EFFECT OF BISMUTH SUBNITRATE TEAT CANAL SEALANT WITH AMPICILLIN-CLOXACILLIN COMBINATION IN CONTROL OF BOVINE MASTITIS IN SELECTED FARMS IN KENYA

NASHON WAVUMBAH WANJALA (BVM, University of Nairobi)

A thesis submitted in partial fulfilment of the requirements for Master of Science in Veterinary Epidemiology and Economics of the University of Nairobi

DEPARTMENT OF PUBLIC HEALTH, PHARMACOLOGY AND TOXICOLOGY

FACULTY OF VETERINARY MEDICINE

UNIVERSITY OF NAIROBI

Declaration

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

Signature: ____________________________ Date: __________________

NASHON WAVUMBAH WANJALA (BVM)

Reg No.: J56/88610/2016

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

Dr Gerald M. Muchemi (BVM, MSc, PhD)

Department of Public Health, Pharmacology and Toxicology, University of Nairobi.

Signature: ____________________________ Date: __________________

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

Department of Clinical Studies, University of Nairobi.

Signature: ____________________________ Date: __________________

Dedication

I dedicate this work to my beloved siblings Nick, Noel and Phoebe for their inspiration in my entire life. This work is also dedicated to my dad Wanjala Mark and mum Irene Rebecca.

ACKNOWLEDGEMENT

I acknowledge the University of Nairobi through the Office of the Dean, Faculty of Veterinary Medicine for awarding me a scholarship and facilities that made my work easier and smoother. I can't thank my supervisors enough because without them this work would not have run to the result level. I acknowledge Prof. Gitau George and the Bimeda® Animal Health Company for having funded the entire project. Dr Muchemi Gerald's thoroughness and strictness made my work smarter. I appreciate both of them for being there for me, even when I felt things were becoming tough. Their encouragement embedded in their experiential skills made me whole as a student and a person to work for the society. In the same spirit, Dr Dennis Makau gave me strong insight and I appreciate his dedication.

I appreciate the expertise I was accorded by the staff of the Department of Public Health, Pharmacology and Toxicology Laboratory led by the principal technologist Mr Nduhiu, Mr Bett, Mr Rono and Miss Pacho. Their skills and experience were invaluable. They made my lab life a wonderful working and learning moment.

I also acknowledge the level of professionalism offered to me by members of the library at the College of Agriculture and Veterinary Sciences. Specifically I appreciate Miss Zilpha for her guidance while writing both my proposal and thesis.

I wish to acknowledge the prayers, financial support and advice of my dad, siblings and classmates.

Last but not least I acknowledge the Lord Almighty for His providence, guidance and sustenance throughout my academic life. Without Jehovah's hand all this would not have been accomplished.

Table of Contents

Declaration ii
Dedication iii
ACKNOWLEDGEMENT iv
LIST OF TABLES viii
LIST OF FIGURES x
LIST OF PLATES xi
ABSTRACT xii
CHAPTER 1: INTRODUCTION 1
1.1 Background to the study 1
1.2 Control measures for bovine mastitis 2
1.2 Problem statement 3
1.3 Justification 3
1.4 Objectives 4
1.4.1 General objective 4
1.4.2 Specific objectives 4
1.5 Hypothesis 4
CHAPTER 2: LITERATURE REVIEW 5
2.1 Aetiology of mastitis 5
2.2 Classification of mastitis 5
2.3 Epidemiology of bovine mastitis 6
2.4 Clinical signs of mastitis 7
2.5 Diagnosis of mastitis 7
2.5.1 Physical examination 7
2.5.2 California Mastitis Test (CMT) 8
2.5.3 Somatic cell count 9
2.5.4 Polymerase chain reaction (PCR) 9
2.5.5 Bacterial culture 10
2.5.6 White slide test 10
2.6 Control of mastitis 10
2.6.1 Management 10

2.6.2 Antibiotic containing dry cow therapy 11
2.6.3 Culling of mastitic cows 11
2.6.4 Internal teat sealants during dry period 11
CHAPTER 3: MATERIALS AND METHODS 13
3.1 Study area 13
3.2 Study design 14
3.2.1 Sample size determination and allocation into study groups 14
3.3 Criteria for cow selection into the study 15
3.4 Udder and hind leg hygiene scoring 16
3.5 Administration of reference and test products 18
3.6 Animal follow-up 18
3. 7 Milk sample collection, transportation 19
3.8 Culture and identification of bacteria 19
3.9 Data management and analysis 20
4.0 RESULTS 21
4.1 Herd demographics and profiles 21
4.2 Prevalence of mastitis 22
4.3 Mastitis cases in the treatment and control groups in Chemusian and Gicheha Farms in 2017/2018 23
4.3.1 Overall mastitis 23
4.3.2 Clinical mastitis 25
4.3.3 Subclinical mastitis 27
4.4 Quarter level prevalence of mastitis 28
4.4.1 Chemusian farm 28
4.4.2 Gicheha farm 30
4.5 Factors influencing occurrence of bovine mastitis 32
4.5 Bacterial pathogens causing mastitis 34
5.0 DISCUSSION 37
6.0 CONCLUSIONS AND RECOMMENDATIONS 40
6.1 Conclusions 40
6.2 Recommendations 40
7.0 REFERENCES 41

8.0 APPENDICES 57
Appendix 8.1: Dry cow preparations used in the study 57
Appendix 8.2: Pictures of Lab Work 58
Appendix 8.3: Farm and herd characteristics and findings on mastitis for Chemusian and Gicheha farms as at 2017/2018 61

LIST OF TABLES

Table 2.5.2 Summary of California Mastitis Test scores and their interpretation 9

Table 4.1 Herd profile in Chemusian farm in 2017/2018 21

Table 4.2 Herd profile in Gicheha farm in 2017/2018 22

Table 4.3 Number of cases and percentage of various categories of bovine mastitis in Chemusian and Gicheha Farms in 2017/2018 23

Table 4.4a Relationship between overall mastitis and type of treatment used in Chemusian Farm in 2017/2018 24

Table 4.4b Relationship between overall mastitis and type of treatment used in Gicheha Farm in the year 2017/2018 25

Table 4.5a Relationship between clinical mastitis and type of treatment used in Chemusian Farm in the year 2017/2018 26

Table 4.5b Relationship between clinical mastitis and type of treatment used in Gicheha Farm in the year 2017/2018 26

Table 4.6a Relationship between subclinical mastitis and type of treatment used in Chemusian Farm in 2017/2018 27

Table 4.6b Relationship between subclinical mastitis and type of treatment used in Gicheha Farm in the year 2017/2018 28

Table 4.7a Distribution of CMT scores in various quarters for cows in Chemusian Farm in 2017/2018 29

Table 4.7b : Comparison of occurrence of mastitis between hind and forequarters for cows in Chemusian Farm in 2017/2018... 30

Table 4.8a : Distribution of CMT scores in various quarters for cows in Gicheha Farm in 2017/2018 31

Table 4.8b : Comparison of occurrence of mastitis between hind and forequarters for cows in Gicheha Farm in 2017/2018……………………………………………………………….32

Table 4.9a showing univariate analalysis of various factors influencing occurrence of mastitis in Chemusian and Gicheha farms in 2017/2018………………………………………………..33

Table 4.9b showing the multiple logistic regression model of factors influencing occurrence of

mastitis……………………………………………………………………………………….34

Table 4.10a : Table showing proportion of various bacteria isolated from mastitic milk from Chemusian and Gicheha Farms in 2017/2018…………………………………………………..35

Table 4.10b : Table showing bacterial distribution between Test and Control groups in Chemusian and Gicheha Farms 36

LIST OF FIGURES

Figure 3.1 Map showing the location of study areas, Chemusian and Gicheha Farms in Kenya. 14

Figure 3.2 Udder cleanliness score. Picture number corresponds to the score for udder and leg by Schreiner & Ruegg, 2010b 17

LIST OF PLATES

Plate 8.1a : Test product,Bismuth subnitrate (Boviseal®)) tube 57

Plate 8.1b : Boviseal® pressed from the tube.. 57

Plate 8.1c : Standard product, Ampicillin+Cloxacillin (Bovaclox®)... 57

Plate 8.2a : Positive sample of *Streptococcus agalactiae* (CAMP positive) on Blood Agar 58

Plate 8.2b : Positive samples for various bacteria for storage 59

Plate 8.2c : Catalase test for *Staphylococcus aureus* 60

ABSTRACT

This study presents results on effect of a combination of bismuth subnitrate teat canal sealant (Boviseal®) and the antibiotic Ampicillin+Cloxacillin (Bovaclox® DC) when used during the dry period on occurrence of mastitis 100 days post-calving. The specific objectives of this study were: to determine the effect of Boviseal® teat canal sealant in combination with Bovaclox® DC in control of dairy cow mastitis 100 days after calving; to determine bacterial pathogens causing mastitis in the selected farms and to determine risk factors associated with mastitis in dairy cows. This controlled field trial was carried out in two dairy farms in Kenya: Chemusian Farm in Nakuru County and Gicheha Farm in Kiambu County. A total of 156 dairy cows were sampled during the dry period for use in the study. They were randomly placed into either of the two study groups: Control group that received the antibiotic -Bovaclox® DC and the Test group that received the Bovaclox® DC followed by an internal teat sealant- Boviseal®. The cows were followed during the entire dry period (60 days to calving) and 100 days post-calving for development of mastitis.

Cows in the control group were more susceptible to mastitis 100 days post-calving compared to cows in the treatment group ($P<0.001$, RR=4.4, OR=17.7). Coagulase negative Staphylococci (CNS) were the most common pathogens isolated from mastitic milk at 34.6 % followed by *Micrococcus* spp. (9.0%), *Streptococcus agalactiae* (3.8%), *Staphylococcus aureus* (1.9%) and *Escherichia coli* (0.6%). A multiple logistic regression at $P\leq0.05$ showed that cows in Gicheha farm whose barn floor was earthen, those cows in the control group and hindquarters were risk factors for mastitis (RR=1.5, 4.4 and 1.18 respectively). The study thus recommends the use of Bovaclox® DC - Boviseal® dry cow combination and maintenance of good hygienic practices in animal barns and instruments of milking for control of bovine mastitis.

CHAPTER 1: INTRODUCTION

1.1 Background to the study

The livestock industry contributes to the growth of the economy of not only Kenya, but the entire world (Muthami, 2011; Mihret *et al*., 2017). Kenya has approximately 17.5 million cattle, with exotic breeds being 3.5million and indigenous breeds 14million (KNBS, 2010). The growth of the dairy sector is limited by various factors including; livestock diseases, poor access to the market, inadequate veterinary and livestock extension services providers and poor nutrition among others (Karanja, 2003; Munyori and Karanja, 2014). One of the major production diseases affecting the dairy cattle is mastitis (Barlow, 2011; *Gitau et al*., 2014; Gomes and Henriques, 2016b).

Mastitis is defined as inflammation of the udder, mainly due to infection. In the dairy industry, mastitis is the most costly production disease (Viguier *et al*., 2009; Gomes and Henriques, 2016a). The disease is caused by various pathogens, ranging from bacterial, fungal to viral organisms. Of major importance are the bacteria, both gram positive such as *Staphylococcus* spp. and *Streptococcus* spp. and gram negative such as the coliforms such as *Escherichia coli* (Blowey and Edmondson, 2010; Girma *et al*., 2012; Belayneh *et al*., 2013).Viral infections such as foot and mouth disease and bovine herpes directly cause mastitis or erode the skin of the udder and predispose it to secondary bacterial infections resulting into mastitis (Wellenberg *et al* ., 2002). Based on clinical features, mastitis can be classified as either clinical or subclinical (Fox, 2009; Mdegela *et al*., 2009). Mastitis can also be classified either as environmental or contagious mastitis (Fox, 2009; National Mastitis Council, 2015). Environmental mastitis is caused by pathogens commonly isolated from the environment of the cow, which includes milking machine, barn floor, soil, walkways, pasture and any surface with which the cow may be in contact. Organisms that cause environmental mastitis include *Staphylococcus* species (excluding *Staphylococcus aureus*), *Streptococcus* species (excluding *Streptococcus agalactiae*), coliforms such as *Escherichia coli* and *Enterobacter* species, *Pseudomonas*, *Proteus*, Yeast and *Prototheca* among others. Contagious mastitis is caused by pathogens that spread from cow to cow. These pathogens primarily inhabit the udder and teat of cows. The major pathogens responsible for this type of mastitis are *Streptococcus agalactiae*, *Corynebacterium bovis*, *Staphylococcus aureus* and *Mycoplasma* (Gonzalez and Wilson., 2003; Breen *et al*., 2009) Factors that predispose cattle to mastitis include breed, increased milk production, increase in parity, poor hygienic status of the cow environment among others (Breen *et al*., 2009; Ramírez *et al*., 2014)

Mastitis not only reduces milk quality and quantity at lactation, but may occasionally result in fatalities of the affected animal (Gomes and Henriques., 2016a). Of greater importance is the zoonotic threat of some bovine mastitis causing microorganisms such as *Mycobacterium tuberculosis*, *Staphylococcus aureus* and *Listeria monocytogenes* among others (Mwinyelle and Alhassan, 2014; Vishnupriya *et al*., 2014; Sharma *et al*., 2017).

1.2 Control measures for bovine mastitis

Several interventions have been applied in the control, prevention and treatment of mastitis in dairy cows. One of the most important preventive and control measures is proper hygiene of the cow environment (Lam *et al*., 2013). The most common approach to treatment and prevention of mastitis worldwide is through the use of antimicrobials such as tetracyclines, sulphonamides and lincosamides among others (Oliver and Murinda, 2012). Due to prolonged use and misuse of these agents, antimicrobial resistance has increased in both livestock and humans (Oliver and Murinda, 2012; WHO, 2014). This fact has called for the use of more environmentally friendly inventions such as vaccines, internal teat sealants, recombinant mucolytic proteins e.g. lysostaphin and nanoparticles (Sankar, 2016).

The use of internal teat sealant containing bismuth subnitrate in controlling bovine mastitis has been practised in various jurisdictions worldwide. Several studies have shown its efficacy in reducing prevalence of mastitis in dairy cows. The product is efficacious against clinical and subclinical mastitis as well as reducing the level of milk somatic cell counts (Cook *et al.*, 2005; Compton *et al.*, 2014). Rabiee and Lean (2013) demonstrated that use of bismuth subnitrate alone or in combination with antibiotic dry cow therapy pre-calving reduces incidence of clinical mastitis post-calving by 29% and 48% respectively.

There are no documented studies in Kenya with regard to the use of bismuth subnitrate or its combination with an antibiotic containing dry cow intramammary in prevention and control of mastitis. This study investigated the effect of combining an antibiotic-containing dry cow intramammary and an internal teat sealant in controlling dry cow mastitis and mastitis up to 100 days post-calving.

1.2 Problem statement

Usually the teat canal remains patent during the early dry period, regardless of antibiotic use during this period. Provided the canal is open, pathogens easily enter the udder usually resulting into an infection.

1.3 Justification

Bismuth subnitrate closes the teat canal during the dry period thus limiting entry and colonisation of mastitis causing pathogens in the udder. This greatly reduces incidence of both dry cow and post-calving mastitis (Woolford *et a*l., 1998).The treatment of clinical mastitis using intramammary (IMM) tubes usually takes 3-5 days consecutively. This means that a total of 12 to 20 tubes are used during this period. With the cost of each IMM tube being Ksh. 100-200 (USD 1-2) it implies that a farmer spends Ksh. 1200-4000 (12-40 USD) to acquire these tubes only. The cost of bismuth subnitrate tubes is Ksh. 400 (4 USD) for four tubes and Ksh. 400-600 (4-6 USD) for four tubes of dry therapy containing Ampicillin and Cloxacillin. These latter products can be administered by farmers thus lowering the professional charges demanded by vets when they treat mastitis cases. After calving, the teat sealant is milked from the teat either by the farmer or the calf.

1.4 Objectives

1.4.1 General objective

To demonstrate the benefit of bismuth subnitrate teat canal sealant in combination with Ampicillin+Cloxacillin dry cow therapy in controlling bovine mastitis in selected farms in Kenya.

1.4.2 Specific objectives

1. To determine the effect of bismuth subnitrate teat canal sealant in combination with Ampicillin+Cloxacillin dry cow therapy in control of dairy cow mastitis 100 days after calving.

2. To determine bacterial pathogens causing mastitis in the selected farms.

3. To determine risk factors associated with mastitis in dairy cows in the selected farms

1.5 Hypothesis

A combination of bismuth subnitrate teat canal sealant and Ampicillin-Cloxacillin dry cow therapy reduces the prevalence of mastitis 100 days post-calving in dairy cows.

CHAPTER 2: LITERATURE REVIEW

2.1 Aetiology of mastitis

Mastitis is caused by multiple factors which include bacterial orgaisms, fungi and viruses. Various bacterial organisms have been isolated in cases of mastitis. Contagious organisms such as *Streptococcus agalactiae*, *Staphylococcus aureus* and *Mycoplasma* spp. mainly cause subclinical mastitis (Dieser *et al*., 2014). Environmental mastitis is commonly caused by *E.coli*, *Klebsiella* species and *Pseudomonas* spp. *S.aureus* has been documented as the most commonly isolated pathogen causing mastitis (Middleton *et al*., 2009; Pereira *et al*., 2011; Gitau *et al*., 2014). Several fungal organisms have been incriminated as pathogens causing mastitis. They include the genera *Aspergillus*, *Candida* and *Malassezia* among others (Motaung *et al*., 2017; Song *et al*., 2017). Viral organisms including foot and mouth disease and bovine herpes virus have also been identified in mastitis cases (Motaung *et al*., 2017).

2.2 Classification of mastitis

Based on clinical features, mastitis can be classified as either clinical or subclinical (Salvador *et al*., 2014; Sankar, 2016). Clinical mastitis presents signs that can be easily detected by observation. In acute phase the udder is swollen, reddened, hot, and painful and loses its functionality e.g. reduced milk production and change in milk quality. Other signs of clinical mastitis are anorexia, fever and swollen lymph nodes, especially the supramammary (Olde Riekerink *et al*., 2008). In subclinical mastitis there are no obvious signs of disease that are presented by animal, making its diagnosis more difficult (Mellenberger and Roth, 2009).

Mastitis can also be classified either as environmental or contagious mastitis based on aetiology (Garcia, 2004; Down *et al*., 2016). Environmental mastitis is caused by pathogens commonly isolated from the environment of the cow, which include milking machine, cow's bedding, soil, walkways, pasture and any surface with which the cow may be in contact. Organisms that cause environmental mastitis include *Staphylococcus* spp. (excluding *Staph.aureus*), *Streptococcus* species (excluding *Strep.agalactiae*), coliforms such as *Escherichia coli* and *Enterobacter* species, *Pseudomonas*, *Proteus*, Yeast and *Prototheca* among others (Deb *et al*., 2013). Contagious mastitis is caused by pathogens that spread from cow to cow. These pathogens primarily inhabit the udder and teat of cows. The major pathogens responsible for this type of mastitis are *Streptococcus agalactiae*, *Corynebacterium bovis*, *Staphylococcus aureus* and *Mycoplasma* (Abebe *et al*., 2016; Ikiz *et al*., 2013; Petersson-Wolfe *et al*., 2011; Swartz *et al*., 2016).

2.3 Epidemiology of bovine mastitis

Mastitis is an economically important production disease with a worldwide distribution. Losses due to mastitis are attributable to reduced milk production, culling of chronic cases, mortality, labour and veterinary costs (Sankar, 2016). Being the commonest form of mastitis, prevalence of subclinical mastitis in Kenya ranges between 30 and 65% (Gitau *et al*., 2014; Mureithi & Njuguna, 2016).

There are various risk factors for mastitis, ranging from cow factors, environmental, herd management and equipment at the farm (Bharti *et al*., 2015; Naseemunnisa *et al*., 2017; Olde Riekerink *et al*.,, 2007). The number of pathogens on the udder during the dry period influence occurrence of mastitis in the subsequent lactation. The pathogens may enter the udder parenchyma but not necessarily cause dry cow mastitis. During the subsequent lactation, mastitis may easily occur not because of new infection but following udder colonisation by the pathogens during the dry period (Green *et al*., 2008). Hygiene at the farm is one of the most important management factors influencing occurrence of mastitis. Cows spend 40-65% of their time lying down, thus environmental mastitis is positively correlated to dirty cow environment (Schreiner & Ruegg, 2010b). A study by Reneau e*t al*., (2005) demonstrated that hygiene of the udder and hind limbs is significantly associated with somatic cell counts, and occurrence of mastitis in dairy cows. Other factors that have been demonstrated to be positively associated with occurrence of mastitis are traumatic injury to the quarters, wetness of the environment, an increasing parity, high somatic cell count due to various causes and hyperkeratosis of the teat ends among others (Nyman *et al*., 2007; Breen *et al*., 2009; Rahman *et al*., 2009).

Mastitis is an expensive disease because it lowers both the quantity and quality of milk produced by infected cows. According to Yalçin (2000) subclinical mastitis costs between £69-228 per cow per year in Scotland. Jones and Bailey (2009) reported that mastitis costs the United States dairy industry approximately $1.7-2 billion annually.

2.4 Clinical signs of mastitis

Acute and peracute forms of mastitis present obvious signs of disease such as fever, anorexia and dullness. At the local level, the udder is swollen, warm, reddened, painful and abnormal milk. Chronic mastitis may present abscesses, gangrene or even sloughing of the teat (Kandeel *et al*., 2018). Subclinical mastitis does not present obvious clinical signs.

2.5 Diagnosis of mastitis

2.5.1 Physical examination

This involves visual examination of the cow, udder, milk and palpation. Clinical cases of mastitis present signs such as dullness, fever, anorexia, swelling of the udder, redness, pain, warmth and abnormal milk e.g. flakes, blood tinged milk, watery milk and so on depending on the causative agent (Riekerink *et al*., 2008; Kandeel *et al*., 2018). This procedure is not applicable for subclinical mastitis because there are no clinical signs.

2.5.2 California Mastitis Test (CMT)

This is a useful test developed in 1957 by Schalm and Noorlander. Several studies have demonstrated that the test is rapid, economically affordable and accurate in detecting subclinical mastitis (Dingwell *et al*., 2003; Fosgate *et al*., 2013). A more simplified way of performing and interpreting the results of a CMT has been described by Mellenberger and Roth (2009). In the procedure, a squirt of milk, about 2 ml from each quarter is placed in each of four shallow cups in the CMT paddle. An equal amount of the commercial CMT reagent is added to each cup. A gentle circular motion is applied to the mixtures in a horizontal plane for 15 s. The CMT results are scored as N (negative), T (trace), 1 (subclinical mastitis), 2 and 3 (clinical mastitis) based on gel formation and colour change, as summarised in the table 2.5.2.

Table 2.5.2 Summary of California Mastitis Test scores and their interpretation

CMT SCORE	Somatic cell count	Gelling	Interpretation
N(Negative)	1,000	None	Healthy quarter
T(Trace)	300,000	Very mild	Subclinical mastitis
1	900,000	Mild	Subclinical mastitis
2	2,700,000	Moderate	Clinical mastitis
3	8,100,000	Heavy, almost solidifies	Clinical mastitis

2.5.3 Somatic cell count

Somatic cell count increases in cases of mastitis. Majority of the somatic cells are leukocytes, which are cells that respond to an infection. The more the number of these cells the more severe the mastitis (Sharma *et al*., 2011; Kashongwe *et al*., 2017;). In the United States dairy industry, a bulk tank somatic cell count of less or equal to 200,000 cells/mL is recommended for good quality milk (Losinger, 2005).

2.5.4 Polymerase chain reaction (PCR)

Since its discovery in 1980s, PCR remains one of the most important diagnostic procedures in the field of medicine. It has a high specificity and sensitivity (usually 100%) thus an excellent method for diagnosis of bovine mastitis (Kramer and Coen, 2001 ; Koskinen *et al*., 2010). The technique is based on amplification of the DNA molecule to several copies that can be easily detected. Several organisms causing mastitis can be detected via this noble technique. There exist probes for rapid detection of Escherichia *coli*, *Staphylococcus aureus*, *Streptococcus* *agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus parauberis*, and *Streptococcus uberis* (Riffon *et al*., 2001; Shome *et al*., 2011).

2.5.5 Bacterial culture

Culturing of mastitic milk remains to be one of the most important and frequently used laboratory methods for isolating the causative agents (Viguier *et al*., 2009). The bacteria isolated can be subjected to various antibiotics to test for any resistance (Getahun *et al*., 2008). Different mastitis causing microorganisms grow well in different culture media, in which they can be identified. For example, the selective media for *Staphylococcus aurueus*, one of the most important pathogens causing mastitis, is Mannitol salt agar in which the organism produces small colonies surrounded by yellow zones (Bautista-Trujillo *et al*., 2013; Kateete *et al*., 2010; Pumipuntu *et al*., 2017).

2.5.6 White slide test

The principle behind this technique is the detection of the high leukocyte population in mastitic milk. The procedure involves mixing about 5 drops of milk and a drop of 4% sodium hydroxide on a glass slide using a rod. For healthy milk there is no thickening. Mastitic milk thickens and flakes appear (Sears and McCarthy, 2003).

2.6 Control of mastitis

2.6.1 Management

Good hygienic practices are of great value in controlling environmental mastitis (Dosogne *et al*., 2002; Deb *et al*., 2013; Garoussi *et al*., 2017). Cows with udder hygienic scores between 3 and 5 (on a scale of 1 to 5) are more likely to have major pathogens such as *E.coli* and *S.aureus* and hence a higher chance of contracting mastitis (Schreiner and Ruegg, 2003). Hygiene revolves around maintaining a clean resting and sleeping area for the cows, clean and disinfected milking machines, cleaning the hands and udder/teats using antiseptics before milking (Breen *et al*., 2009; Lakew *et al*., 2009).

2.6.2 Antibiotic containing dry cow therapy

There exists a significant correlation between intramammary infections during the dry period and occurrence of clinical and subclinical mastitis in the subsequent lactation (Green *et al*., 2008). Use of antibiotic is one of the best ways of controlling contagious mastitis (Bradley *et al*., 2011; Scherpenzeel *et al*., 2014). Different antibiotics show different efficacies towards various disease causing pathogens (Gundelach *et al*., 2011). However antimicrobial resistance has been on the rise due to prolonged, suboptimal and misuse of antibiotics in food animals (Oliver *et al*., 2011). The goodness of antibiotic containing dry cow therapy is that it can be used together with an internal teat sealant to control mastitis (Newton *et a*l., 2008; Bradley *et al*, 2010).

2.6.3 Culling of mastitic cows

Some agents of mastitis such as *Brucella* and *Mycoplasma* and *Staph.aureus* have proven resistant to the commonly available antibiotics. An alternative is slaughter of the cows that test positive because if left these animals serve as carriers to the healthy herd (Nicholas *et al*., 2016; Vakkamäki *et al* 2017).

2.6.4 Internal teat sealants during dry period

A study by Huxley *et al*., (2002) in the United Kingdom to compare the efficacy of an internal teat sealant containing bismuth subnitrate and a long acting antibiotic preparation containing cephalonium in controlling mastitis during the dry period and in the first 100 days postcalving showed that anomals with the teat sealant had significantly fewer number of new intramammary infections (IMI), mainly due to *Escherichia coli*, other Entero-bacteriaceae and environmental pathogens. Bismuth subnitrate teat canal sealant has also been proven to significantly reduce postcalving intramammary mastitis in heifers if it is used during the dry period. When administered in heifers, the sealant greatly lowers the incidence of mastitis caused by both gram negative and gram positive bacteria. Almost 70% of clinical and subclinical mastitis is prevented by use of the sealant (Parker *et al*., 2007). Parker *et al*., (2008) demonstrated that the product reduces the risk of new IMI with any disease-causing microorganism, prevalence of postcalving IMI and risk of mastitis caused by *Streptococcus uberis* by an average of 70%. These advantages were not realised when an antibiotic (tylosin) was administered intramuscularly. However, the study demonstrated that the teat canal sealant had no significant curative effect on precalving IMI.

Other preventive methods include use of nanoparticles, bacteriophages and cytokines (Sankar, 2016).

CHAPTER 3: MATERIALS AND METHODS

3.1 Study area

The study was carried out in two dairy farms in Kenya; namely Chemusian and Gicheha, as shown in Figure 3.1. These farms were conveniently selected because they had dairy cows for drying, which were target animals for this study. In both farms, records on individual animals including age, parity, barn floor type and disease management are computer-stored thus easily retrievable. Each of the farms had a resident veterinarian and animal health assistants who helped in monitoring of animal health during the entire study period.

Chemusian farm is located in Rongai Constituency, Nakuru County. It is approximately 19.7 km West of Nakuru Town and approximately 200kms west of the capital city Nairobi. The farm has about 1000 dairy cows mainly of two breeds, the Friesian and Ayrshire.

Gicheha farm is located in Kiambu County. It is located approximately 25kms north of the capital city of Nairobi. The farm has about 500 dairy cows consisting mainly Friesians and a few Ayrshire and Guernsey breeds.

Figure 3.1 Map showing the location of the study areas, Chemusian and Gicheha Farms in Kenya (Source: Author)

3.2 Study design

The study was a controlled field trial.

3.2.1 Sample size determination and allocation into study groups

Sample was calculated as follows, using the formula by Naing *et al*. (2006):

$$n = \frac{Z^2 P(1-P)}{d^2}$$

Where n= sample size,

Z= Z statistic for a level of confidence (which is 1.96 at 95% CL)

P= expected prevalence or proportion (which is estimated at 0.5 since no study has been conducted on prevalence of mastitis in both farms)

d= precision (= 0.05).

Using this formula, the sample size for each farm was 384 animals. However, since the farms had a finite population of cows for drying off (200 for Chemusian and 50 for Gicheha) , the sample size was adjusted using the formula by Naing *et al*., (2006):

$$n' = \frac{NZ^2P(1-P)}{d^2(N-1)+Z^2P(1-P)}$$

Where

n' = sample size with finite population correction,

N= Population size (200 for Chemusian and 50 for Gicheha),

Z= Z statistic for a level of confidence (1.96 at 95% CL),

P= Expected proportion (0.5)

d= Precision (0.05).

The calculated values were 133 and 44 cows for Chemusian and Gicheha farms respectively. In each farm, the cows were allocated to either of the two groups: test group and a control group. A simple random approach was used for allocation of cows into either group. The test group received bismuth subnitrate (Boviseal®- Bimeda® Animal Health, Ireland) and antibiotic Ampicillin+Cloxacillin (Bovaclox® DC-Norbrook Laboratories Ltd-UK) while the control group received the antibiotic Ampicillin+Cloxacillin alone. These animals were followed for development of mastitis from the day of dry off to 100 days post-calving.

3.3 Criteria for cow selection into the study

All the cows in the study were healthy on physical and historical assessment. The animals were in their first or subsequent lactation with no case of mastitis in the current lactation. The California Mastitis Test (CMT) was used to check the health status of the udder and only the animals with a score of 0, indicating absence of mastitis were recruited into the study. The cows were in their dry period (60 days to calving) as indicated in the farm records.

3.4 Udder and hind leg hygiene scoring

Before administration of the study products, the cleanliness of individual cow's hind leg and udder were scored using the method proposed by Schreiner and Ruegg (2010a) and Schreiner & Ruegg (2010b) as shown in Figure 3.2. In brief, a score of 1 indicated clean udder and hind legs while a score of 2 indicated a clean udder with slightly dirty hind legs. A score of 3 indicated slight dirt on both the udder and hind legs while a score of 4 indicated a grossly dirty udder and hind legs as shown in Figure 3.2. In this study, scores 1 and 2 were merged to indicate a clean score while 3 and 4 were merged to indicate a dirty udder and hind legs.

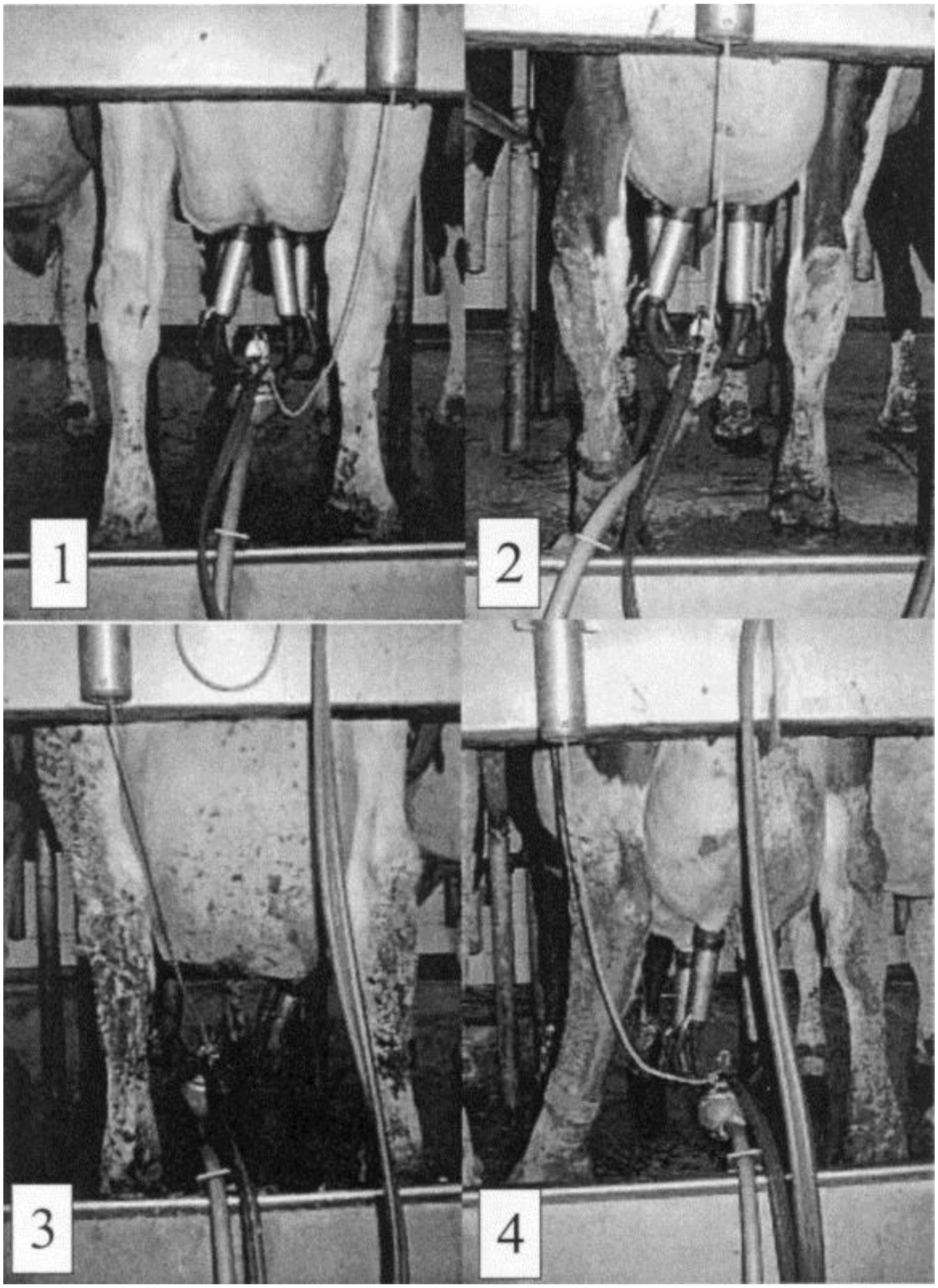

Figure 3.2 Udder cleanliness score. Picture number corresponds to the score for udder and leg by Schreiner & Ruegg, 2010b

3.5 Administration of reference and test products

For each farm, the sampled cows were allocated into either of the two study groups: Test group or Control group. The test group animals were infused with Ampicillin+Cloxacillin (Bovaclox® DC) and bismuth subnitrate (Boviseal®) intramammary while the control group received intramammary Ampicillin+Cloxacillin alone. These products were infused aseptically according to the National Mastitis Council (2015) recommendations. Once the cow was physically restrained in a crush, the teats were cleaned using cotton swabbed in70% surgical spirit. The udder was milked before the products were administered.

3.6 Animal follow-up

Animals in the study were followed up for development of mastitis from the day of dry off to 100 days post-calving. With each cow physically restrained in a standing position, each quarter was examined for any clinical abnormalities including signs of inflammation of teat canal /teat cistern/udder cistern on the following occasions: prior to the administration of Test and Reference Products; study Day 7, 14, 30 (post administration of the Test and Reference Product); immediately prior to calving and on each day post calving until day 100. Any abnormal clinical observation including signs of inflammation was recorded in the data capture form. In the event that a case of clinical mastitis was suspected, such that clots or abnormalities are found in the foremilk, 8 squirts were stripped to empty the teat cistern. At this stage milk would be coming from the udder tissue. If at this stage the abnormalities / clots had disappeared, then this was not clinical mastitis. If the abnormalities persisted, the cow was deemed to have clinical mastitis and treated as such.

3. 7 Milk sample collection, transportation

Milk samples were collected for bacteriological examination if quarter or udder was clinically diagnosed as having mastitis through CMT and visual examination of the milk. Milk was also collected from each cow in the study at any day after calving (within 100 days post-calving) to determine the prevalence of subclinical mastitis for those animals which had not developed clinical mastitis. The milk sample was collected aseptically as per National Mastitis Council (2015). In summary, 5ml composite milk was stripped into a properly labeled sterile test tube for each cow. The samples were transported in a cool box with ice packs to the University of Nairobi, Department of Public Health, Pharmacology and Toxicology laboratory for immediate bacteriological culture or stored at 4^{0}C for culture within 48 hours.

3.8 Culture and identification of bacteria

The culture and identification were carried out using standard methods (Quinn & Markey, 2003). The preparation of sheep blood agar (Himedia, India) and MacConkey agar (Oxoid, UK) were done according to guidelines by the manufacturer. The agars were left to set and stored in a refrigerator until use. 100μl of the milk sample was inoculated onto both sheep blood agar and MacConkey agar and incubated at 37^{0}C for 24-48 hours. The morphology of the bacterial colonies obtained was checked for the colony size, shape, texture, and colour. Red blood cell hemolysis in the sheep blood agar was checked for by identification of the changes in the media around and under the colonies. Plates with no growth after 48 hours were recorded as no growth while those with mixed growth were subcultured to obtain pure colonies. The gram-staining technique was used to differentiate between Gram-positive and Gram-negative bacteria and also to note the microscopic shape of the bacteria. Biochemical tests were used to further identify bacteria according to standard methods. Identification of bacteria was primarily made on the basis of colony morphology, haemolytic characteristics, and gram-stain reaction, coagulase and catalase, indole, methyl red, voges proskauer, citrate and Christie–Atkins–Munch-Petersen tests (Quinn & Markey, 2003)

3.9 Data management and analysis

All data collected were entered, cleaned and stored in MS Excel 2010 (Microsoft, Sacramento, California, USA). The data were analysed using Stata13.1 software (StataCorp LLC, College station, Texas, USA). The outcome of the study was presence/absence of bovine mastitis while the explanatory variables included type of treatment during dry period, animal age, barn floor type, breed, farm, and lactation number, daily milk production, milking frequency, management system, hind leg and udder hygiene score. The data were subjected to descriptive data analysis in which proportions of various variables such as microorganisms causing mastitis, breed, milking frequency, management system and mastitis cases were computed. Chi square test or Fisher's exact test were used to evaluate level of association for each independent variable and the outcome using statistical frequency tables. Odds ratio (OR) and relative risk (RR) were calculated from the frequency tables in order to find out if a particular variable was a risk factor for mastitis at P value ≤ 0.05.

4.0 RESULTS

4.1 Herd demographics and profiles

Farm summary for Chemusian and Gicheha farms is outlined in Table 4.1 and Table 4.2 respectively. A total of 156 cows were recruited into the study. Thirty two (20.51%) of these were from Gicheha Farm and 124 (79.49%) were from Chemusian Farm. One hundred and thirty nine (89.1%) of these cows were Friesians while 17 (10.9%) were Ayrshires. Both farms practice semi-intensive dairy farming system with machine milking being the method for milking the cows. Gicheha Farm milks the cows three times a day while Chemusian does it twice a day.

Table 4.1 Herd profile in Chemusian farm in 2017/2018

Factor	Factor levels	Number of animals	Percentage
Management system	Semi-intensive	124	100
Milking method	Machine	124	100
Barn floor type	Concrete	124	100
Milking frequency (per day)	Two times	124	100
Breed	Friesian	113	91.1
	Ayrshire	11	8.9
Udder/hind leg hygiene score	≤2 (clean)	66	53.2
	>2 (dirty)	58	46.8
Age (years)	≤5	63	50.8
	>5	61	49.2
Lactation number/parity	≤2	55	44.4
	>2	69	55.6

Table 4.2 Herd profile in Gicheha farm in 2017/2018

Factor	Factor levels	Number of animals	Percentage
Management system	Semi-intensive	32	100
Milking method	Machine	32	100
Barn floor type	Earthen	32	100
Milking frequency (per day)	Three times	32	100
Breed	Friesian	25	78.13
	Ayrshire	7	21.87
Udder/hind leg hygiene score	≤2 (clean)	10	31.3
	>2 (dirty)	22	68.7
Age (years)	≤5	9	28.1
	>5	23	71.9
Lactation number/parity	≤2	7	21.9
	>2	25	78.1

4.2 Prevalence of mastitis

In Chemusian farm, the prevalence of mastitis (clinical and subclinical) was 46.77% (58/124). Out of this, 10.48% (13/124) was clinical and 36.29% (45/124) was subclinical mastitis. As shown in Table 4.3, the prevalence of mastitis (clinical and subclinical) in Gicheha farm was 65.63% (21/32). Out of this, 25% (8/32) was clinical and 40.63% (13/32) was subclinical mastitis.

Table 4.3 Number of cases and percentage of various categories of bovine mastitis in Chemusian and Gicheha Farms in 2017/2018

Farm	Mastitis category	Number of cases (percentage)
Chemusian	Overall mastitis	58 (46.77)
	Clinical	13 (10.48)
	Subclinical	45 (36.29)
Gicheha	Overall mastitis	21 (65.63)
	Clinical	8 (25)
	Subclinical	13 (40.63)

4.3 Mastitis cases in the treatment and control groups in Chemusian and Gicheha Farms in 2017/2018

4.3.1 Overall mastitis

There was a significant difference in the prevalence of mastitis between cows in the treatment and control groups in both farms at $p \leq 0.05$. In Chemusian farm, the prevalence of mastitis in the test group was 16.13% (10/62) compared to 79.03% (49/62) in the control group ($p<0.001$) as shown in Table 4.4a. The relative risk was 4, implying that cows in the control group were four times more likely to develop mastitis compared to those in the test group.

Table 4.4a Relationship between overall mastitis and type of treatment used in Chemusian Farm in 2017/2018

GROUP	Overall mastitis			χ^2	P value	Relative risk (odds ratio)
	Positive	Negative	Total			
Control	49	13	62	49.18	<0.001	4(19.6)
Test	10	52	62			
Total	59	65	124			

In Gicheha farm, the prevalence of mastitis was 37.5% (6/16) in the test group compared to 93.8% (15/16) in the control group (p=0.001) as shown in Table 4.4b. The relative risk ratio was 10, implying that cows in the control group were ten times more likely to develop mastitis compared to those in the test group.

Table 4.4b Relationship between overall mastitis and type of treatment used in Gicheha Farm in the year 2017/2018

GROUP	Overall mastitis			χ^2	P value	Relative risk (odds ratio)
	Positive	Negative	Total	11.22	0.001	10 (25)
Control	15	1	16			
Test	6	10	16			
Total	21	11	32			

4.3.2 Clinical mastitis

The prevalence of clinical mastitis in Chemusian farm was 10.48% (13/124) as shown in Table 4.5a. In this farm, the prevalence of clinical mastitis in the test group was 1.61% (1/62) compared to 19.35% (12/62) in the control group. There was a significant difference in the prevalence of clinical mastitis between the test and control group (p=0.001). Cows in the control group were 1.22 times more likely to develop clinical mastitis compared to those in the test group.

Table 4.5a Relationship between clinical mastitis and type of treatment used in Chemusian Farm in the year 2017/2018

GROUP	Clinical mastitis			χ^2	P value	Relative risk (odds ratio)
	Positive	Negative	Total	10.40	0.001	1.22 (14.6)
Control	12	50	62			
Test	1	61	62			
Total	13	111	124			

In Gicheha farm, the prevalence of clinical mastitis was 18.75% (3/16) in the test group compared to 31.25% (5/16) in the control group. There was no statistical association between clinical mastitis and treatment group as shown in Table 4.5b.

Table 4.5b Relationship between clinical mastitis and type of treatment used in Gicheha Farm in the year 2017/2018

GROUP	Clinical mastitis			Fisher's exact	P value
	Positive	Negative	Total	0.685	0.414
Control	5	11	16		
Test	3	13	16		
Total	8	24	32		

4.3.3 Subclinical mastitis

The prevalence of subclinical mastitis in Chemusian farm was 36.29% (45/124) as shown in Table 4.6a below. In this farm, the incidence of subclinical mastitis in the test group was 14.52% (9/62) compared to 58.06% (36/62) in the control group. There was a significant difference in the prevalence of subclinical mastitis between the test and control group (p=0.001). The relative risk was 2; implying that cows in the control group were 2 times more likely to develop subclinical mastitis compared to those in the test group.

Table 4.6a Relationship between subclinical mastitis and type of treatment used in Chemusian Farm in 2017/2018

GROUP	Subclinical mastitis			χ^2	P value	Relative risk (odds ratio)
	Positive	Negative	Total	25.43	0.001	2 (8.2)
Control	36	26	62			
Test	9	53	62			
Total	45	79	124			

The prevalence of subclinical mastitis in Gicheha farm was 40.63% (13/32) as shown in Table 4.6b below. The prevalence of subclinical mastitis was 18.75% (3/16) in the test group compared to 62.5% (10/16) in the control group. There was a significant difference in the prevalence of subclinical mastitis between the test and control group (p=0.012). The relative risk was 2.2; implying that cows in the control group were 2.2 times more likely to develop subclinical mastitis compared to those in the test group.

Table 4.6b Relationship between subclinical mastitis and type of treatment used in Gicheha Farm in the year 2017/2018

GROUP	Subclinical mastitis			χ^2	P value	Relative risk (odds ratio)
	Positive	Negative	Total	6.35	0.012	2.2 (7.2)
Control	10	6	16			
Test	3	13	16			
Total	13	19	32			

4.4 Quarter level prevalence of mastitis

4.4.1 Chemusian farm

The CMT results for Chemusian farm are summarized in Table 4.7a. Over seventy percent (358/496) of the quarters were healthy throughout the study period and 27.22% (138/496) had either subclinical or clinical mastitis.

Table 4.7a Distribution of CMT scores in various quarters for cows in Chemusian Farm in 2017/2018

Mastitis level	CMT score	Quarter			
		Fore right	Fore left	Hind right	Hind left
Healthy quarter	0	92(74.19%)	99(79.84%)	82(66.13%)	85(68.55%)
Subclinical mastitis	1	26(20.97%)	19(15.32%)	31(25%)	28(22.58%)
Clinical mastitis	2	2(1.61%)	4(3.23%)	4(3.22%)	2(1.61%)
	3	4(3.23%)	2(1.61%)	7(5.65%)	9(7.26%)

Of the 138 quarters that had mastitis (either clinical or subclinical) 58.7% were hind quarters and 41.3% were fore quarters as shown in Table 4.7b. There was a significant difference prevalence of mastitis between fore and hind quarters (p=0.016). Hind quarters were 1.1 times more likely to develop mastitis compared to forequarters.

Table 4.7b: Comparison of occurrence of mastitis between hind and forequarters for cows in Chemusian Farm in 2017/2018

Quarter	Mastitis			χ^2	P value	Relative risk (odds ratio)
	Positive	Negative	Total	5.78	0.016	1.1(1.6)
Hind	81	167	248			
Fore	57	191	248			
Total	138	358	496			

4.4.2 Gicheha farm

The CMT results for Gicheha farm are summarized in Table 4.8a. Fifty percent (64/128) of the quarters were healthy throughout the study period and 50% (64/128) had either subclinical or clinical mastitis.

Table 4.8a: Distribution of CMT scores in various quarters for cows in Gicheha Farm in 2017/2018

Mastitis level	CMT score	Quarter			
		Fore right	Fore left	Hind right	Hind left
Healthy quarter	0	18(56.25%)	20(62.5%)	14(43.75%)	12(37.5%)
Subclinical mastitis	1	8(25%)	5(15.63%)	8(25%)	11(34.38%)
Clinical mastitis	2	4(12.5%)	4(12.5%)	4(12.5%)	0(0%)
	3	2(6.25%)	3(9.37%)	6(18.75%)	9(28.13%)

Of the 64 quarters that had mastitis (either clinical or subclinical) 59.38% were hind quarters and 40.63% were fore quarters as shown in Table 4.8b. There was a significant difference prevalence of mastitis between fore and hind quarters (p=0.034). Hind quarters were 1.5 times more likely to develop mastitis compared to forequarters**.**

Table 4.8b: Comparison of occurrence of mastitis between hind and forequarters for cows in Gicheha Farm in 2017/2018

Quarter	Mastitis			χ^2	P value	Relative risk (odds ratio)
	Positive	Negative	Total			
Hind	38	26	64	4.5	0.034	1.5(2.1)
Fore	26	38	64			
Total	64	64	128			

4.5 Factors influencing occurrence of bovine mastitis

The factors influencing occurrence of mastitis under the study were subjected to a univariate logistic regression at $P \leq 0.2$. Significant factors were farm, breed, barn floor and quarter position as shown in Table 4.9a.

Table 4.9a showing univariate analysis of various factors influencing occurrence of mastitis in Chemusian and Gicheha Farms in 2017 and 2018

OVER	Coef.	Std. Err.	z	P>|z|	[80% Conf.	Interval]
FARM	.743477	.4133545	1.80	0.072	.2137419	1.273212
_cons	-.8403268	.517555	-1.62	0.104	-1.5036	-.1770534
UHYG	.1000835	.3205572	0.31	0.755	-.3107271	.510894
_cons	1.35e-15	.2294157	0.00	1.000	-.2940081	.2940081
Brd	.7221347	.5281966	1.37	0.172	.0452236	1.399046
_cons	-.7511223	.6049516	-1.24	0.214	-1.526399	.0241544
Ageyrs	.1986707	.3216546	0.62	0.537	-.2135463	.6108877
_cons	-.0555699	.2357932	-0.24	0.814	-.3577511	.2466114
GROUP	2.874372	.4070413	7.06	0.000	2.352727	3.396016
_cons	-1.519826	.2950484	-5.15	0.000	-1.897946	-1.141706
BARN	.743477	.4133545	1.80	0.072	.2137419	1.273212
_cons	-.0968498	.1798159	-0.54	0.590	-.3272932	.1335936
QUARTER	.5313147	.1732043	3.07	0.002	.3093445	.7532849
_cons	.4835667	.1165535	4.15	0.000	.3341974	.632936

Those variables with significant association with mastitis at $P \leq 0.2$ were subjected to multivariate logistic regression at $P \leq 0.05$ in order to get a parsimonious model. These factors were breed, barn floor, group and farm as shown in the Table 4.9b. Barn floor and group were the two variables that significantly explained the difference in the prevalence of mastitis. The variables farm and barn floor were collinear, since cows in Chemusian farm slept on a concrete floor while those in Gicheha farm slept on an earthen floor.

Table 4.9b showing the multiple logistic regression model of factors influencing occurrence of mastitis

OVER	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
Brd	.3479394	.7089551	0.49	0.624	-1.041587	1.737466
BARN	-1.221103	.5261026	-2.32	0.020	-2.252246	-.1899613
FARM	0	(omitted)				
GROUP	3.057013	.4431469	6.90	0.000	2.188461	3.925565
_cons	-1.743975	.8796458	-1.98	0.047	-3.468049	-.0199008

4.5 Bacterial pathogens causing mastitis

The most common bacterial pathogens isolated from mastitic milk were coagulase negative Staphylococci (CNS) (34.6 %) and *Micrococcus* spp. (9.0%). Other bacteria isolated were *Streptococcus agalactiae* (3.8%), *Staphylococcus aureus* (1.9%); *Escherichia coli* (0.6%) and various bacterial mixtures as shown in Table 4.10a.

Table 4.10a: Table showing proportion of various bacteria isolated from mastitic milk from Chemusian and Gicheha Farms in 2017/2018

Organism	Number	Percentage
CNS	54	34.6
Micrococcus spp.	14	9.0
Streptococcus agalactiae	6	3.8
S.aureus	3	1.9
CNS+*Strep. agalactiae*	3	1.9
E.coli	1	0.6
Micrococcus+E.coli	1	0.6
Micro+Strep. agactiae	1	0.6
No growth	73	46.8
Total	156	100

Pathogen distribution between the test and control groups is as shown in Table 4.10b. In summary, more bacteria pathogens were isolated from the control group than the test group (P<0.001).

Table 4.10b: Table showing bacterial distribution between Test and Control groups in Chemusian and Gicheha Farms

Group	Presence of organism					Relative risk (Odds ratio)
	YES	NO	TOTAL	Pearson χ^2	P value	
Control	61	17	78	39.16	<0.001	3.3(9.1)
Test	22	56	78			
Total	83	73	156			

5.0 DISCUSSION

From the current study, a combination of Ampicillin+Cloxacillin (Bovaclox® DC) and bismuth subnitrate was more effective in controlling bovine mastitis 100 days postcalving compared to Bovaclox® DC alone. This was in agreement with studies done in by Newton *et al*., (2008), Runciman *et al*. (2010), Berry & Hillerton, 2010, Golder *et al*. (2016) and Bates *et al*. (2016) who used the sealant with other antibiotics. In this study, animals in the control group were 4.4 times more likely to develop mastitis within 100 days postcalving compared to 1.9 times obtained from a study by Golder *et al*., (2016). The bismuth subnitrate teat sealant complements the antibiotic function of Bovaclox® DC. It seals the teat canal thus limiting the number of bacteria and other mastitis causing pathogens entering the udder tissue. This explains why the test group animals (received both bismuth subnitrate and Bovaclox® DC) had a lower prevalence of mastitis compared to the control group.

The prevalence of mastitis (clinical and subclinical) from the current study was 51.8% (47.58% in Chemusian and 65.63% in Gicheha), slightly lower than that documented by Mekibib *et al*., (2010) of 71.0% in Holeta town of Ethiopia and 74.7% reported by Abebe *et al*., (2016). The prevalence of subclinical and clinical mastitis was 37.18% (36.29% in Chemusian and 40.63% in Gicheha) and 14.74% (10.48% in Chemusian and 25% in Gicheha) respectively. This agreed with several studies previously done that showed that subclinical mastitis is usually higher compared to clinical mastitis in a ratio of even up to 1:40 (Shaheen *et al*., 2016). Mureithi & Njuguna (2016) had reported a prevalence of 64% for subclinical mastitis in herds within Thika sub county of Kenya. In a study by Gitau *et al.,*(2014) in Mukurueini and Nakuru Districts of Kenya, the prevalence of clinical and subclinical mastitis was 0.7% and 32.4% respectively. Ndirangu *et al*., (2017) reported the prevalence of clinical and subclinical mastitis in Sahiwals of

KALRO-Naivasha of Kenya as 6% and 54% respectively. Prevalence of subclinical mastitis is higher than that of clinical mastitis because subclinical mastitis is not easily detected at farm level by both farmers and animal health care providers. Therefore most farms do not pay attention to it because there are no obvious financial costs attributed to it thus limited control measures are paid to curb it.

From the current study, the most common bacteria isolated from mastitic milk were the Coagulase-negative staphylococci. This was in agreement with a study done by Pitkälä *et al*., (2004), Pyörälä & Taponen, (2009) and Vakkamäki *et al*., (2017) in Finland and Mpatswenumugabo *et al*., (2017) in Ethiopia showing that the group is an emerging cause of subclinical mastitis. According to Sánchez *et al*. (2018) coagulase negative staphylococci are the most prevalent mastitis causing pathogens in Anaime Canyon, a dairy region in Colombia. This disagreed with a study done by Gitau *et al*., (2014) whose findings showed that *Staphylococcus aureus* is the commonest bacteria causing bovine mastitis in Mukurueini and Nakuru Districts of Kenya. Generally organisms in the staphylococcal group are the main pathogens causing mastitis in dairy cows as also documented by Ndirangu *et al*., (2017) in a study carried out at the Kenya Agricultural & Livestock Research Organization (KALRO) in Naivasha, Kenya. Coagulase negative staphylococci are emerging mastitis causing pathogens that are becoming the most prevalent pathogens isolated in mastitic milk in many countries (Taponen & Pyörälä, 2009).

The current study demonstrated that management factors in different farms contribute to the difference in prevalence of mastitis. Gicheha farm whose barn floor was earthen, and with more animals having dirty udders had more prevalence of mastitis compared to Chemusian farm wose barn floor was concrete, thus easy to clean.

From the current study, the hind quarters were more likely to develop both clinical and subclinical mastitis compared to forequarters. This was in agreement with a study done by Vulić, (2000) and Khan & Muhammad (2005) in Faisalabad in Pakistan, Joshi & Gokhale, 2006, Tripathi *et al*., (2018) in India on cross breed cows, Hussain *et al*., (2018) in a study on dairy buffaloes in Pakistan. This may be partly due to the fact that hind quarters are more frequently dirtied from dung and the floor. Furthermore, hind quarters are more vulnerable to direct trauma due to their proximity to the ground compared to forequarters.

This study showed that cows sleeping on concrete floor are less susceptible to mastitis compared to those sleeping on earthen floors. This is in agreement with a study by Kayesh *et al*. (2014) who reported 36.69% and 23.7% prevalence in subclinical mastitis for cows sleeping on earthen and concrete floors respectively in Bangladesh. This is because concrete floors are easier to clean, thus environmental pathogens are washed off more easily than earthen floors.

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Based on the current study, the following conclusions can be made:

1. Use of bismuth subnitrate teat canal sealant and Ampicillin+Cloxacillin combination during the dry period significantly reduces occurrence of mastitis compared to use of Ampicillin+Cloxacillin dry cow therapy alone
2. Subclinical mastitis is more common than clinical mastitis in both Chemusian and Gicheha Farms
3. Coagulase negative staphylococci pathogens are the most common mastitis causing pathogens in both Gicheha and Chemusian farm
4. Bovine hind quarters are more prone to mastitis compared to forequarters
5. Apart from the type of dry cow treatment used, farm, type of barn floor and position of the quarter, all other factors under study: age, lactation number, breed, udder hygiene score were not associated with bovine mastitis

6.2 Recommendations

I. The study recommends the use of a combination of bismuth subnitrate teat canal sealant Ampicillin+Cloxacillin dry cow therapy for the reduction of the prevalence of bovine mastitis 100 days post-calving.

II. It is very important for farms to observe hygiene of milking machines, the barn floor and the general environment of the cow in order to reduce prevalence of mastitis, especially from environmental mastitis

7.0 REFERENCES

Abebe, R., Hatiya, H., Abera, M., Megersa, B., and 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*. https://doi.org/10.1186/s12917-016-0905-3

Barlow, J. (2011). Mastitis therapy and antimicrobial susceptibility: A multispecies review with a focus on antibiotic treatment of mastitis in dairy cattle. *Journal of Mammary Gland Biology and Neoplasia*. https://doi.org/10.1007/s10911-011-9235-z

Bates, A. J., Chambers, G., and Laven, R. A. (2016). Comparison of cephalonium alone and in combination with an internal teat sealant for dry cow therapy in seasonally calving dairy cows. *New Zealand Veterinary Journal*. https://doi.org/10.1080/00480169.2015.1093970

Bautista-Trujillo, G. U., Solorio-Rivera, J. L., Rentería-Solórzano, I., Carranza-Germán, S. I., Bustos-Martínez, J. A., Arteaga-Garibay, R. I., and Valdez-Alarcón, J. J. (2013). Performance of culture media for the isolation and identification of Staphylococcus aureus from bovine mastitis. *Journal of Medical Microbiology*. https://doi.org/10.1099/jmm.0.046284-0

Belayneh, R., Belihu, K., and Wubete, A. (2013). Dairy cows mastitis survey in Adama Town, Ethiopia. *Journal of Veterinary Medicine and Animal Health*. https://doi.org/10.5897/JVMAH2013.0222

Berry, E. A., and Hillerton, J. E. (2010). Effect of an Intramammary Teat Seal and Dry Cow Antibiotic in Relation to Dry Period Length on Postpartum Mastitis. *Journal of Dairy Science*. https://doi.org/10.3168/jds.s0022-0302(07)71560-6

Bharti, P., Bhakat, C., Pankaj, P. K., Bhat, S. A., Arul Prakash, M., Thul, M. R., and Puhle Japheth, K. (2015). Relationship of udder and teat conformation with intra-mammary infection in crossbred cows under hot-humid climate. *Veterinary World*. https://doi.org/10.14202/vetworld.2015.898-901

Blowey, R., and Edmondson, P. (2010). *Mastitis Control in Dairy Herds*. *Control*. https://doi.org/10.1017/CBO9781107415324.004

Bradley, A. J., Breen, J. E., Payne, B., and 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*. https://doi.org/10.3168/jds.2010-3192

Bradley, A. J., Breen, J. E., Payne, B., Williams, P., and 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*. https://doi.org/10.3168/jds.2009-2725

Breen, J. E., Green, M. J., and Bradley, A. J. (2009). Quarter and cow risk factors associated with the occurrence of clinical mastitis in dairy cows in the United Kingdom. *Journal of Dairy Science*. https://doi.org/10.3168/jds.2008-1369

Compton, C. W. R., Emslie, F. R., and McDougall, S. (2014). Randomised controlled trials demonstrate efficacy of a novel internal teat sealant to prevent new intramammary infections in dairy cows and heifers. *New Zealand Veterinary Journal*. https://doi.org/10.1080/00480169.2014.898201

Cook, N. B., Pionek, D. A., and Sharp, P. (2005). An assessment of the benefits of Orbeseal when used in combination with dry cow antibiotic therapy in three commercial dairy herds.

Bovine Pract.

Deb, R., Kumar, A., Chakraborty, S., Verma, A. K., Tiwari, R., Dhama, K., and Kumar, S. (2013). Trends in diagnosis and control of bovine mastitis: a review. *Pakistan Journal of Biological Sciences : PJBS*. https://doi.org/10.3923/pjbs.2013.1653.1661

del Pilar Sánchez Bonilla, M., Murillo, N. P. G., and Almanza, I. J. P. (2018). Prevalence of bovine mastitis in the anaime canyon, a colombian dairy region, including etiology and antimicrobial resistance. *Revista de Investigaciones Veterinarias Del Peru*. https://doi.org/10.15381/rivep.v29i1.14084

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

Dingwell, R. T., Leslie, K. E., Schukken, Y. H., Sargeant, J. M., and Timms, L. L. (2003). Evaluation of the California mastitis test to detect an intramammary infection with a major pathogen in early lactation dairy cows. *Canadian Veterinary Journal*. https://doi.org/2003-044

Dosogne, H., Vangroenweghe, F., and Burvenich, C. (2002). Potential mechanism of action of J5 vaccine in protection against severe bovine coliform mastitis. *Veterinary Research*. https://doi.org/10.1051/vetres:2001001

Down, P. M., Bradley, A. J., Breen, J. E., Hudson, C. D., and Green, M. J. (2016). Current management practices and interventions prioritised as part of a nationwide mastitis control plan. *Veterinary Record*. https://doi.org/10.1136/vr.103203

Fosgate, G. T., Petzer, I. M., and Karzis, J. (2013). Sensitivity and specificity of a hand-held milk electrical conductivity meter compared to the California mastitis test for mastitis in dairy cattle. *Veterinary Journal*. https://doi.org/10.1016/j.tvjl.2012.07.026

Fox, L. K. (2009). Prevalence, incidence and risk factors of heifer mastitis. *Veterinary Microbiology*. https://doi.org/10.1016/j.vetmic.2008.09.005

Garcia, A. (2004). Contagious vs. Environmental Mastitis. *Dairy Science, ExEx 4028*(January).

Garoussi, M. T., Atarie, S., Vodjgani, M., Gharagozloo, F., and Vand e ussefi, J. (2017). The prevalence and control of bovine mastitis in Leptospira outbreak. *Comparative Clinical Pathology*. https://doi.org/10.1007/s00580-016-2367-1

Getahun, K., Kelay, B., Bekana, M., and Lobago, F. (2008). Bovine mastitis and antibiotic resistance patterns in Selalle smallholder dairy farms, central Ethiopia. *Tropical Animal Health and Production*. https://doi.org/10.1007/s11250-007-9090-5

Girma, S., Mammo, A., Bogele, K., Sori, T., Tadesse, F., and Jibat, T. (2012). Study on prevalence of bovine mastitis and its major causative agents in West Harerghe zone, Doba district, Ethiopia. *Journal of Veterinary Medicine and Animal Health*. https://doi.org/10.5897/JVMAH12.016

Gitau, G. K., Bundi, R. M., Vanleeuwen, J., and Mulei, C. M. (2014). Mastitogenic bacteria isolated from dairy cows in Kenya and their antimicrobial sensitivity. *Journal of the South African Veterinary Association*. https://doi.org/10.4102/jsava.v85i1.950

Golder, H. M., Hodge, A., and 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*. https://doi.org/10.3168/jds.2016-11114

Gomes, F., and Henriques, M. (2016a). Bovine Mastitis: Current Concepts and Future Control Approaches. *Current Microbiology*. https://doi.org/10.1007/s00284-015-0958-8

Gomes, F., and Henriques, M. (2016b). Control of Bovine Mastitis: Old and Recent Therapeutic Approaches. *Current Microbiology*. https://doi.org/10.1007/s00284-015-0958-8

Gonzalez, R. N., and Wilson, D. J. (2003). Mycoplasmal mastitis in dairy herds. *Vet.Clin.North Am.Food Anim.Pract.* https://doi.org/2003-006

Green, M. J., Bradley, A. J., Medley, G. F., and Browne, W. J. (2008). Cow, Farm, and Herd Management Factors in the Dry Period Associated with Raised Somatic Cell Counts in Early Lactation. *Journal of Dairy Science*. https://doi.org/10.3168/jds.2007-0621

Gundelach, Y., Kalscheuer, E., Hamann, H., and Hoedemaker, M. (2011). Risk factors associated with bacteriological cure, new infection, and incidence of clinical mastitis after dry cow therapy with three different antibiotics. *Journal of Veterinary Science*. https://doi.org/10.4142/jvs.2011.12.3.227

Hussain, A., Ahmad, M., Mushtaq, M. H., Chaudhry, M., Khan, M. S., and Reichel, M. (2018). Prevalence of Overall and Teatwise Mastitis and Effect of Herd Size in Dairy Buffaloes, *50*(3), 1107–1112.

Huxley, J. N., Green, M. J., Green, L. E., and Bradley, A. J. (2002). Evaluation of the Efficacy of an Internal Teat Sealer During the Dry Period. *Journal of Dairy Science*. https://doi.org/10.3168/jds.S0022-0302(02)74108-8

Ikiz, S., Başaran, B., Bingöl, E. B., çetin, Ö., Kaşikçi, G., Özgür, N. Y., and Sabuncu, A.

(2013). Presence and antibiotic susceptibility patterns of contagious mastitis agents (staphylococcus aureus and streptococcus agalactiae) isolated from milks of dairy cows with subclinical mastitis. *Turkish Journal of Veterinary and Animal Sciences*. https://doi.org/10.3906/vet-1302-63

Jones, G. M., and Bailey, T. L. (2009). Understanding the Basics of Mastitis. *Virginia Cooperative Extension*. https://doi.org/10.1108/02621711111098406

Joshi, S., and Gokhale, S. (2006). Status of mastitis as an emerging disease in improved and periurban dairy farms in India. In *Annals of the New York Academy of Sciences*. https://doi.org/10.1196/annals.1373.007

Kandeel, S. A., Morin, D. E., Calloway, C. D., and 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*. https://doi.org/10.1111/jvim.14876

Karanja, A. M. (2003). The dairy industry in Kenya: The post-liberalization agenda. *Tegemeo Institute of Agricultural Policy and Development, Egerton University, Kenya.*

Kashongwe, O. B., Bebe, B. O., Matofari, J. W., and Huelsebusch, C. G. (2017). Associations between milking practices, somatic cell counts and milk postharvest losses in smallholder dairy and pastoral camel herds in Kenya. *International Journal of Veterinary Science and Medicine*. https://doi.org/10.1016/j.ijvsm.2017.01.001

Kateete, D. P., Kimani, C. N., Katabazi, F. A., Okeng, A., Okee, M. S., Nanteza, A., and Najjuka, F. C. (2010). Identification of Staphylococcus aureus: DNase and Mannitol salt agar improve the efficiency of the tube coagulase test. *Annals of Clinical Microbiology and Antimicrobials*. https://doi.org/10.1186/1476-0711-9-23

Kayesh, M., Talukder, M., and Anower, A. (2014). Prevalence of subclinical mastitis and its association with bacteria and risk factors in lactating cows of Barisal district in Bangladesh. *International Journal of Biological Research*. https://doi.org/10.14419/ijbr.v2i2.2835

Khan, A. Z., and Muhammad, G. (2005). Quarter-wise comparative prevalence of mastitis in buffaloes and crossbred cows. *Pakistan Veterinary Journal*.

KNBS. (2010). The 2009 Kenya Population and Housing Census. *Home Healthcare Nurse*. https://doi.org/10.1097/00004045-200006000-00012

Koskinen, M. T., Wellenberg, G. J., Sampimon, O. C., Holopainen, J., Rothkamp, a, Salmikivi, L., and Pyörälä, S. (2010). Field comparison of real-time polymerase chain reaction and bacterial culture for identification of bovine mastitis bacteria. *Journal of Dairy Science*. https://doi.org/10.3168/jds.2010-3167

Kramer, M. F., and Coen, D. M. (2001). Enzymatic Amplification of DNA by PCR: Standard Procedures and Optimization. In *Current Protocols in Molecular Biology*. https://doi.org/10.1002/0471142727.mb1501s56

Lakew, M., Tolosa, T., and Tigre, W. (2009). Prevalence and major bacterial causes of bovine mastitis in Asella, South Eastern Ethiopia. *Tropical Animal Health and Production*. https://doi.org/10.1007/s11250-009-9343-6

Lam, T. J. G. M., van den Borne, B. H. P., Jansen, J., Huijps, K., van Veersen, J. C. L., van Schaik, G., and Hogeveen, H. (2013). Improving bovine udder health: A national mastitis control program in the Netherlands. *Journal of Dairy Science*. https://doi.org/10.3168/jds.2012-5958

Losinger, W. C. (2005). Economic impacts of reduced milk production associated with an increase in bulk-tank somatic cell count on US dairies. *Journal of the American Veterinary Medical Association*. https://doi.org/10.2460/javma.2005.226.1652

Mdegela, R. H., Ryoba, R., Karimuribo, E. D., Phiri, E. J., Løken, 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*. https://doi.org/10.4102/jsava.v80i3.195

Mekibib, B., Furgasa, M., Abunna, F., Megersa, B., and Regassa, A. (2010). Bovine mastitis: Prevalence, risk factors and major pathogens in dairy farms of holeta town, central Ethiopia. *Veterinary World*. https://doi.org/10.5455/vetworld.2010.397-403

Mellenberger, R., and Roth, C. J. (2009). California Mastitis Test (CMT) Fact Sheet. *Department of Animal Sciences, Michigan State University and Department of Dairy Science, University of Wisconsin-Madison*, 1–9.

Middleton, J. R., Luby, C. D., and Adams, D. S. (2009). Efficacy of vaccination against staphylococcal mastitis: A review and new data. *Veterinary Microbiology*. https://doi.org/10.1016/j.vetmic.2008.09.053

Mihret, T., Mitku, Fentahun, and Guadu, T. (2017). Dairy Farming and its Economic Importance in Ethiopia: A Review. *World Journal of Dairy & Food Sciences*. https://doi.org/10.5829/idosi.wjdfs.2017.42.51

Motaung, T. E., Petrovski, K. R., Petzer, I. M., Thekisoe, O., and Tsilo, T. J. (2017). Importance of bovine mastitis in Africa. *Animal Health Research Reviews*. https://doi.org/10.1017/S1466252317000032

Mpatswenumugabo, J. P., Bebora, L. C., Gitao, G. C., Mobegi, V. A., Iraguha, B., Kamana, O., and Shumbusho, B. (2017). Prevalence of Subclinical Mastitis and Distribution of Pathogens in Dairy Farms of Rubavu and Nyabihu Districts, Rwanda. *Journal of Veterinary Medicine*. https://doi.org/10.1155/2017/8456713

Munyori, K. J., and Karanja Ngugi, J. (2014). Factors affecting the growth of small and micro enterprises dairy farmers' in kenya: case of Gatundu south farmer's dairy co-operative society ltd. *International Journal of Current Business and Social Sciences | IJCBSS International Journal of Current Business and Social Sciences International Journal of Current Business and Social Sciences | IJCBSS*.

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*.

Muthami, R. B. and D. (2011). *The contribution of livestock to the Kenyan economy*. *IGAD Livestock Policy Initiative Working Paper*.

Mwinyelle, G. B., and Alhassan, A. (2014). Overview of bovine tuberculosis in Ghana. In *Zoonotic Tuberculosis: Mycobacterium bovis and Other Pathogenic Mycobacteria: 3rd Edition*. https://doi.org/10.1002/9781118474310.ch14

Naing, L., Winn, T., and Rusli, B. N. (2006). Practical Issues in Calculating the Sample Size for Prevalence Studies. *Archives of Orofacial Sciences*. https://doi.org/10.1146/annurev.psych.60.110707.163629

Naseemunnisa, M., Mahmood, S. K., and Mohammed, M. S. (2017). Determination of Bovine Mastitis in Cows by Considering the Different Parameters Like Lactation , Age , Breed , Quarters , Herd , and Season Wise in Kurnool , A . P ., *3*(1), 351–355.

National Mastitis Council. (2015). A practical look at contagious mastitis. *Global Milk Quality*. https://doi.org/10.1109/MDT.2004.44

Ndirangu, P. N., Siamba, D., Wesonga, H. O., Mungube, E. O., Maichomo, M. W., and Mugambi, J. M. (2017). Prevalence of bovine mastitis and multi-antibiotic resistant Staphylococcus and Streptococcus species in a research centre farm at Naivasha, Kenya. *Bulletin of Animal Health and Production in Africa*.

Newton, H. T., Green, M. J., Benchaoui, H., Cracknell, V., Rowan, T., and Bradley, a J. (2008). Comparison of the efficacy of cloxacillin alone and cloxacillin combined with an internal teat sealant for dry-cow therapy. *The Veterinary Record*. https://doi.org/10.1136/vr.162.21.678

Nicholas, R. A. J., Fox, L. K., and Lysnyansky, I. (2016). Mycoplasma mastitis in cattle: To cull or not to cull. *Veterinary Journal*. https://doi.org/10.1016/j.tvjl.2016.08.001

Nyman, A. K., Ekman, T., Emanuelson, U., Gustafsson, A. H., Holtenius, K., Waller, K. P., and Sandgren, C. H. (2007). Risk factors associated with the incidence of veterinary-treated clinical mastitis in Swedish dairy herds with a high milk yield and a low prevalence of subclinical mastitis. *Preventive Veterinary Medicine*.

https://doi.org/10.1016/j.prevetmed.2006.10.002

Olde Riekerink, R. G. M., Barkema, H. W., Kelton, D. F., and Scholl, D. T. (2008). Incidence Rate of Clinical Mastitis on Canadian Dairy Farms. *Journal of Dairy Science*. https://doi.org/10.3168/jds.2007-0757

Olde Riekerink, R. G. M., Barkema, H. W., and Stryhn, H. (2007). The effect of season on somatic cell count and the incidence of clinical mastitis. *Journal of Dairy Science*. https://doi.org/10.3168/jds.2006-567

Oliver, S. P., and Murinda, S. E. (2012). Antimicrobial resistance of mastitis pathogens. *Veterinary Clinics of North America - Food Animal Practice*. https://doi.org/10.1016/j.cvfa.2012.03.005

Oliver, S. P., Murinda, S. E., and Jayarao, B. M. (2011). Impact of Antibiotic Use in Adult Dairy Cows on Antimicrobial Resistance of Veterinary and Human Pathogens: A Comprehensive Review. *Foodborne Pathogens and Disease*. https://doi.org/10.1089/fpd.2010.0730

Parker, K. I., Compton, C., Anniss, F. M., Weir, A., Heuer, C., and McDougall, S. (2007). Subclinical and Clinical Mastitis in Heifers Following the Use of a Teat Sealant Precalving. *Journal of Dairy Science*. https://doi.org/10.3168/jds.S0022-0302(07)72622-X

Parker, K. I., Compton, C. W. R., Anniss, F. M., Heuer, C., and McDougall, S. (2008). Quarter-Level Analysis of Subclinical and Clinical Mastitis in Primiparous Heifers Following the Use of a Teat Sealant or an Injectable Antibiotic, or Both, Precalving. *Journal of Dairy Science*. https://doi.org/10.3168/jds.2007-0212

Pereira, U. P., Oliveira, D. G. S., Mesquita, L. R., Costa, G. M., and Pereira, L. J. (2011). Efficacy of Staphylococcus aureus vaccines for bovine mastitis: A systematic review. *Veterinary Microbiology*. https://doi.org/10.1016/j.vetmic.2010.10.003

Petersson-Wolfe, C. S., Mullarky, I. K., and Currin, J. (2011). Klebsiella spp.: A Practical Summary for Controlling Mastitis, 1–2.

Pitkälä, A., Haveri, M., Pyörälä, S., Myllys, V., and Honkanen-Buzalski, T. (2004). Bovine Mastitis in Finland 2001—Prevalence, Distribution of Bacteria, and Antimicrobial Resistance. *Journal of Dairy Science*. https://doi.org/10.3168/jds.S0022-0302(04)73366-4

Pumipuntu, N., Kulpeanprasit, S., Santajit, S., Tunyong, W., Kong-ngoen, T., Hinthong, W., and Indrawattana, N. (2017). Screening method for Staphylococcus aureus identification in subclinical bovine mastitis from dairy farms. *Veterinary World*. https://doi.org/10.14202/vetworld.2017.721-726

Pyörälä, S., and Taponen, S. (2009). Coagulase-negative staphylococci-Emerging mastitis pathogens. *Veterinary Microbiology*. https://doi.org/10.1016/j.vetmic.2008.09.015

Quinn, P. J., and Markey, B. K. (2003). Concise review of Veterinary microbiology. *Blackwell Publishingpress*.

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

Ramírez, N. F., Keefe, G., Dohoo, I., Sánchez, J., Arroyave, O., Cerón, J., and Palacio, L.

G. (2014). Herd- and cow-level risk factors associated with subclinical mastitis in dairy farms from the High Plains of the northern Antioquia, Colombia. *Journal of Dairy Science*. https://doi.org/10.3168/jds.2013-6815

Reneau, J. K., Seykora, A. J., Heins, B. J., Endres, M. I., Farnsworth, R. J., and F. Bey, R. (2005). Association between hygiene scores and somatic cell scores in dairy cattle. *Journal of the American Veterinary Medical Association*. https://doi.org/10.2460/javma.2005.227.1297

Riffon, R., Sayasith, K., Khalil, H., Dubreuil, P., Drolet, M., and Lagacé, J. (2001). Development of a rapid and sensitive test for identification of major pathogens in bovine mastitis by PCR. *Journal of Clinical Microbiology*. https://doi.org/10.1128/JCM.39.7.2584-2589.2001

Runciman, D. J., Malmo, J., and Deighton, M. (2010). The use of an internal teat sealant in combination with cloxacillin dry cow therapy for the prevention of clinical and subclinical mastitis in seasonal calving dairy cows. *Journal of Dairy Science*. https://doi.org/10.3168/jds.2009-2956

Salvador, R. T., Soliven, R. L., Balagan, E. J. Y., Abes, N. S., Gutierrez, C. A., and Mingala, C. N. (2014). Evaluation of a portable somatic cell counter in the diagnosis of bubaline subclinical mastitis. *Thai Journal of Agricultural Science*.

Sankar, P. (2016). New Therapeutic Strategies to Control and Treatment of Bovine Mastitis. *Veterinary Medicine - Open Journal*, *1*(2), e7–e8. https://doi.org/10.17140/VMOJ-1-e004

Scherpenzeel, C. G. M., den Uijl, I. E. M., van Schaik, G., Olde Riekerink, R. G. M.,

Keurentjes, J. M., and Lam, T. J. G. M. (2014). Evaluation of the use of dry cow antibiotics in low somatic cell count cows. *Journal of Dairy Science*. https://doi.org/10.3168/jds.2013-7655

Schreiner, D. A., and Ruegg, P. L. (2003). Relationship Between Udder and Leg Hygiene Scores and Subclinical Mastitis. *Journal of Dairy Science*. https://doi.org/10.3168/jds.S0022-0302(03)73950-2

Schreiner, D. A., and Ruegg, P. L. (2010a). Effects of Tail Docking on Milk Quality and Cow Cleanliness. *Journal of Dairy Science*. https://doi.org/10.3168/jds.s0022-0302(02)74333-6

Schreiner, D. A., and Ruegg, P. L. (2010b). Relationship Between Udder and Leg Hygiene Scores and Subclinical Mastitis. *Journal of Dairy Science*. https://doi.org/10.3168/jds.s0022-0302(03)73950-2

Sears, P. M., and McCarthy, K. K. (2003). Diagnosis of mastitis for therapy decisions. *Veterinary Clinics of North America - Food Animal Practice*. https://doi.org/10.1016/S0749-0720(02)00074-9

Shaheen, M., Tantary, H., and Nabi, S. (2016). A Treatise on Bovine Mastitis: Disease and Disease Economics, Etiological Basis, Risk Factors, Impact on Human Health, Therapeutic Management, Prevention and Control Strategy. *Journal Advances in Dairy Research*. https://doi.org/10.4172/2329-888X.1000150

Sharma, N., Singh, N. K., and Bhadwal, M. S. (2011). Relationship of somatic cell count and mastitis: An overview. *Asian-Australasian Journal of Animal Sciences*. https://doi.org/10.5713/ajas.2011.10233

Sharma, S., Sharma, V., Dahiya, D. K., Khan, A., Mathur, M., and Sharma, A. (2017). Prevalence, Virulence Potential, and Antibiotic Susceptibility Profile of Listeria monocytogenes Isolated From Bovine Raw Milk Samples Obtained From Rajasthan, India. *FOODBORNE PATHOGENS AND DISEASE*. https://doi.org/10.1089/fpd.2016.2118

Shome, B. R., Das Mitra, S., Bhuvana, M., Krithiga, N., Velu, D., Shome, R., and Rahman, H. (2011). Multiplex PCR assay for species identification of bovine mastitis pathogens. *Journal of Applied Microbiology*. https://doi.org/10.1111/j.1365-2672.2011.05169.x

Song, Y., Huang, Y., Hu, G., Guo, X., Cao, H., Zhang, C., and Liu, P. (2017). Microbial diversity in milk from holstein dairy cattle with mastitis in Southern China using illumina MiSeq-based analysis. *Pakistan Veterinary Journal*.

Swartz, T., Student, P. D., Science, D., and Tech, V. (2016). Corynebacterium bovis : A Practical Summary for Controlling Mastitis, 2016.

Taponen, S., and Pyörälä, S. (2009). Coagulase-negative staphylococci as cause of bovine mastitis-Not so different from Staphylococcus aureus? *Veterinary Microbiology*. https://doi.org/10.1016/j.vetmic.2008.09.011

Tripathi, S., Shekhar, S., and Rajora, V. (2018). Etio-prevalence of sub clinical mastitis in crossbred cattle. *Journal of Entomology and Zoology Studies*.

Vakkamäki, J., Taponen, S., Heikkilä, A. M., and Pyörälä, S. (2017). Bacteriological etiology and treatment of mastitis in Finnish dairy herds. *Acta Veterinaria Scandinavica*. https://doi.org/10.1186/s13028-017-0301-4

Viguier, C., Arora, S., Gilmartin, N., Welbeck, K., and O'Kennedy, R. (2009). Mastitis detection: current trends and future perspectives. *Trends in Biotechnology*. https://doi.org/10.1016/j.tibtech.2009.05.004

Vishnupriya, S., Antony, P. X., Mukhopadhyay, H. K., Pillai, R. M., Thanislass, J., Vivek Srinivas, V. M., and Kumar, R. S. (2014). Methicillin resistant staphylococci associated with bovine mastitis and their zoonotic importance. *Veterinary World*. https://doi.org/10.14202/vetworld.2014.422-427

Vulić, B. (2000). The influence of different factors on the results of mastitis tests at the "Hana-Šipovac" dairy cattle farm. *Veterinarski Arhiv*.

Wellenberg, G. J., Van Der Poel, W. H. M., and Van Oirschot, J. T. (2002). Viral infections and bovine mastitis: A review. *Veterinary Microbiology*. https://doi.org/10.1016/S0378-1135(02)00098-6

WHO. (2014). Antimicrobial resistance. Global report on surveillance. *World Health Organization*. https://doi.org/10.1007/s13312-014-0374-3

Woolford, M. W., Williamson, J. H., Day, A. M., and Copeman, P. J. A. (1998). The prophylactic effect of a teat sealer on bovine mastitis during the dry period and the following lactation. *New Zealand Veterinary Journal*. https://doi.org/10.1080/00480169.1998.36044

Yalçin, C. (2000). Cost of Mastitis in Scottish Dairy Herds with Low and High Subclinical Mastitis Problems. *Turkish Journal of Veterinary and Animal Sciences*.

8.0 APPENDICES

Appendix 8.1: Dry cow preparations used in the study

Plate 8.1a: Test product,Bismuth subnitrate (Boviseal®)) tube

Plate 8.1b: Boviseal® pressed from the tube

Plate 8.1c: Standard product, Ampicillin+Cloxacillin (Bovaclox® DC)

Appendix 8.2: Pictures of Lab Work

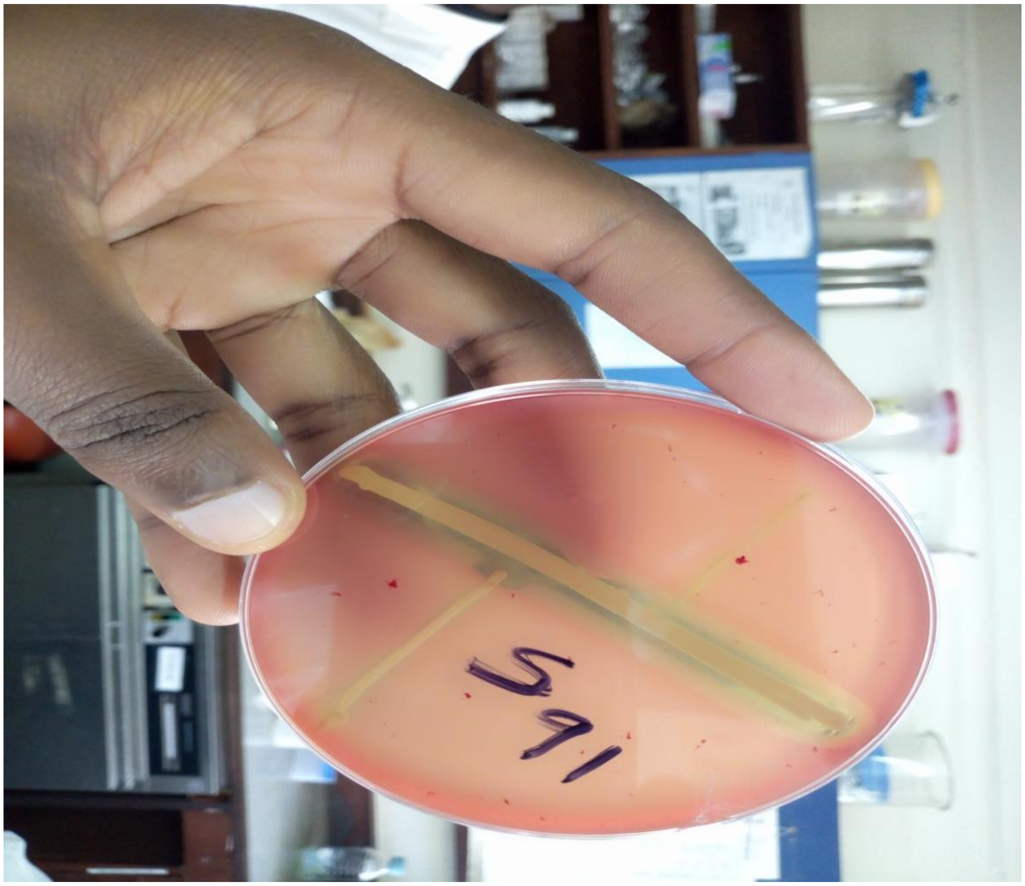

Plate 8.2a: Positive sample of *Streptococcus agalactiae* (CAMP positive) on Blood Agar

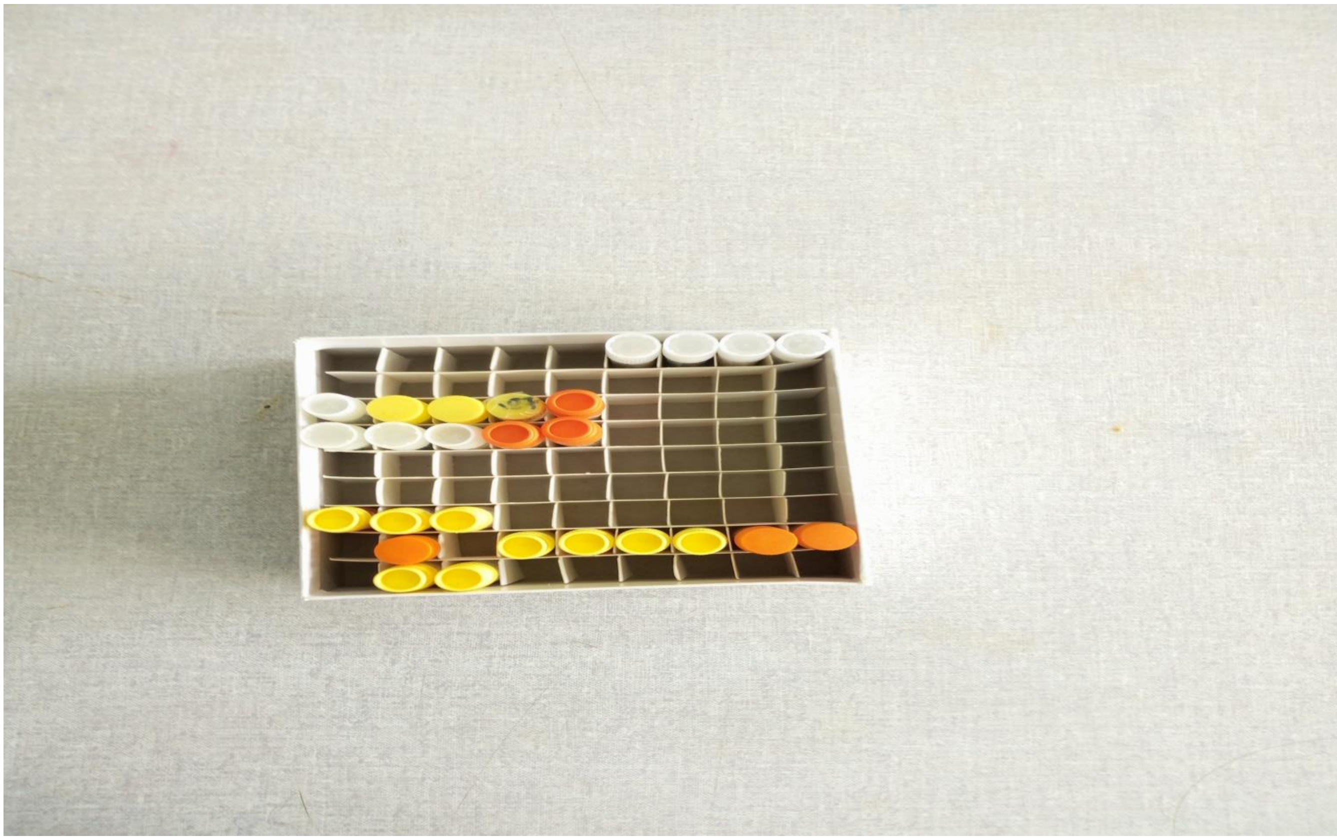

Plate 8.2b: Positive samples for various bacteria for storage

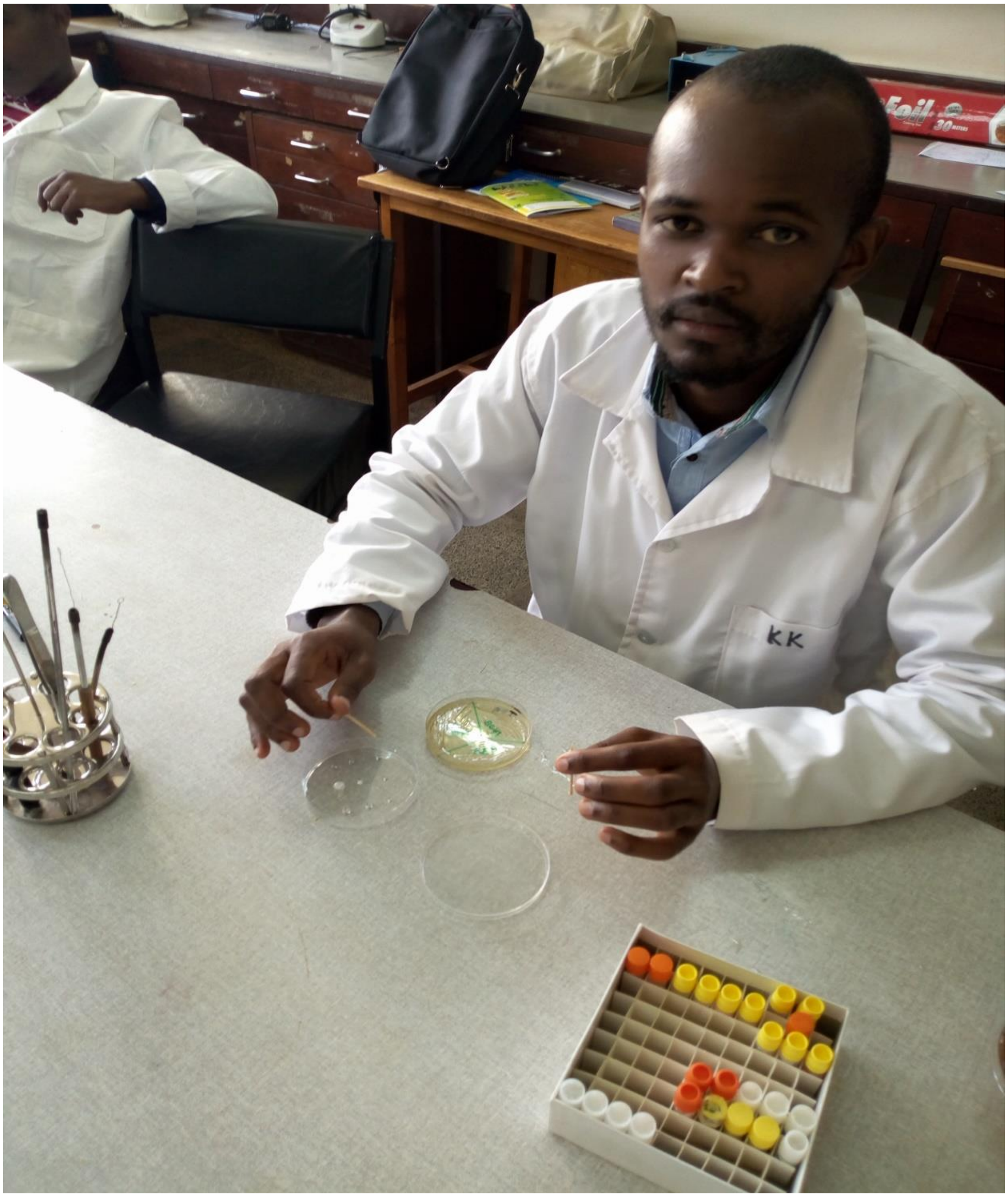

Plate 8.2c: Conducting catalase test for *Staphylococcus aureus*

Appendix 8.3: Farm and herd characteristics and findings on mastitis for Chemusian and Gicheha farms as at 2017/2018

												CMT SCORES				MASTITIS						
FARM NO	**LAB NO**	**FARM**	**MLK**	**Milk prdn**	**AVR MILK**	**Mngt**	**U.HYG**	**Brd**	**Age (yrs)**	**Prty**	**GROUP**	**cmt 1**	**cmt 2**	**cmt 3**	**cmt 4**	**OVER**	**CL**	**SUB**	**Wk**	**mnt**	**ORG**	**BARN**
5330	1	1	1	7625	25	2	0	1	1	1	0	0	0	1	0	1	0	1	12	3	2	0
246	2	1	1	8235	27	2	1	1	1	1	1	0	0	0	0	0	0	0	4	1	5	0
13 CC 125	3	1	1	7015	23	2	0	1	0	0	0	0	0	1	0	1	0	1	4	1	1	0
85	4	1	1	6205	20	2	1	2	1	1	0	0	1	1	0	1	0	1	5	2	1	0
350	5	1	1	7100	24	2	0	1	1	1	0	1	3	3	0	1	1	0	4	1	6	0
14 CC	6	1	1	7930	26	2	1	1	0	0	0	1	0	0	0	1	0	1	4	1	7	0

81																						
421	7	1	1	7900	26	2	1	1	0	0	0	1	1	1	1	1	0	1	12	3	1	0
56	8	1	1	7300	24	2	0	1	0	0	0	0	1	0	0	1	0	1	10	3	5	0
641	9	1	1	6750	23	2	1	1	0	0	1	0	0	0	0	0	0	0	12	3	0	0
331	10	1	1	7200	24	2	0	1	0	0	1	1	0	0	0	1	0	1	5	2	1	0
151	11	1	1	7910	26	2	1	1	1	1	1	1	1	1	1	1	0	1	12	3	5	0
512	12	1	1	8395	28	2	0	1	0	1	1	0	1	0	0	1	0	1	8	2	5	0
522	13	1	1	7300	24	2	0	1	1	1	0	0	0	1	1	1	0	1	4	1	1	0
673	14	1	1	7200	24	2	0	1	1	1	0	0	0	1	0	1	0	1	2	1	1	0
14 CC 31	15	1	1	7500	25	2	0	1	1	1	0	0	1	0	0	1	0	1	4	1	1	0
295	16	1	1	7300	24	2	1	1	1	1	0	0	0	0	0	0	0	0	12	3	0	0
166	1	1	1	76	25	2	0	1	1	1	0	0	0	0	0	0	0	0	1	3	0	0

	7			25															2			
352	18	1	1	7665	26	2	1	1	0	1	1	1	1	0	0	1	0	1	2	1	5	0
14 CC 72	19	1	1	7320	24	2	0	1	0	0	0	1	0	0	0	1	0	1	4	1	1	0
14 CC 73	20	1	1	6205	21	2	0	2	0	0	1	0	0	0	1	1	0	1	4	1	1	0
527	21	1	1	6205	21	2	0	2	1	1	0	0	0	0	0	0	0	0	10	3	1	0
635	22	1	1	6205	21	2	1	1	1	0	0	1	1	0	0	1	0	1	8	2	5	0
610	23	1	1	5490	18	2	1	2	1	1	0	1	1	1	1	1	1	0	8	2	0	0
355	24	1	1	7300	24	2	1	1	1	1	0	0	0	0	0	0	0	0	1	1	0	0
14 CC 25	25	1	1	7665	26	2	1	1	0	0	1	0	0	0	0	0	0	0	7	2	1	0
506	26	1	1	8235	27	2	1	1	1	1	0	1	1	1	1	1	0	1	2	1	1	0
14	2	1	1	65	22	2	1	1	0	0	0	1	1	1	1	1	0	1	5	2	1	0

CC9	7			70																		
118	28	1	1	5490	18	2	0	2	1	1	0	1	1	1	1	1	0	1	2	1	1	0
13CC42	29	1	1	7625	25	2	0	1	0	0	0	0	3	2	1	1	1	0	3	1	5	0
13CC147	30	1	1	6205	21	2	1	1	0	0	0	0	1	1	0	1	0	1	2	1	1	0
555	31	1	1	7015	23	2	1	1	0	0	0	0	1	1	0	1	0	1	7	2	1	0
14CC53	32	1	1	7300	24	2	1	1	0	0	1	0	0	0	0	0	0	0	10	3	0	0
588	33	1	1	7100	24	2	1	1	1	1	0	0	1	0	1	1	0	1	12	3	1	0
552	34	1	1	7000	23	2	1	1	0	0	1	0	0	0	0	0	0	0	12	3	0	0
587	35	1	1	6935	23	2	1	1	1	1	0	0	3	1	0	1	1	0	8	2	1	0
14CC	36	1	1	5840	19	2	1	1	0	0	0	0	0	0	0	0	0	0	10	3	0	0

13																						
14100	37	1	1	6970	23	2	1	1	1	1	0	0	0	1	0	0	0	0	9	3	1	0
467	38	1	1	8760	29	2	0	1	1	1	0	0	1	1	0	1	0	1	2	1	1	0
13CC111	39	1	1	8200	27	2	0	1	1	1	1	1	1	0	0	0	0	0	4	1	0	0
14CC26	40	1	1	6840	23	2	0	2	1	0	1	0	0	0	0	0	0	0	4	1	0	0
13CC126	41	1	1	6570	22	2	0	2	0	0	0	0	0	0	0	0	0	0	4	1	0	0
49	42	1	1	9124	30	2	0	1	1	1	0	1	1	1	1	1	0	1	2	1	6	0
14CC107	43	1	1	8540	28	2	0	1	0	0	0	1	1	1	1	1	0	1	2	1	1	0
14CC96	44	1	1	6570	22	2	1	1	0	0	0	1	0	0	0	1	0	1	2	1	2	0
845	4	1	1	76	25	2	1	1	1	1	1	0	0	0	0	0	0	0	1	3	0	0

	5			25															2			
462	46	1	1	7300	24	2	0	1	1	1	1	0	0	0	0	0	0	0	2	1	2	0
13 CC 132	47	1	1	8030	27	2	1	1	0	0	1	0	1	0	0	1	0	1	8	2	6	0
14 CC 71	48	1	1	7625	15	2	1	2	1	0	1	0	0	0	0	0	0	0	3	1	0	0
14 CC 24	49	1	1	6570	22	2	1	2	0	0	0	1	2	3	1	1	1	0	5	2	2	0
	64	1	1	7100	24	2	1	1	1	1	0	0	0	0	0	0	0	0	5	2	0	0
641	65	1	1	6000	20	2	1	1	1	1	0	3	3	3	2	1	1	0	7	2	1	0
14 CC 127	66	1	1	7200	24	2	1	1	1	1	1	1	1	3	1	1	1	0	7	2	0	0
462	67	1	1	7205	24	2	1	1	0	0	1	0	0	0	0	0	0	0	6	2	0	0
13 CC	68	1	1	6500	22	2	1	1	0	0	1	0	0	0	0	0	0	0	6	2	0	0

114																						
12 CC 85	69	1	1	7300	24	2	0	1	1	1	1	0	0	0	0	0	0	0	6	2	0	0
149	70	1	1	9000	30	2	0	1	1	1	0	0	0	0	0	0	0	0	5	2	0	0
12 CC 139	71	1	1	8030	27	2	0	1	1	1	1	0	0	0	0	0	0	0	5	2	0	0
14 CC 54	72	1	1	7300	24	2	0	1	0	0	1	0	0	0	0	0	0	0	4	1	0	0
94	73	1	1	8395	28	2	1	1	1	1	0	2	2	3	2	1	1	0	4	1	0	0
13 CC 80	74	1	1	7300	24	2	0	1	0	1	1	0	1	0	0	1	0	1	8	2	1	0
14 CC 53	75	1	1	6205	21	2	0	1	1	1	1	0	0	0	0	0	0	0	4	1	0	0
14 CC 116	76	1	1	6570	22	2	0	1	1	0	0	0	0	0	0	1	0	0	4	1	1	0

295	77	1	1	7300	24	2	0	1	1	1	0	3	3	3	3	1	1	0	7	2	1	0
599	78	1	1	6100	20	2	0	1	1	1	0	0	0	0	0	0	0	0	5	2	0	0
14CC23	79	1	1	6570	22	2	0	1	0	0	0	0	0	0	0	0	0	0	5	2	0	0
14CC43	80	1	1	6800	23	2	1	1	0	0	1	0	0	0	0	0	0	0	5	2	0	0
529	81	1	1	6800	23	2	1	1	1	1	0	0	0	0	0	0	0	0	5	2	0	0
583	82	1	1	7900	26	2	1	1	1	1	0	1	0	0	0	1	0	1	5	2	1	0
14CC81	83	1	1	7930	26	2	1	1	0	0	1	0	0	0	0	0	0	0	5	2	0	0
611	84	1	1	5678	19	2	0	1	1	1	0	1	1	0	0	1	0	1	5	2	1	0
13CC138	85	1	1	6570	22	2	0	1	0	0	1	0	0	0	0	0	0	0	5	2	5	0
N0	8	1	1	80	27	2	0	1	0	0	0	0	0	1	0	1	0	1	3	1	8	0

2	6			30																		
14 CC 58	87	1	1	6570	22	2	0	1	0	1	1	0	0	0	0	0	0	0	3	1	0	0
14 CC 105	88	1	1	8540	28	2	0	1	0	0	1	0	0	0	0	0	0	0	10	3	0	0
13 CC 43	89	1	1	7290	24	2	0	1	1	1	1	0	0	0	0	0	0	0	10	3	0	0
13 CC 84	90	1	1	8400	28	2	1	1	0	1	1	0	0	0	0	0	0	0	5	2	0	0
438	91	1	1	7625	25	2	1	1	1	1	1	0	0	0	0	0	0	0	5	2	5	0
13 CC 21	92	1	1	8030	27	2	0	1	0	1	1	0	0	1	0	1	0	1	12	3	5	0
457	93	1	1	6100	20	2	0	1	0	1	1	0	0	0	0	0	0	0	5	2	0	0
521	94	1	1	7300	24	2	0	1	1	1	1	0	1	1	0	1	0	1	5	2	3	0
14	9	1	1	61	20	2	0	1	0	0	1	0	0	0	0	0	0	0	3	1	1	0

CC80	5			00																		
194	96	1	1	8394	28	2	0	1	1	1	1	0	0	0	0	0	0	0	3	1	0	0
399	97	1	1	7625	25	2	0	1	1	1	0	2	2	3	2	1	1	0	3	1	1	0
13CC3	98	1	1	7665	26	2	0	1	0	0	1	0	0	0	0	0	0	0	8	2	0	0
676	99	1	1	8540	28	2	0	1	1	1	0	0	1	0	0	1	0	1	5	2	1	0
544	100	1	1	7625	25	2	0	1	1	1	0	3	3	3	2	1	1	0	8	2	5	0
153	101	1	1	6570	22	2	0	1	1	1	0	1	0	0	0	1	0	1	2	1	1	0
106	102	1	1	7200	24	2	1	1	0	0	0	0	0	0	0	0	0	0	8	2	0	0
698	103	1	1	8720	29	2	1	1	0	1	1	0	0	0	0	0	0	0	10	3	0	0

13CC02	104	1	1	7665	26	2	1	1	0	0	1	0	0	0	0	0	0	0	12	3	0	0
125	105	1	1	7930	26	2	0	1	0	0	0	1	0	1	1	1	0	1	3	1	5	0
13CC130	106	1	1	8030	27	2	0	1	0	0	1	0	0	0	0	0	0	0	3	1	0	0
14CC127	107	1	1	7300	24	2	0	1	0	0	1	0	0	0	0	0	0	0	3	1	0	0
667	108	1	1	8760	29	2	0	1	1	1	1	0	0	0	0	0	0	0	3	1	0	0
149	109	1	1	9125	30	2	0	1	1	1	1	0	0	0	0	0	0	0	3	1	0	0
678	110	1	1	6800	23	2	0	1	0	0	1	0	0	0	0	0	0	0	3	1	0	0
14CC	11	1	1	8540	28	2	1	1	0	0	1	0	0	0	0	0	0	0	3	1	0	0

105	1																					
696	112	1	1	7625	25	2	1	1	1	1	1	0	0	0	0	0	0	0	7	2	0	0
567	113	1	1	7200	24	2	0	1	1	1	1	0	0	0	0	0	0	0	7	2	0	0
624	114	1	1	6205	21	2	0	1	1	1	1	0	0	0	0	0	0	0	7	2	0	0
424	115	1	1	8395	28	2	1	1	1	1	0	0	0	1	0	1	0	1	3	1	1	0
615	116	1	1	7200	24	2	1	1	0	0	1	0	0	0	0	0	0	0	7	2	0	0
13CC8	117	1	1	7300	24	2	0	1	0	0	1	0	0	0	0	0	0	0	7	2	0	0
14CC150	118	1	1	6420	21	2	0	1	1	1	1	0	0	0	0	0	0	0	7	2	0	0
11	1	1	1	72	24	2	0	1	1	1	1	0	0	0	0	0	0	0	7	2	0	0

CC72	119			00																		
13CC154	120	1	1	6300	21	2	0	1	0	1	0	0	1	0	0	1	0	1	3	1	1	0
14CC56	121	1	1	7665	26	2	0	1	0	0	1	0	0	0	0	0	0	0	4	1	0	0
303	122	1	1	6935	23	2	1	1	1	1	1	0	0	0	0	0	0	0	4	1	0	0
14CC18	123	1	1	7300	24	2	1	1	0	0	0	0	0	0	0	1	1	0	4	1	0	0
541	124	1	1	8100	27	2	0	2	1	1	1	0	0	0	0	0	0	0	4	1	0	0
568	125	1	1	7625	25	2	1	1	1	0	1	0	0	0	0	0	0	0	4	1	0	0
173	126	1	1	7625	25	2	0	1	0	1	1	0	0	0	0	0	0	0	4	1	0	0

709	127	1	1	6540	22	2	1	1	1	0	1	0	0	0	0	0	0	0	4	1	0	0
14CC57	128	1	1	6570	22	2	1	1	0	0	0	1	0	0	0	1	0	1	4	1	1	0
548	136	1	1	6500	22	2	1	1	0	0	0	3	3	3	3	1	1	0	4	1	1	0
SALAWA	137	1	1	7300	24	2	0	2	0	1	0	0	1	2	0	1	0	1	4	1	1	0
13CC152	138	1	1	7350	25	2	0	1	0	0	0	1	2	1	1	1	0	1	8	2	1	0
13CC105	139	1	1	7000	23	2	1	1	0	1	1	0	0	0	0	0	0	0	8	2	0	0
13CC80	140	1	1	7200	24	2	1	1	0	0	1	0	0	0	0	0	0	0	7	2	0	0
13	1	1	1	72	24	2	1	1	0	0	0	0	1	1	1	1	0	1	4	1	1	0

CC159	41			00																	
99	142	1	1	7000	23	2	1	1	0	1	1	0	0	0	0	0	0	0	6	2	0	0
14CC127	143	1	1	7300	24	2	1	1	0	0	0	1	1	1	1	1	0	1	4	1	1	0
512	144	1	1	7200	24	2	1	1	0	1	0	1	1	1	1	1	0	1	4	1	1	0
12CC94	145	1	1	6800	23	2	0	1	1	1	0	0	1	1	0	1	0	1	4	1	1	0
AW1349	146	2	1	6494	22	2	1	1	1	1	0	0	0	1	0	1	0	1	4	1	4	1
WN1412	147	2	1	5768	19	2	1	2	1	1	0	2	2	3	2	1	0	1	4	1	1	1
AW161	148	2	1	7243	24	2	1	1	1	1	0	1	3	3	3	1	0	1	4	1	1	1

1ST LADY	149	2	1	5200	17	2	1	1	0	0	0	1	1	1	0	1	0	1	4	1	5	1
WN941	150	2	1	6615	22	2	1	1	1	1	0	1	1	1	0	1	0	1	4	1	5	1
WN2032	151	2	1	6803	21	2	1	1	1	1	1	2	3	3	2	1	1	0	4	1	1	1
WB61	152	2	1	5869	20	2	1	1	1	1	0	1	2	3	1	1	0	1	4	1	1	1
WN2849	153	2	1	6801	23	2	1	2	1	1	0	2	3	1	2	1	0	1	4	1	1	1
WB262	154	2	1	5086	27	2	1	1	0	0	1	0	0	1	0	1	0	1	4	1	1	1
CND986	155	2	1	6309	21	2	1	1	1	1	1	1	1	1	1	1	0	1	4	1	1	1
PE	1	2	1	58	29	2	1	2	1	1	0	0	1	1	1	1	0	1	4	1	4	1

MBA69	56			00																		
GF1640	129	2	1	6895	23	2	0	1	1	1	1	2	2	3	2	1	0	1	10	3	1	1
WN2167	130	2	1	7272	24	2	0	1	1	1	0	1	3	3	1	1	1	0	8	2	0	1
WB181	131	2	1	6300	21	2	0	2	1	1	0	0	0	0	0	0	0	0	4	1	0	1
311	132	2	1	7100	24	2	0	1	0	1	1	0	0	0	0	0	0	0	4	1	0	1
WB40	133	2	1	6500	22	2	0	1	1	1	0	0	0	1	0	1	1	0	9	3	1	1
12	134	2	1	6100	20	2	1	1	0	0	1	0	0	0	0	0	0	0	4	1	0	1
SERE	13	2	1	6570	22	2	1	1	0	0	1	0	0	0	0	0	0	0	4	1	1	1

NGETI	5																					
WN2061	50	2	1	6272	21	2	0	1	1	1	1	0	0	0	0	0	0	0	5	2	0	1
WN1132	51	2	1	6833	23	2	0	2	0	0	0	0	1	0	0	1	1	0	5	2	1	1
BW322	52	2	1	7561	25	2	1	1	1	1	1	0	0	0	0	0	0	0	4	1	0	1
916	53	2	1	7874	26	2	1	1	1	1	1	0	1	1	0	1	1	0	4	1	2	1
89	54	2	1	8591	29	2	0	2	0	0	0	1	2	3	0	1	1	0	4	1	0	1
559	55	2	1	7625	25	2	0	1	1	1	0	3	3	3	3	1	1	0	8	2	1	1
WN1519	56	2	1	8102	27	2	1	1	1	1	1	0	0	0	0	0	0	0	4	3	0	1
WN1068	57	2	1	6287	21	2	0	1	1	1	1	0	0	0	0	0	0	0	5	2	0	1
W	5	2	1	67	22	2	1	1	1	1	1	3	3	3	3	1	1	0	4	1	2	1

N1277	8			45																		
2812	59	2	1	7300	24	2	1	1	0	0	1	0	0	0	0	0	0	0	8	2	0	1
442	60	2	1	6100	20	2	1	1	1	1	1	0	0	0	0	0	0	0	8	2	0	1
SUZZY	61	2	1	7000	23	2	1	1	1	1	0	0	1	1	0	1	0	1	8	2	1	1
WN723	62	2	1	6423	21	2	1	2	0	1	0	1	1	1	1	1	0	1	8	2	4	1
2332	63	2	1	6766	23	2	1	1	1	1	1	0	0	0	0	0	0	0	8	2	0	1

KEY

Farm	**Breed =Brd**	**Group**
1-Chemusian	1-Friesian	1-Test
2-Gicheha	2-Ayrshire	2-Control

Organism=ORG	1=Coagulase negative Staph	3=*E.coli*
0=none	2=*S.agaactiae*	4=*S.aureus*

5=*Micrococcus* spp   7=*Micrococcus*+*Strep*

6=CNS+*S.agalactiae*   8=*Micrococcus*+*E.coli*

CMT

1=right fore   3=left hind

2=right hind   4=left fore

CMT result

0=negative   1=subclinical mastitis   2&3=clinical mastitis

Milking method=MLK

1=machine   2=hand

Management system (mngt)

1=intensive   2=semi intensive

Barn floor type (BARN)

0=concrete   1=earthen

Parity=prty

0= less than 2   1>2

Udder hygiene score=U.HGY

0=clean   1=dirty