooth ring	85	70.2
	Rough ring	23	19
Teat placement	Same level	22	18.2
	Deep front	99	81.8
Quarter treatment (n=121)	ITS	59	49
	DCT+ ITS	62	51

4.4 Bacteriology results

Out of the 121 total CMT-positive samples during the first visit, 101 were culture-positive (83.5%), while 20 were culture-negative. The most isolated organism was *Staphylococcus aureus* with 54.6% (66/121), followed by coagulase-negative *Staphylococcus* at 22% (27/121), *Streptococcus* species at 3.3% (4/121), *Actinomyces* species 1.6% (2/121), and both *Escherichia coli* and *Pseudomonas* species at 0.8% (1/121) each (Table 4.8).

Of the 97 post-calving milk samples, 55 were culture-positive while 42 were culture-negative. The most isolated organism during the second visit was *Staphylococcus aureus* with 42.3% (41/97), followed by *Escherichia coli* at 6.2% (6/97)), *Coagulase-negative Staphylococcus* at 3.1% (3/97), *Streptococcus* species and *Pseudomonas* species each at 2.1% (2/97), and lastly *Actinomyces species* at 1.0% (1/97) (Table 4.8).

At the cow level (Table 4.9), 81.3% (26/32) of the recruited cows (with at least one CMT-positive quarter) had *Staphylococcus aureus* in one or more quarters at dry-off. Similarly, 40.6%, 6.3%, 12.3%, 3.1% and 3.1% of cows had coagulase-negative *Staphylococcus*, *Actinomyces* species, *Streptococcus, Escherichia coli* and *Pseudomonas* species, respectively. Post-calving, of the 26 cows tested, 65.4%, 11.5%, 3.9%, 19.2%, 7.7% and 7.7% of cows had *Staphylococcus aureus,* coagulase-negative *Staphylococcus*, *Actinomyces* species, *Escherichia coli*, *Pseudomonas* species and *Streptococcus* species, respectively, in one or more quarters. Out of the 32 cows with CMT-positive quarters at dry-off and 26 of these cows remaining in the study post-calving, 30 (93.8%) and 19 (73.1%) cows had at least one quarter confirmed infected by culture results.

Table 4.8 Proportions of bacteria isolated at the quarter level from 32 cows on 20 smallholder dairy farms in Kiambu County during the first visit (n = 121) and second visit (n = 97), from September 2019 - March 2020

Bacteria isolate	**Dry-off (%)**	**Post-calving (%)**	**Difference (%)**
Staphylococcus aureus	54.5 (66/121)	42.3 (41/97)	12.2
Coagulase-negative *Staphylococcus*	21.1 (27/121)	3.1 (3/97)	18
Actinomyces species	1.8 (2/121)	1.0 (1/97)	0.8
Streptococcus species	2.8 (4/121)	2.1 (2/97)	0.7
Escherichia coli	0.9 (1/121)	6.2 (6/97)	-5.3
Pseudomonas species	0.9 (1/121)	2.1 (2/97)	1.1

Table 4.9 Proportions of bacteria isolated at the cow level from 20 smallholder dairy farms in Kiambu County during the first visit (n=32) and second visit (n=26), from September 2019 - March 2020

Variable	Category	Frequency	Proportion	Difference
Staphylococcus aureus	Dry-off	26	81.3	15.9
	Post calving	17	65.4	
Actinomyces species	Dry-off	2	6.3	2.4
	Post calving	1	3.9	
Coagulase-negative Staphylococcus	Dry-off	13	40.6	29.1
	Post calving	3	11.5	
Escherichia coli	Dry-off	3	3.1	-16.1
	Post calving	5	19.2	
Pseudomonas species	Dry-off	1	3.1	-4.6
	Post calving	2	7.7	
Streptococcus species	Dry-off	4	12.5	4.8
	Post calving	2	7.7	

Proportion represents percentages and the difference is between the percentages at dry-off and post-calving

4.5 Effectiveness of dry cow therapy in treating existing udder infections

The trial results showed that dry cow therapy and ITS (treatment group) significantly reduced the proportions of *Staphylococcus aureus* quarter infections from 67.9% at dry-off to 44.0% post-calving, while the quarter-level infections of *S. aureus* in the control group (ITS only) only reduced from 47.2% at dry-off to 40.4% post-calving. The treatment reductions were substantial considering the difficulty in eliminating cases of mastitis with *Staphylococcus aureus*.

Coagulase-negative *Staphylococcus* infections in the intervention group at the quarter-level lowered from 17.9% at dry-off to 2.0% post-calving (Table 4.10). However, there was also a significant reduction in coagulase-negative *Staphylococcus* infections in the control group from 24.5% at dry-off to 4.3% post-calving, therefore, these reductions in both groups should be attributed to self-cure phenomenon

The results further showed that there was only a slight increase in proportions of *Escherichia coli*, and in *Streptococcus, Pseudomonas* and *Actinomyces* species in the DCT and ITS group (Table 4.10). The DCT+ITS group did seem to cure all of the *Streptococcus* and *Actinomyces.* There was no *E. coli* or *Pseudomonas* species infections in the control group at dry-off, but there were a small number of these pathogens isolated post-calving in the control group, similar to the proportions in the intervention group; therefore, there were no significant differences between in proportions of pathogens isolated

The interaction terms that explored whether the association between treatment group and infection depended on visit (dry-off vs post-calving) was found to be non-significant at p-value of <0.05, overall and for any specific pathogen.

Table 4.80 Proportions of bacterial isolates at dry-off and post-calving, by treatment group and control group, in 97 quarters from 26 cows on 19 smallholder dairy farms in Kiambu County, Kenya, from September 2019 - March 2020

Isolate	**DCT + ITS**		**ITS only**	
	Dry-off %	**Post-calving %**	**Dry-off %**	**Post-calving %**
Staphylococcus aureus	**67.9 (38/56)**	**44.0 (22/50)***	47.2 (24/53)	40.4 (19/47)
Coagulase-negative *Staphylococcus*	**17.9 (10/56)**	**2.0 (1/50)***	**24.5 (13/53)**	**4.3 (2/47)***
Escherichia coli	1.8 (1/56)	6.0 (3/50)	0 (0/53)	6.3 (3/47)
Pseudomonas spp.	1.8 (1/56)	2.0 (1/50)	0 (0/53)	2.1 (1/47)
Streptococcus spp.	0 (0/56)	4.0 (2/50)	5.7 (3/53)	0 (0/47)
Actinomyces spp.	1.7 (1/56)	2.0 (1/50)	1.8 (1/53)	0 (0/47)

* Significant difference ($p<0.05$) between dry-off percent and post-calving percent within the group

ITS: Internal Teat Sealant

DCT: Dry cow therapy

4.6 Factors associated with *Staphylococcus aureus* isolated from milk cultures

On univariable analysis, the following variables met the P-value cut-off (p<0.25) for univariable logistic regression analysis with *S. aureus* infections among CMT-positive cows at dry-off and post-calving: bedding type (p = 0.0799), DCT use (p = 0.1046), teat dip use (p = 0.1330), who was in charge of farm management (p = 0.1046), cow breed (p = 0.1073), treatment group (p = 0.0730), teat placement (p = 0.0132), lower leg cleanliness (p = 0.0167), udder cleanliness (p = 0.0037), current level of production (p = 0.0733) and use of disinfectant (p = 0.1120) (Table 4.11).

The factors associated with *S. aureus* isolated from the milk cultures in the final multivariable analysis (Table 4.12) included: type of bedding in cow cubicle (p < 0.001), udder cleanliness score (p = 0.004), lower leg cleanliness score (p = 0.022), lactation stage (p = 0.002) and production level (p < 0.001). Interpreting the associations, there were 0.35 times lower odds of *S. aureus* infection post-calving than at dry-off. The odds of *S. aureus* infection were 0.14 times lower when milk production was over 2.5 kg/day than when it was under 2.5 kg/day. There were 0.29 times lower odds of *S. aureus* infection when udders had scant dirtiness versus when they were clean. However, compared to "clean" lower leg cleanliness, scant and moderate dirtiness had 8.7 and 4.5 times higher odds of *S. aureus* infection, respectively. Finally, compared to no bedding, mattresses and sawdust/grass/leaves/crop waste had 0.14 and 0.06 times lower odds of *S. aureus* infection, respectively. The area under the ROC curve for this final model was 0.816, indicating a good overall goodness-of–fit of the model to the observed data (Figure 4.1).

Table 4.91 Univariable mixed logistic regression factors associated (P<0.15) with *Staphylococcus aureus* isolated from 218 milk cultures of 32 cows from 20 smallholder farms in Kiambu County, Kenya, from September 2019 - March 2020

Variable	Category	Coefficient	95% CI	P-value
Bedding type	None	Reference		0.080*
	Mattress	-1.3928	-2.6711 - 0.1144	0.033
	Sawdust/grass/leaves/crop waste	-1.3295	-2.9273 - 0.2683	0.103
Dry cow therapy	No	Reference		
	Yes	-0.8948	-2.0621 - 0.2727	0.105
Teat dip	No	Reference		
	Yes	-1.4297	-2.9627 - 0.1033	0.133
Person in charge of farm management	Husband	Reference		0.105*
	Wife	1.9425	-.02048 - 3.9054	0.052
	Children	1.8022	-0.0443 - 3.6486	0.056
	Employee	-0.6290	-4.1525 - 2.8945	0.726
Breed	Friesian	Reference		0.107*
	Ayrshire	-0.9998	-2.4093 - 0.4098	0.164
	Jersey	-2.1281	-4.5190 - 0.2627	0.081
Treatment	DCT +ITS	Reference		
	ITS	0.5860	-0.0552 - 1.2271	0.073
Time of sampling	Dry-off	Reference		
	Post-calving	-0.8448	-1.5131 - -0.1765	0.013
Teat placement	Centrally placed quarter	Reference		
	Base of teats on extreme inside of quarter	-0.8878	-2.3343 - 0.5587	0.229
Lower leg cleanliness	Clean	Reference		0.017*
	Scant	2.3680	0.7094 - 4.0267	0.005
	Moderate	1.3631	-0.1672 - 2.8933	0.081

Udder cleanliness	Clean	Reference		
	Scant	-0.7569	-2.1479 - 0.6341	0.004
Current milk production	0 is <2.5 kg/day	Reference		
	1 is ≥2.5 kg/day	-1.5455	-2.5905 - -0.5005	0.073
Use of disinfectant in cleaning udder	No	Reference		
	Yes	-1.3169	-2.9392 - 0.3054	0.112

---

* Global p-value for the whole categorical variable.

Table 4.102 Final mixed logistic regression model of factors associated (P<0.15) with *Staphylococcus aureus* isolated from 218 milk cultures of 32 cows from 20 smallholder farms in Kiambu County, Kenya, from September 2019 - March 2020

Variable	Category	Coefficient	Odd ratio	95% CI	P-value
Type of bedding	None	Reference			<0.001*
	Mattress	-1.8662	0.1412	-2.7331 - -0.9993	0.001
	Sawdust/grass/leaves /crop waste	-2.5618	0.0597	-3.7505 - -1.3732	0.001
Lower leg cleanliness	Clean	Reference			0.002*
	Scant	2.1306	8.6949	0.9500 - 3.3111	0.001
	Moderate	1.4470	4.4907	0.30245 - 2.5915	0.013
Udder cleanliness	Clean	Reference			
	Scant	-1.0711	0.2947	-1.9844 - -0.1578	0.022
Time of sampling	Dry-off	Reference			
	Post-calving	-1.0482	0.3454	-1.7239 - -0.3725	0.002
Current milk production	0 is <2.5 kg/day	Reference			
	1 is ≥2.5 kg/day	-1.8501	0.1389	-2.6582 - -1.0420	<0.001

* Global p-value for the whole categorical variable.

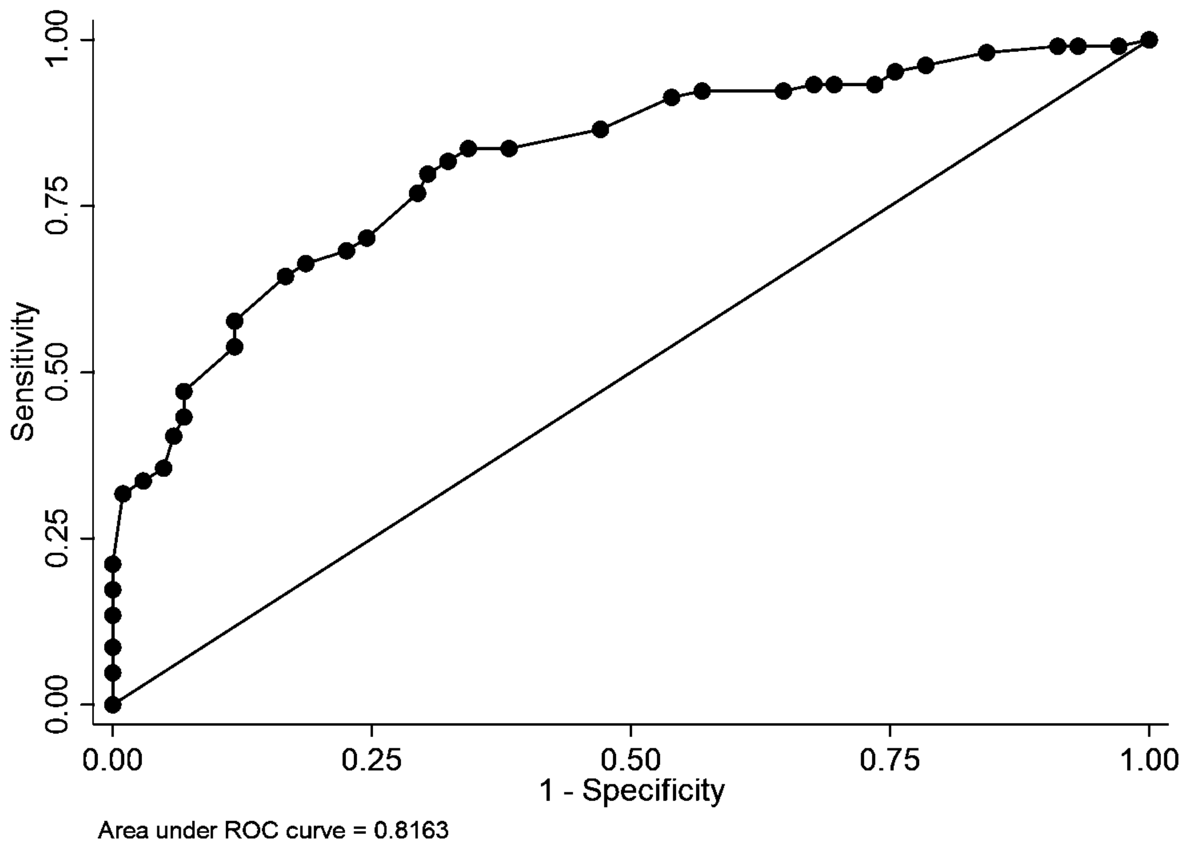


Figure 4.1 Area under receiver operating curve (ROC) showing the goodness-of-fit for the final model of the factors associated with *Staphylococcus aureus* isolated from 218 milk cultures of 32 cows on 20 smallholder farms in Kiambu County, Kenya, from September 2019 - March 2020

CHAPTER FIVE: DISCUSSION

The study revealed that subclinical mastitis due to *S. aureus* at dry-off in smallholder dairy farms in Kiambu County was a substantive problem. Among the CMT-positive cows at dry-off cultured, the most commonly isolated organism at the quarter-level was *Staphylococcus aureus* (57.8%), followed by coagulase-negative *Staphylococcus* (21.1%), *Streptococcus* species (2.8%), *Actinomyces* species (1.8%) and *Escherichia coli* and *Pseudomonas* species each at 0.9%. For comparison purposes, there are few studies reporting bacterial causes of subclinical mastitis at dry-off in a representative sample of smallholder dairy farms in tropical countries. In Thailand, Leelahapongsathon *et al*. (2016) reported coagulase-negative *Staphylococcus* as the major organism in infected cows at dry-off with 41.4%, followed by *Corynebacterium* species at 33.3%, *Streptococcus dysgalactiae* at 5.4%, *Streptococcus bovis*, *Staphylococcus aureus* and *Streptococcus uberis* each at 2.7%, *Streptococcus agalactiae*, *Bacillus species* and *Salmonella species each* at 0.9% and other environmental *Streptococci* at 9.0%. The few *S. aureus* infections reported could have been due wide adoption of DCT among those Thai farmers.

There are subclinical mastitis studies for farms in tropical countries at other times of lactation. Our results were in agreement with Ondiek *et al.,* (2013) who reported quarter-level infection of *S. aureus* at 58.8% in a different District of Kenya. but higher proportions of *Streptococcus* species at 11.8%, mixed infections of *E. coli*, *Streptococcus* and *Staphylococcus* at 20.5% and *E. coli* at 8.9%. In Iraq, *Staphylococcus aureus* was also the most frequently isolated mastitis pathogen at 37.5%, followed by *Streptococcus agalactiae* at 20.6%, *Escherichia coli* at 16.9% and *Corynebacterium pyogenes* at 9.4% (Majeed, 2016). However, this was in contrast to the findings by Dieser *et al.,* (2014) who reported the most commonly isolated organism in Argentina as coagulase-negative *Staphylococci* (52.1%), followed by *Staphylococcus aureus* at

21.3% and *Streptococcus* species at 8.8%. It was also in contrast with findings in Zimbabwe and Tanzania where *coagulase-negative Staphylococcus* was leading at 27.6% and 74%, *Escherichia coli* at 25.2%, *Staphylococcus aureus* at 15.5% and 20.4% and *Streptococcus* species at 1.6% (Mdegela *et al*., 2009: Katsande *et al*., 2013). The high number of *Staphylococcus aureus* could be attributed to lack of teat dipping after milking, lack of DCT practice, and failure to cull chronically infected cows (Abebe *et al*., 2016).

Treatment with dry cow intramammary antibiotics and internal teat sealant showed a significant reduction in the proportion of *S. aureus* isolates from 67.9% of CMT-positive quarters at dry-off to 44.0% at post-calving (35% cure). This result was in agreement with findings by Mutze *et al*. (2012) who reported that there was a reduction of prevalence by 25-75% in the first 100 days of lactation in quarters that received internal teat sealants and a long acting antibiotic. However, our cure rate for *Staphylococcus* aureus was low as compared to the cure rate of 77% reported in a meta-analysis by Halasa *et al.,* (2009a), although this higher cure rate was for all *Staphylococcus* species, not just *S. aureus*. The *S. aureus* organisms develop micro-abscesses around themselves, thus making treatment difficult since antibiotics cannot penetrate the micro-abscesses without ample concentration and duration of exposure, especially if the infection is long-standing leading to thick walls of the micro-abscesses (Petersson-Wolfe, 2010). As a result, lactational and DCT has been reported to have less efficiency against *Staphylococcus aureus* as compared to Streptococcal bacteria for many years (Halasa *et al*., 2009a; Ziv *et al*., 1981).

In this study, the proportions of coagulase-negative *Staphylococcus* reduced from 17.9% to 2.0%, but there was also significant reduction in coagulase-negative *Staphylococcus* in the control group from 24.5% to 4.3%, and these reductions could be attributed to self-cure phenomenon. This self-cure result was in agreement with findings in various studies that received DCT + ITS (Godden *et al*., 2003; Newton et al., 2008; Bradley *et al*., 2011).

The proportions of *Escherichia coli*, *Pseudomonas* species, *Streptococcus* species and *Actinomyces* species increased slightly in the treatment group, however, the proportion of *Escherichia coli* and *Pseudomonas* species also increased equally slightly in the control group. These new infections could be explained by management practices during the dry period or post-calving which might have exposed the quarters to new infections. Dry cow intramammary antibiotics are effective in management of existing intramammary infections and control of new infections in early dry period, but will not prevent new infections towards the end of dry period due to reduction of antibiotics levels below the minimum inhibitory concentration (Bradley and Green, 2000; Berry and Hillerton, 2002b; Halasa *et al*., 2009b). Bradley *et al*., (2010) also reported new intramammary cases of *Escherichia coli* and other *Enterobacteriaceae* in quarters that had been given intramammary antibiotics at dry-off. In a meta-analysis study, it was reported that quarters that received DCT were 0.61 times less likely to develop new intramammary infections of all types of organisms (particularly *Staphylococcus* and *Streptococcus* species, but not *E. coli*) during the dry period as compared to untreated quarters (Halasa *et al*., 2009b).

In the control group, there was a slight drop in the proportion of *Staphylococcus aureus* infections in CMT-positive quarters from 47.2% to 40.4%, *Streptococcus* species from 5.7% to 0, and *Actinomyces* species from 1.8% to 0. These reductions could be attributed to self-cure, particularly the *Streptococcus* species, as Huxley *et al*., (2002) also reported cure rates in quarters that received teat sealants only. Halasa *et al*., (2009a) also reported spontaneous average cure rates of 46% and 52% in two control groups of untreated quarters in a meta-analysis.

Udder cleanliness and level of production at dry-off were negatively associated with occurrence of *S. aureus* mastitis in the smallholder dairy farms. However, poor lower leg cleanliness was positively associated with *S. aureus* mastitis. This positive association is supported by the findings of Abebe *et al.*, (2016) who reported that udder and lower leg hygiene were strongly associated with cases of subclinical mastitis by *Staphylococcus* species. It is unclear why we found lower odds of *S. aureus* infection with clean udders than with scant dirty udders, but we know that *S. aureus* is a contagious infection, and perhaps the clean udders are a result of cleaning with a cloth on more than one cow, or a cloth spreading the infection between an infected teat and uninfected teat on the same cow.

In Ethiopia, Abebe *et al.*, (2016) noted that the high prevalence of *S. aureus* in dairy farms were associated with failure to cull chronic carriers, lack of post-milking teat dipping and dry cow therapy, and use of the hand-milking method where, in most cases, milkers wash hands before milking the first cow only and do not rewash hands between cows. Although pre-milking and post-milking teat dipping was initially meant to control environmental pathogens, it has been also shown to reduce incidences of *S. aureus* infections (Dufour *et al*., 2012). Lack of use of gloves during milking have also been shown to increase the incidences of *S. aureus* infections and its use greatly increases the odds of its elimination (Dufour *et al*., 2012). Testing for mastitis pathogens in newly acquired cows before joining the herd is also critical in the control of mastitis caused by *S. aureus* and other contagious mastitis infections, and it has been shown that open herds that do not practice this precaution have higher cases of this organism (Costa *et al*., 2016).

The current study negatively linked the level of production at dry-off with occurrence of subclinical *S. aureus* mastitis, and this was in agreement with findings in Thailand by Leelahapongsathon *et al.*, (2016), although that study used all types of subclinical mastitis as the outcome variable, not just *S. aureus*, because *S. aureus* infections were uncommon. Conversely, Islam *et al.*, (2011) reported that cows in Bangladesh that were producing ≤2 litres per day had prevalence of <20.75% while those producing over 2 litres had ≥30.95% prevalence. However, this Bangladeshi result is likely because the prevalence of mastitis is highly dependent on stage of lactation, with early lactation, when milk production is high, being a common time for new cases of mastitis.

The study had some limitations that included lack of antimicrobial sensitivity results, which was because Covid-19 pandemic that led to the shut down the bacteriology lab during the planned time for sensitivity analyses. Furthermore, it would have also been very helpful to carry out PCR on the isolated organisms in order to determine if the strains at dry-off were the same as those at post-calving, differentiating unsuccessful treatment from new infections. Future research into the prevalence of subclinical mastitis at dry-off and the efficacy of DCT to treat mastitis at dry-off should ensure antimicrobial sensitivity and molecular analysis of the isolates in order to understand better the sensitivity profiles and development of new infections during the dry period. The small number of herds and cows in this study could have led to poor representativeness of the descriptive results, and lower power to detect significant associations to *S. aureus* infections at dry-off as compared to a large population.

CHAPTER SIX: CONCLUSION AND RECOMMENDATION

6.1: Conclusion

1. Subclinical *S. aureus* mastitis is among the most common type of infection at dry-off (83.5% of CMT-positive quarters and 93.8% of CMT-positive cows were infected with *S. aureus*) in smallholder dairy farms in Kiambu County, Kenya.
2. Dry cow therapy significantly reduced the proportion of quarters infected with *S. aureus* from 67.9% at dry-off to 44.0% post-calving (35% reduction), but did not significantly reduce other infections, although proportions of other isolates were low.
3. Among cows with CMT-positive quarters at dry-off, the odds of *S. aureus* infection were lower with milk production over 2.5 kg/day, at post-calving rather than at dry-off, and when udders had scant dirtiness versus when they were clean. However, compared to "clean" lower legs, scant and moderate lower leg dirtiness had higher odds of *S. aureus* infection. Finally, compared to no bedding, mattresses and sawdust/grass/leaves/crop waste had lower odds of *S. aureus* infection.

6.2: Recommendation

1. A large study should be carried out in other tropical counties to establish and compare the types and occurrences of subclinical mastitis at dry-off in other smallholder dairy farming contexts.

2. Use of dry cow therapy should be adopted my smallholder dairy farms in order to address the challenges caused by subclinical mastitis, particularly *S. aureus* infections.

3. The identified risk factors, along with other important mastitis prevention practices such as proper teat dipping and separate towels for each cow, should be included in management change recommendations to reduce the occurrence and impacts of *S. aureus* mastitis.

REFERENCES

Abebe, R., Hatiya, H., Abera, M., Megersa, B., & Asmare, K., (2016). Bovine mastitis: prevalence, risk factors and isolation of *Staphylococcus aureus* in dairy herds at Hawassa milk shed, South Ethiopia. *BMC Veterinary Research*, ***12***(1), 270.

Aghamohammadi, M., Haine, D., Kelton, D. F., Barkema, H. W., Hogeveen, H., Keefe, G. P., & Dufour, S., (2018). Herd-level mastitis-associated costs on Canadian dairy farms. *Frontiers in Veterinary Science*, ***5***, 100.

Ayano A A., Hiriko F, Simyalew A M, and Yohannes A., (2013). Prevalence of subclinical mastitis in lactating cows in selected commercial dairy farms of Holeta District. *Journal of Veterinary Medicine and Animal Health*, **5** (3), 67-72.

Berry, E. A., & Hillerton, J. E., (2002). The effect of selective dry cow treatment on new intramammary infections. *Journal of Dairy Science*, ***85***(1), 112-121.

Bhutto, A. L., Murray, R. D., & Woldehiwet, Z., (2012). California mastitis test scores as indicators of subclinical intra-mammary infections at the end of lactation in dairy cows. *Research in Veterinary Science*, ***92***(1), 13-17.

Bradley, A. J., & Green, M. J., (2000). A study of the incidence and significance of intramammary enterobacterial infections acquired during the dry period. *Journal of Dairy Science*, ***83***(9), 1957-1965.

Bradley, A. J., Breen, J. E., Payne, B., & Green, M. J., (2011). A comparison of broad-spectrum and narrow-spectrum dry cow therapy used alone and in combination with a teat sealant. *Journal of Dairy Science*, ***94***(2), 692-704.

Bradley, A. J., Breen, J. E., Payne, B., Williams, P., & Green, M. J. (2010). The use of a cephalonium containing dry cow therapy and an internal teat sealant, both alone and in combination. *Journal of Dairy Science*, ***93***(4), 1566-1577.

Bradley, A. J., De Vliegher, S., Green, M. J., Larrosa, P., Payne, B., van de Leemput, E. S., & White, V., (2015). An investigation of the dynamics of intramammary infections acquired during the dry period on European dairy farms. *Journal of Dairy Science*, ***98***(9), 6029-6047.

Cameron, M., Keefe, G. P., Roy, J. P., Stryhn, H., Dohoo, I. R., & McKenna, S. L., (2015). Evaluation of selective dry cow treatment following on-farm culture: Milk yield and somatic cell count in the subsequent lactation. *Journal of Dairy Science*, ***98***(4), 2427-2436.

Church, G. T., Fox, L. K., Gaskins, C. T., Hancock, D. D., & Gay, J. M., (2008). The effect of a shortened dry period on intramammary infections during the subsequent lactation. *Journal of Dairy Science*, ***91***(11), 4219-4225.

Crispie, F., Flynn, J., Ross, R. P., Hill, C., & Meaney, W. J., (2004). Dry cow therapy with a non-antibiotic intramammary teat seal-a review. *Irish Veterinary Journal*, ***57***(**7**), 412.

Da Costa, L. B., Rajala-Schultz, P. J., & Schuenemann, G. M. (2016). Management practices associated with presence of Staphylococcus aureus in bulk tank milk from Ohio dairy herds. *Journal of Dairy Science*, ***99***(2), 1364-1373.

Deb, R., Kumar, A., Chakraborty, S., Verma, A. K., Tiwari, R., Dhama, K., & Kumar, S., (2013). Trends in diagnosis and control of bovine mastitis: a review. *Pakistan Journal of Biological Sciences*, ***16***(23), 1653-61.

Dieser, S.A., Vissio, C., Lasagno, M.C., Bogni, C.I., Larriestra, A.J & Odierno, L.M., (2014). Prevalence of pathogens causing subclinical mastitis in Argentinean dairy herds. *Pakistan Veterinary Journal*, **34**(1): 124-126.

Duarte, C. M., Freitas, P. P., & Bexiga, R., (2015). Technological advances in bovine mastitis diagnosis: an overview. *Journal of Veterinary Diagnostic Investigation*, ***27***(6), 665-672.

Dufour, S., I. R. Dohoo, H. W. Barkema, L. Descoteaux, T. J. Devries,K. K. Reyher, J. P. Roy, and D. T. Scholl., (2012). Manageable risk factors associated with the lactational incidence, elimination, and prevalence of *Staphylococcus aureus* intramammary infections in dairy cows. *Journal of. Dairy Science*. **95**:1283–1300.

Eldesouky, I., Mohamed, N., Khalaf, D., Salama, A., Elsify, A., Ombarak, R., & Alshabrawy, M., (2016). Candida mastitis in dairy cattle with molecular detection of Candida albicans. *Kafkas Üniversitesi Veteriner FakÜltesi Dergisi*, ***22***(3), 461-4.

Ganguly, S., Shoukat, S., Wani, H., Ali, U. and Ali, M., (2018). Chapter-6 Mastitis and Its Diagnosis: A Review.

Gao, J., Barkema, H. W., Zhang, L., Liu, G., Deng, Z., Cai, L., & Han, B., (2017). Incidence of clinical mastitis and distribution of pathogens on large Chinese dairy farms. *Journal of Dairy Science*, ***100***(6), 4797-4806.

Gitau, G. K., Bundi, R. M., Vanleeuwen, J., & Mulei, C. M., (2014). Mastitogenic bacteria isolated from dairy cows in Kenya and their antimicrobial sensitivity. *Journal of the South African Veterinary Association*, ***85***(1), 01-08.

Godden, S., Rapnicki, P., Stewart, S., Fetrow, J., Johnson, A., Bey, R., & Farnsworth, R. (2003). Effectiveness of an internal teat seal in the prevention of new intramammary infections during the dry and early-lactation periods in dairy cows when used with a dry cow intramammary antibiotic. *Journal of Dairy Science*, ***86***(12), 3899-3911.

Golder, H. M., Hodge, A., & Lean, I. J., (2016). Effects of antibiotic dry-cow therapy and internal teat sealant on milk somatic cell counts and clinical and subclinical mastitis in early lactation. *Journal of Dairy Science*, ***99***(9), 7370-7380.

Gomes, F., Saavedra, M. J., & Henriques, M., (2016). Bovine mastitis disease/pathogenicity: evidence of the potential role of microbial biofilms. *Pathogens and Disease*, ***74***(3).

Green, M. J., Green, L. E., Medley, G. F., Schukken, Y. H., & Bradley, A. J. (2002). Influence of dry period bacterial intramammary infection on clinical mastitis in dairy cows. *Journal of dairy science*, ***85***(10), 2589-2599.

Green, M., Huxley, J., & Bradley, A., (2002). A rational approach to dry cow therapy: 1. Udder health priorities during the dry period. *In Practice*, ***24***(10), 582-587.

Gurjar, A., Gioia, G., Schukken, Y., Welcome, F., Zadoks, R., & Moroni, P. (2012). Molecular diagnostics applied to mastitis problems on dairy farms. *Veterinary Clinics of North America: Food Animal Practice*, ***28***(3), 565-576.

Halasa, T., Nielen, M., Whist, A. C., & Østerås, O., (2009). Meta-analysis of dry cow management for dairy cattle. Part 2. Cure of existing intramammary infections. *Journal of Dairy Science*, ***92***(7), 3150-3157.

Halasa, T., Østerås, O., Hogeveen, H., van Werven, T., & Nielen, M., (2009a). Meta-analysis of dry cow management for dairy cattle. Part 1. Protection against new intramammary infections. *Journal of Dairy Science*, ***92***(7), 3134-3149.

Hillerton and Booth (2018). The Five-Point Mastitis Control Plan -A Revisory Tutorial. *In National Mastitis Control Annual Meeting Proceedings*.

Hossain, M. K., Paul, S., Hossain, M. M., Islam, M. R., & Alam, M. G. S., (2017). Bovine mastitis and its therapeutic strategy doing antibiotic sensitivity test. *Austin Journal of Veterinary Science Animal Husbandry*, ***4***(1), 1030.

Huxley, J. N., Green, M. J., Green, L. E., & Bradley, A. J. (2002). Evaluation of the efficacy of an internal teat sealer during the dry period. *Journal of Dairy Science*, ***85***(3), 551-561.

Ilonka van der Wagt (2017). Cost-effectiveness of selective dry cow therapy. Retrieved on 14 October 2019 from https://edepot.wur.nl/424566

Iraguha, B., Hamudikuwanda, H., & Mushonga, B., (2015). Bovine mastitis prevalence and associated risk factors in dairy cows in Nyagatare District, Rwanda. *Journal of the South African Veterinary Association*, ***86***(1), 1-6.

Islam, M. A., Islam, M. Z., Rahman, M. S., & Islam, M. T., (2011). Prevalence of subclinical mastitis in dairy cows in selected areas of Bangladesh. *Bangladesh Journal of Veterinary Medicine*, ***9***(1), 73-78.

Ismail, Z. B., (2017). Mastitis vaccines in dairy cows: Recent developments and recommendations of application. *Veterinary World*, ***10***(9), 1057.

Ismail, Z. B., Muhaffel, M. M., & Abu-Basha, E., (2018). The effect of dry cow therapy using systemic tylosin in combination with common intramammary medications on mastitis rate, cull rate, somatic cell count, and milk production in dairy cows affected with subclinical mastitis. *Veterinary World*, ***11***(9), 1266

Jones, G. M. (2009). Proper dry cow management critical for mastitis control. Retrieved on 14 October 2019 from https://www.pubs.ext.vt.edu/404/404-212/404-212.html

Kadlec, K., Entorf, M., & Peters, T., (2019). Occurrence and Characteristics of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* in Quarter Milk Samples from Dairy Cows in Germany. *Frontiers in Microbiology*, ***10***, 1295. doi:10.3389/fmicb.2019.01295

Kandeel, S. A., Morin, D. E., Calloway, C. D., & Constable, P. D., (2018). Association of California Mastitis Test Scores with intramammary infection status in lactating dairy cows admitted to a veterinary teaching hospital. *Journal of Veterinary Internal Medicine*, ***32***(1), 497-505.

Kashifa, M., Rizwanb, M., Alib, M., Ahmadc, T., & Durranid, A. Z., (2016). Control of Mastitis through dry cow therapy: A review. *Veterinaria*, ***4***(2), 13-16.

Katsande, S., Matope, G., Ndengu, M., and Pfukenyi, D M., (2013). Prevalence of mastitis in dairy cows from smallholder farms in Zimbabwe. *Onderstepoort Journal of Veterinary Research*, **80** (1), 00-00.

Kibebew, K. (2017). Bovine mastitis: a review of causes and epidemiological point of view. *Journal of Biology, Agriculture and Healthcare*, ***7***(2), 2017.

Klaas, I. C., & Zadoks, R. N. (2018). An update on environmental mastitis: Challenging perceptions. *Transboundary and emerging diseases*, ***65***, 166-185.

Krömker, V., & Leimbach, S. (2017). Mastitis treatment—reduction in antibiotic usage in dairy cows. *Reproduction in Domestic Animals*, ***52***, 21-29.

Lakew, B. T., Fayera, T., & Ali, Y. M., (2019). Risk factors for bovine mastitis with the isolation and identification of Streptococcus agalactiae from farms in and around Haramaya district, eastern Ethiopia. *Tropical Animal Health and Production*, 1-7.

Lakshmi, R. (2016). Bovine mastitis and its diagnosis. *International Journal of Applied Research* 2016; **2**(6): 213-216

Leelahapongsathon, K., Piroon, T., Chaisri, W., & Suriyasathaporn, W., (2016). Factors in dry period associated with intramammary infection and subsequent clinical mastitis in early postpartum cows. *Asian-Australasian Journal of Animal Sciences*, ***29***(4), 580.

Leelahapongsathon, K., Schukken, Y. H., & Suriyasathaporn, W., (2014). Quarter, cow, and farm risk factors for intramammary infections with major pathogens relative to minor pathogens in Thai dairy cows. *Tropical Animal Health and Production*, ***46***(6), 1067-1078.

Lundberg, A. (2015). Genotypes, Spread, and Infection Outcome of Three Important Udder Pathogens. Retrieved on 14 October 2019 from https://www.semanticscholar.org/paper/Mastitis-in-Dairy-Cows-Genotypes%2C-Spread%2C-and-of-Lundberg/7f271d1f9ce0a174ea49cb345e8a9e1eaf45d55d

Lyhs, U., Kulkas, L., Katholm, J., Waller, K. P., Saha, K., Tomusk, R. J., & Zadoks, R. N. (2016). Streptococcus agalactiae Serotype IV in Humans and Cattle, Northern Europe[1]. *Emerging infectious diseases*, ***22***(12), 2097–2103. doi:10.3201/eid2212.151447

Majeed, H. M. (2016). Study on Prevalence of Bovine Mastitis and Its Major Causative Agents in Salahadin City, Iraq. *Journal of Tikrit University For Agriculture Sciences*, ***16***(2), 7-14.

Mandrekar, J. N. (2010). Receiver operating characteristic curve in diagnostic test assessment. *Journal of Thoracic Oncology*, ***5***(9), 1315-1316.

Martins, S. A. M., Martins, V. C., Cardoso, F. A., Germano, J. H., Rodrigues, M. C., Duarte, C. M., & de Freitas, P. P., (2019). Biosensors for on-farm diagnosis of mastitis. *Frontiers in Bioengineering and Biotechnology*, ***7***, 186.

Mdegela, R. H., Ryoba, R., Karimuribo, E. D., Phiri, E. J., Loken, T., Reksen, O. and Urio, N. A., (2009). Prevalence of clinical and subclinical mastitis and quality of milk on smallholder dairy farms in Tanzania. *Journal of the South African Veterinary Association*, **80** (3), 163-168.

Mureithi, D.K. and Njuguna M, N., (2016). Prevalence of subclinical mastitis and associated risk factors in dairy farms in urban and peri-urban areas of Thika sub-county, Kenya. *Livestock Research for Rural Development* **28** (2) 2016

Mütze, K., Wolter, W., Failing, K., Kloppert, B., Bernhardt, H., & Zschöck, M., (2012). The effect of dry cow antibiotic with and without an internal teat sealant on udder health during the first 100 d of lactation: a field study with matched pairs. *Journal of Dairy Research*, ***79***(4), 477-484.

Neelam, Jain, V.K., Bhagwan, J. and Kumar, P., (2017). A review on mastitis control through dry cow therapy, *International. Journal of. Pure and Applied. Bioscience.* **5**(5): 1427-1432

Newton. H. T., Green, M. J., Benchaoui, H., Cracknell, V., Rowan, T & Bradley A.J., (2008). Comparison of the efficacy of cloxacillin alone and cloxacillin combined with an internal teat sealant for dry-cow therapy. *Veterinary Record* 162 678–684

Norberg, E., Hogeveen, H., Korsgaard, I. R., Friggens, N. C., Sloth, K. H. M. N., & Løvendahl, P. (2004). Electrical conductivity of milk: ability to predict mastitis status. *Journal of Dairy Science*, ***87***(4), 1099-1107.

Oliveira, C. S. F., Hogeveen, H., Botelho, A. M., Maia, P. V., Coelho, S. G., & Haddad, J. P. A., (2015). Cow-specific risk factors for clinical mastitis in Brazilian dairy cattle. *Preventive Veterinary Medicine*, ***121***(3-4), 297-305.

Oliveira, L., & Ruegg, P. L., (2014). Treatments of clinical mastitis occurring in cows on 51 large dairy herds in Wisconsin. *Journal of Dairy Science*, *97*(**9**), 5426-5436.

Ondiek, J. O., Ogore, P. B., Shakala, E. K., & Kaburu, G. M., (2013). Prevalence of bovine mastitis, its therapeutics and control in Tatton Agriculture Park, Egerton University, Njoro District of Kenya. *Basic Research Journal of Agricultural Science and Review*, ***2***(1), 15-20.

Pantoja, J. C., C. Hulland, & P. L. Ruegg., (2009). Somatic cell count status across the dry period as a risk factor for the development of clinical mastitis in the subsequent lactation. *Journal of Dairy Science*. **92**:139-148.

Parker, K. I., Compton, C. W. R., Anniss, F. M., Weir, A. M., & McDougall, S. (2007). Management of dairy heifers and its relationships with the incidence of clinical mastitis. *New Zealand veterinary journal*, ***55***(5), 208-216.

Petersson-Wolfe, C. S., Mullarky, I. K., & Jones, G. M., (2010). Staphylococcus aureus mastitis: cause, detection, and control. Retrieved on 14, October 2019 from https://www.pubs.ext.vt.edu/content/dam/pubs_ext_vt_edu/404/404-229/404-229_pdf.pdf

Rabiee, A. R. & Lean, I.J., (2013). The effect of internal teat sealant products (Teat seal and Orbeseal) on intramammary infection, clinical mastitis, and somatic cell counts in lactating dairy cows: A meta-analysis. *Journal of. Dairy Science*. **96**:6915–6931 http://dx.doi.org/ 10.3168/jds.2013-6544

Rathod, P., Shivamurty, V. & Anant, A. R., (2017). Economic Losses due to Subclinical Mastitis in Dairy Animals: A Study in Bidar District of Karnataka. *The Indian Journal of Veterinary Sciences & Biotechnology*. ***13(1):***37-41.

Reshi, A. A., Husain, I., Bhat, S. A., Rehman, M. U., Razak, R., Bilal, S., & Mir, M. R., (2015). Bovine mastitis as an evolving disease and its impact on the dairy industry. *International Journal of Current. Research and Review*,***7*** (5), 48-55.

Richards, S., Vanleeuwen ,J., Peter, S G., Wichtel, J., Kamunde, C., Uehlinger, F & Gitau G., (2019): Impact of mineral feeding on reproductive efficiency on smallholder dairy farms in Kenya. *Livestock Research for Rural Development.* ***31****, (80)*. Retrieved August 17, 2019, from http://www.lrrd.org/lrrd31/6/srich31080.html

Riekerink, R. O., Barkema, H. W., Kelton, D. F., & Scholl, D. T. (2008). Incidence rate of clinical mastitis on Canadian dairy farms. *Journal of Dairy Science*, ***91***(4), 1366-1377.

Romero, J., Benavides. E & Meza C., (2018). Assessing Financial Impacts of Subclinical Mastitis on Colombian Dairy Farms. *Frontiers in Veterinary. Science.* ***5****:273.doi: 10.3389/fvets.2018.00273*

Scherpenzeel, C. G. M., Hogeveen, H., Maas, L., & Lam, T. J. G. M., (2018). Economic optimization of selective dry cow treatment. *Journal of Dairy Science*, ***101***(2), 1530-1539.

Shaheen, M., Tantary, H. A., & Nabi, S. U., (2016). A treatise on bovine mastitis: disease and disease economics, etiological basis, risk factors, impact on human health, therapeutic management, prevention and control strategy. *Advances in Dairy Research*, 1-10.

Sharma, N., Singh, N. K., & Bhadwal, M. S., (2011). Relationship of somatic cell count and mastitis: An overview. *Asian-Australasian Journal of Animal Sciences,* ***24*** (3), 429-438.

Sharma, N., Singh, N. K., Singh, O. P., Pandey, V., & Verma, P. K., (2011). Oxidative stress and antioxidant status during transition period in dairy cows. *Asian-Australasian Journal of Animal Sciences*, ***24***(4), 479-484.

Sztachańska, M., Barański, W., Janowski, T., Pogorzelska, J., & Zduńczyk, S., (2016). Prevalence and etiological agents of subclinical mastitis at the end of lactation in nine dairy herds in North-East Poland. *Polish Journal of Veterinary Sciences*, ***19*** (1), 119-124.

The Merck veterinary manual.(2016). 11th edition Whitehouse Station, NJ :Merck & Co., Inc.

Tremblay, Y. D., Lamarche, D., Chever, P., Haine, D., Messier, S., & Jacques, M., (2013). Characterization of the ability of coagulase-negative staphylococci isolated from the milk of Canadian farms to form biofilms. *Journal of Dairy Science*, ***96*** (1), 234-246.

Unnerstad, H. E., Lindberg, A., Waller, K. P., Ekman, T., Artursson, K., Nilsson-Öst, M., & Bengtsson, B., (2009). Microbial aetiology of acute clinical mastitis and agent-specific risk factors. *Veterinary Microbiology*, ***137***(1-2), 90-97.

Vanhoudt, A., van Hees-Huijps, K., van Knegsel, A. T. M., Sampimon, O. C., Vernooij, J. C. M., Nielen, M., & van Werven, T. (2018). Effects of reduced intramammary antimicrobial use during the dry period on udder health in Dutch dairy herds. *Journal of dairy science*, ***101***(4), 3248-3260.

Waldner, D. N., (2007). Dry cow therapy for mastitis control. Division of Agricultural Sciences and Natural Resources, Oklahoma State University. Retrieved on 14, October 2019 from http://www.dairyweb.ca/Resources/USWebDocs/DryCowTreatment.pdf

Wanjala, N. W., Gitau, G. K., Muchemi, G. M., & Makau, D. N., (2020). Effect of Bismuth Subnitrate Teat Canal Sealant with Ampicillin-Cloxacillin Combination in Control of Bovine Mastitis in Selected Farms in Kenya. *International Journal of Veterinary Science, **9**(3), 331-336*

Zdanowicz, M., Shelford, J. A., Tucker, C. B., Weary, D. M., & Von Keyserlingk, M. A. G., (2004). Bacterial populations on teat ends of dairy cows housed in free stalls and bedded with either sand or sawdust. *Journal of Dairy Science,* ***87***(6), 1694-1701.

Ziv, G., Storper, M., & Saran, A., (1981). Comparative efficacy of three antibiotic products for the treatment and prevention of sub-clinical mastitis during the dry period. *Veterinary Quarterly*, ***3***(2), 74-79.

APPENDIX

Appendix 1: Mastitis Study Questionnaires - Kiambu Dry Cow Therapy Study - August - November 2019

Questionnaire number_________ Date______________

Farmer name______________________________ Tel. No__________________

Location____________

1. Age bracket of the farmer___________
2. Gender of the principal farmer_______________
3. Level of education of the farmer: completed

☒Primary ☐ SecondaryTertiary/College

4. Number of adults staying (>1week) in the homestead_________
5. Marital status of the principal farmer____________
6. Who manages the operations of the farm?

☐ Wife ☐ Husband ☐ Children ☐ Relative ☐ Employee

7. For how long have you been keeping dairy cattle? _______
8. Total area of land used for dairy farming (acres)________
9. Total land area rented for dairy farming__________
10. Number of cows in the farm

Dry cows_ Milking cows____Heifers _____Calves _____ Adult bulls_____

11. Average daily milk production from the farm_______

12. Percent of total income coming from milk production (1-100%) ______

13. Which dairy cooperative do you belong? ___________

14. What are the common cow diseases in the farm?

   a. ____________

   b. _____________

   c. _____________

   d. _____________

15. Number of cubicles for cows (observe/ask*)*_____

16. Roof status over the cow cubicle (observe/ask)

☐Intact ☐Leaking holes present ☐Incomplete ☐No roof

17. Type of floor on cow cubicle (observe/ask)

☐Concrete ☐Dirt ☐Wooden ☐Other (please specify ________________)

18. Type of beddings used on the cow cubicle (observe and ask)

☐Grass/Leaves☐Sawdust/wood shavings☐Crop waste

☐Other (please specify ________________)

19. Frequency of adding new beddings on cow cubicle: Once every _____{days}

20. Level of manure accumulation in udder area of the cow cubicle (observe)

☐Clean☐Scant☐Moderate☐Excessive

21. Do sometime cows lie backwards in the cubicle? a. Yes ☐b. No ☐

22. Frequency of cleaning the cow cubicle: Once every _____{days}

23. Nature of the floor on the walk alley (observe)

☐Concrete☐Stones☐Dirt☐Wooden

24. Do cows sometime lie in the walk alley? a. Yes ☐b. No ☐

25. Frequency of cleaning the walk alley: Once every _____{days}

26. How do you milk your cows

☐Hand ☐Machine ☐Both

27. If hand, how do you do it

☐Pull ☐Squeeze ☐Both

28. How are cows currently fed?

29. ☐Zero grazing ☐Open grazing ☐Both Do you clean the udder pre- milking

☐Yes ☐No

30. If yes, what temperature of water do you use

☐Warm ☐Cold

31. If yes, do you add any disinfectant into the water?

☐Yes ☐No

32. Is the udder dried before milking?

☐Yes ☐No

33. If yes, what material do you use to dry the udder

☐Cloth ☐Paper

34. If cloth, is a different cloth used for each milking cow

☐Yes ☐No

35. Do you wash your hands before milking?

☐Yes ☐No

36. If you have >1 milking cow, do you wash your hands between milking cows?

☐Yes ☐No

37. Do you wash out your wipe cloth between milkings?

☐Yes ☐No

38. Do you hang the wash cloth up to dry out between milkings?

☐Yes ☐No

39. Do you use a teat dip (i.e. disinfectant) on all milking cows after each milking?

☐Yes ☐No

40. Do you apply milking Jelly prior to milking? ☐Yes ☐No

41. Is the cow fed fresh feed after milking to keep her standing for 1 hour______

42. Number of milkings per cow per day______

43. Name the constraints to better profitability on the farm *(score them 1-5)*

Udder infections☐ Hardware disease☐ Tick-borne☐ Infectious diseases☐

Nutrition☐ Marketing☐ Availability of good breeding sires/quality semen

44. Have you had case of mastitis in the farm in the last 12 months?

☐Yes ☐No

45. If yes, how was it managed? _______________________

46. Who treats your animals for mastitis?

☐Vet ☐AHA ☐Self ☐Neighbor/friend

47. What common signs of mastitis have you seen____________________________________

48. If your cow(s) had mastitis, how was the response for mastitis after management?

☐Decreased milk production ☐Constant milk production ☐Increased milk production

49. For how long did the infection(s) take to resolve (in general)

☐One week ☐Above one week ☐Never resolved

50. How do you dry your cows?

a. Abrupt (i.e. milk twice a day and then not at all)

b. Gradual (i.e. milk once a day for a period of time)

c. Other

51. Do you practice dry cow therapy?

☐Yes ☐No

52. If yes, is it applied to all drying cows irrespective of presence of mastitis or no?

☐Yes ☐No

53. Do you use teat sealants in drying cows?

☐Yes ☐No

54. If yes, are they used together with dry cow antibiotic therapy

☐Yes ☐No

DRY COW INFORMATION

ANIMAL LEVEL INFORMATION

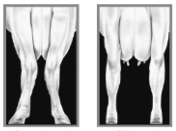
1. What is the estimated age of the cow______ years
2. What is the parity of the cow_____
3. *What is the breed of the cow__________ (observe/ask)*
4. *Body condition score_____ (observe)*
5. What is the current level of milk production per cow per day_____
6. What is the last calving date or month of the milking cow___________
7. Has the cow had mastitis since the last calving? Yes ____ No______
8. If yes, how many episodes of mastitis occurred? ______
9. If yes, which quarters?____________________
10. If yes, when during the current lactation did the mastitis occur (months)? ______________
11. If yes, when was the last treatment given (need date or month)? ______________
12. What is the cleanliness score of the milking cow (do the cleanliness score)
    a. Lower leg____
    b. Upper leg & Flank____
    c. Udder____
13. Lameness score______

14. Hind limb conformation

Hock conformation (side view)

Extremely sickled Intermediate set in hock Posty and straight

Hock conformation (Rear view)



Severe toe-out No toe-out

Foot angle

Extreme low angle Intermediate angle Extremely steep angle

QUARTER LEVEL INFORMATION

Udder:

1. Udder depth (0,1,2)

Very deep udder floor well below hocks Udder floor above hocks Extreme height of udder floor above hocks

2. Udder cleft (0,1,2)

Weak cleft Intermediate Extremely strong cleft

3. Udder height (low, intermediate, high)___________

4. Udder width (observed from rear- narrow, intermediate, wide) ___________

5. Fore udder attachment (loose, intermediate, strong)______________

6. Udder balance (deep rear, same level, deep front)_______

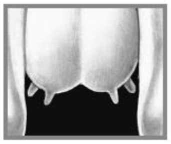
Teat:

7. Does milk leak from the teat (ask and observe) Yes☐ No☐

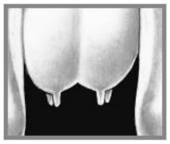
8. Teat end score (N, S, R, VR ring)_____________________

9. Teat length (cm)

10. Teat placement



Extremely wide placement
on outside of quarter



Centrally placed
on quarter

Base of teats on
extreme inside of quarter