PREVALENCE OF MASTITIS AND ANTIMICROBIAL RESISTANCE AMONG DAIRY CATTLE IN UASIN GISHU COUNTY – KENYA

BY

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DECLARATION

Declaration by student:

This thesis is my original work and, to the best of my knowledge, has not been presented for a degree in any other university. No part of this work may be reproduced without prior permission of the author or the university.

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DEDICATION

We give all the glory to the almighty God who gives us life and academic ability. To my old parents Mr. Joash Nyakiti Ngoge and Mrs. Dorcas Aloo Nyakiti who brought me up and have given me material and moral support all through my life. To my dear wife Elizabeth and children Christopher, Sheilla, Roy, Wendy, Sandra, Joy and Cynthia who had to endure periods of neglect during the research and thesis writing period.

ABSTRACT

A random sample consisting of one hundred and fifty lactating dairy cows; fifty from each of the three study sites, of different breeds, parities, stages of lactation and average daily milk yields from several farms spread across Uasin Gishu County, Kenya were tested in a study of mastitis. We evaluated the use by forty randomly chosen dairy farmers of routine testing of foremilk and teat dipping as mastitis control measures. Mastitis causative microbes were identified both by cultural morphology and biochemical tests. Culture and sensitivity tests were done to determine their *in vitro* resistance to various antimicrobial agents. The Draminski Mastitis Detector was used to screen individual udder quarters of every cow sampled for mastitis. Readings below 300 units were recorded as suspect for subclinical mastitis whilst those with visible changes to the udder and /or the milk from a strip cup were recorded as having clinical mastitis. At the sampled population level the prevalence of mastitis was found to be 50.7% of which 24.7 % had clinical mastitis while 17.3% had subclinical mastitis. The remaining 8.7% of the samples had both clinical and subclinical mastitis. The prevalence of mastitis at quarter level was 21.8% and of these, 11.5% were clinical while 10.3% were subclinical. Out of the 76 positive samples obtained at screening, 72 samples had bacterial growth/ isolates while 4 had none. Of those samples with growth 66 grew one type of microbe while 6 grew mixed infections. Six genera of bacteria and one of yeasts were isolated. The most common bacterial or fungal genus isolated was *Staphylococcus sp* 31.6%, followed by *Escherichia sp* 22.4%, *Klebsiella sp* 18.4%, *Streptococcus sp* 17.1%, *Corynebacterium sp* 2.6%, *Pseudomonas sp* 1.3% and *Candida sp* 1.3%. Resistance by the isolated microbes was greatest to the two sulphonamides; sulphamethoxazole 17.3% and cotrimoxazole 17.3% followed by chloramphenicol 16.6%, nalidixic acid 15.9%, ampicillin 15.2%, tetracycline 11.2%, streptomycin 5.1%, kanamycin 2.8% and gentamicin 2.2% in that decreasing order. However there was no significant difference in the mean resistance across the bacterial genera to ampicillin, nalidixic acid, chloramphenicol, sulfamethoxazole and cotrimoxazole. Tetracycline and streptomycin were next with medium resistance but with no statistical difference between them. The group to which there was least resistance was kanamycin and gentamicin. The incidences of mastitis were found to increase as parity/age of cow increased; with parities between 4 and 10 having the highest number of cases. The breed of cow was found to have no influence on the incidence of mastitis. Cows in early lactation (the first 2 months post calving) had the highest incidence compared to those in mid and late lactation. The cows with higher milk production had higher mastitis incidences compared to those with lower production. There was, among the forty respondent farmers, widespread ignorance about routine management practices that can be used to control the incidences of mastitis at milking such as regular testing, pre and post milking teat dipping in suitable germicides and the timing of fresh feeding after milking. It was concluded that there is widespread lack of knowledge by the farm managers about the cow factors, the environmental factors and management factors that exacerbate mastitis within the farms, hence the high prevalence of mastitis and high resistance to antimicrobials among the causative microorganisms. It was recommended that there is need for capacity building by veterinarians and other dairy stakeholders to alleviate this. The overall objective is to increase the production of clean wholesome milk of high market value which ultimately increases profits to the farmers and all stakeholders in the dairy subsector and hence help alleviate poverty.

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ACRONYMS AND ABBREVIATIONS

ADMY	-	Average Daily Milk Yield
AMR	-	Antimicrobial Resistance
CMT	-	California Mastitis Test
DLPO	-	District Livestock Production Officer
DMD	-	Draminski Mastitis Detector
DVO	-	District Veterinary Officer
EADD	-	Eastern African Dairy Development
G	-	Gram reaction
GOK	-	Government of Kenya
IFAD	-	International Fund for Agricultural Development
KCC	-	Kenya Cooperative Creameries
MIC	-	Minimum Inhibitory Concentration
ml.	-	Milliliter
NMC	-	National Mastitis Council
SCC	-	Somatic Cell Count
V.I.L	-	Veterinary Investigation Laboratory
RNA	-	Ribonucleic Acid
Sp	-	Species
%	-	Percentage
UK	-	United Kingdom
β	-	Beta
≥	-	Greater than or equal to
≤	-	Less than or equal to
<	-	Less than

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CHAPTER ONE

INTRODUCTION

1.1 Background Information

Mastitis in dairy cattle is inflammation of the udder tissue. It occurs when white blood cells (leucocytes) are released into the mammary gland usually in response to an invasion by bacteria of the teat canal (Blood *et al*. 2006). Affected milk secreting tissue and ducts in the mammary gland are damaged due to toxins produced by bacteria. Mastitis can also occur as a result of chemical, mechanical or thermal injury. The mammary gland with mastitis produces little or no milk. The udder sac or affected quarter may be hot to the touch, painful, swollen, hard, tight and usually firm (Harmon 1994).

The mammary infections are described as being sub clinical or clinical mastitis. Sub clinical mastitis is the presence of an infection without apparent signs of local inflammation or systemic involvement that can be detected by visual examination or by a strip cup. Although transient episodes of abnormal milk or udder inflammation may appear, these infections are for the most part asymptomatic and if the infection persists for at least two months then they are termed as being chronic. Once established, many of these infections persist for the entire lactation period or the life of the cow (Kirk 2010). Mastitis is a major cause of economic losses to the dairy industry. Detection is best done by examination of milk for somatic cell counts (predominantly neutrophils) using the California Mastitis Test (CMT) or the automated methods such as the Draminski electronic mastitis detector (National Mastitis Council 1996).

Somatic cell counts (SCC) are positively correlated with the presence of infection. Although variable (especially if determined on a single analysis), cows with a SCC of

≥ 280,000 cells/ml (> a linear score of 5) have a >80% chance of being infected. Likewise, the higher the SCC in a herd bulk tank, the higher the prevalence of infection in the herd. Causative agents are best identified by bacterial culture of milk.

Clinical mastitis is an inflammatory response to infection causing visibly abnormal milk (e.g. colour, fibrin clots). As the extent of the inflammation increases, changes in the udder (swelling, heat, pain, and redness) also become more apparent. The most common cause of mastitis in dairy cattle is bacterial infections especially *Streptococcus agalactiae, Staphylococcus aureus* and *Escherichia coli*. *E. coli* is especially important in housed or confined cattle. Many other bacterial species can cause mastitis in cattle. This potentially fatal mammary gland infection is the most common disease in dairy cattle in the Uasin Gishu County accounting for 77.5% of all reported diseases of large animals during the three year period (GOK- V.I.L. - Eldoret Annual Reports 2008-2011).

It is thus a major problem to dairy farmers in the area under study as clinical mastitis causes economic loss due to treatment costs, lost quarters, perhaps dying cows and most importantly, discarded milk. Sub clinical mastitis on the other hand reduces milk production and quality but is not noticeable until detected with a somatic cell count or by instruments that detect changes in electrical resistance of milk (Kirk 2010).

The mainstay of treating bacterial mastitis is the use of antibiotics administered parenterally (injected into the body), or more commonly as an intra-mammary infusion directly into the affected gland or quarter. For those infused into the gland their success in treating mastitis depends on the degree of binding of the drug to mammary tissues and secretions, its ability to pass through the lipid phase of milk and the degree of ionization. For antibiotics administered parenterally the rate of diffusion

into the udder tissue from the bloodstream is greater in damaged than in normal tissue (Blood *et. al*, 2006).

In most countries surveys of the incidence of mastitis, irrespective of cause, show comparable figures of about 40% morbidity amongst dairy cows and an udder quarter infection rate of about 25%. A major survey of dairy herds in Britain revealed an udder quarter infection rate, in terms of positive cell count, of 27%, but an actual quarter infection rate, as indicated by infection with a significant pathogen, of only 9.6% (Blood *et al*. 2006). Mastitis is one of the most common and costly diseases of dairy cattle (Rodernberg 2012). Annual reports of 2011-2014 from the regional Veterinary Investigation Laboratory (VIL) - Eldoret indicates that confirmed cases of mastitis are 77.5% of all diagnosed diseases of large animals during the period. In recent years there has been an increase in the occurrence of antimicrobial resistance (AMR) to the standard antibiotics and sulphonamides commonly used for mastitis treatment (Regional V.I.L. Annual Report, (2008-2011), Call *et. al.* (2008).

Antimicrobial resistance (AMR) is a major concern to physicians, veterinarians, farmers and consumers worldwide because resistance can render some diseases untreatable. This is because whenever we treat an animal or human with an antimicrobial drug, a certain selection pressure is placed on the microbial population that could ultimately select for AMR. From a public health perspective, because animal products become food, there is concern about AMR pathogens disseminating from the livestock sector into the human population (Oliver *et al.*2011). This could occur by direct contact with animals, through environmental contamination or through the food chain. This public concern has led to increased pressure to reduce antimicrobial usage in livestock throughout the world. Understanding AMR and the

prudent usage of antimicrobials in livestock is therefore important for everyone involved in the industry (Waller *et al.* 2011).

1.2 Problem Statement

Dairy cattle mastitis is important because it affects the udder which is the organ that synthesizes milk (the raw material for the whole dairy industry). It has also become the most commonly reported disease of dairy cattle in the area according to the VIL-Eldoret reports of 2008-2011. An understanding of its occurrence, prevalence, etiology, risk factors, antimicrobial resistance, treatment and control is therefore of great importance to many a stakeholder especially in Uasin Gishu county.

1.3 Justification Of The study

There is a need to understand the factors that contribute to the increased occurrence of mastitis in order to control it. In order to recommend prudent use of the antimicrobials available for treating mastitis we need to develop a profile of sensitivity/resistance by the microbes isolated from milk sample obtained from cases in the area.

Understanding the level of prevalence of sub clinical mastitis will guide control measures and also create awareness of its existence and the silent losses that it causes to the farmer and by extension the whole dairy industry.

1.4 Significance Of The study

Knowledge of the prevalence of clinical and subclinical mastitis, the antimicrobial sensitivity picture of the identified microbes and the phenotypic as well as the genotypic factors that determine mastitis in the area provides the farm managers/farmers and professionals with information necessary for the control and treatment of this important disease in the area..

1.5 Objectives Of The Study

1.5.1 General objectives

The study investigated the prevalence and factors that affect both clinical and subclinical mastitis in the study area as well as the antimicrobial profile of the isolated mastitis causative microbes.

1.5.2 Specific objectives

i.) To determine the prevalence of mastitis in the areas studied in Uasin- Gishu County through on-farm survey sampling.

ii.) To assess the degree to which farmers undertook mastitis control management practices such as routine testing of foremilk at milking.

iii.) To investigate the influence of breed, age/ parity, stage of lactation, and average daily milk yield of cow on the incidence of mastitis.

iv.) To isolate and identify the genera of micro-organisms that, commonly, caused mastitis among lactating dairy cows in the area under study.

v.) To ascertain the presence of antimicrobial resistant mastitis causing microbes in the affected cows.

1.6 Assumptions

Three study areas were chosen as representative of the larger Uasin-Gishu County and it was assumed that the data was uniform for the rest of the county. The dairy cattle management systems were also assumed to be largely the same in the areas of study.

1.7 The Null Hypothesis (Ho)

i.) Mastitis does not exist among dairy cattle in Uasin Gishu County - Kenya.

ii.) Farmers do not undertake any routine management practices aimed at controlling mastitis.

iii.) There is no association between the breed, age/parity, stage of lactation or average daily milk yield of cow and the incidence of mastitis in the area.

iv.) Microbial mastitis does not occur among dairy cattle in the area.

v.) There are no antimicrobial resistant mastitis- causing pathogens in the area under study.

1.8 The Alternative Hypothesis (H_1)

i.) Mastitis exists among dairy cattle in Uasin Gishu County, Kenya.

ii.) Farmers in the area do undertake routine management practices aimed at controlling mastitis.

iii.) The incidence of mastitis is affected by the breed, parity, stage of lactation and the average daily milk yield of the cows.

iv.) Mastitis caused by microbes occurs among dairy cattle in the area.

v.) There is antimicrobial resistant mastitis in cattle in the area studied.

CHAPTER TWO

LITERATURE REVIEW

2.1 History and Prevalence of Mastitis

The world's understanding of mastitis has been developed in several stages in the past one hundred years. It was Peterson who in 1938 first found that pathogenic microorganisms caused mastitis (Petersen, 1938). Antimicrobials became available for use in animal production including in the treatment of some but not all mastitis causing pathogens in 1945 although majority of pathogens were identified earlier around 1940. (Downham, and Christie, 1946, Edwards, 1968). This encouraged further research into the other potential management and husbandry practices that exacerbated the occurrence of mastitis.

In the 1960s, the multi-factorial aetiology of bovine mastitis was commonly recognized by Neave (1959) and Fell (1964).Today, according to Blood *et al.*(2006), mastitis is considered to be a multi-factorial disease, closely related to the production system and the environment in which the cows are kept. Mastitis risk factors or disease determinants can be classified into three groups: host, pathogen and environmental determinants.

2.2 Identification Of Mastitis And Mastitis Causing Bacteria

This disease can be identified by abnormalities in the udder such as swelling, heat, redness, hardness or pain. Other indications are abnormalities in milk such as a watery appearance, flakes, clots or pus.

Many bacterial species are known to cause bovine mastitis including *Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus agalactiae, Streptococcus uberis, Brucella melitensis, Corynebacterium bovis,*

Mycoplasma species, *Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Enterobacter aerogenes, Pasteurella species, Proteus species, Prototheca zopfii, Prototheca wickerhamii* (Jones and Bailey 2010). Fungal infections include *Trichosporon* sp., *Aspergillus fumigatus, A. nidulans,* and *Pichia* sp. Yeast infections include *Candida* sp., *Cryptococcus neoformans, Saccharomyces* sp. and *Torulopsis* sp. Two algae types are also known to cause mastitis; Prototheca trispora and P. zopfii (Blood *et. al.*, 2006).

An understanding of whether the infectious causes of mastitis are contagious or environmental is very crucial in planning of measures to control it once we identify the causative agents from suspected cases. The contagious agents do spread from one cow to another primarily during milking while the environmental agents infect cows mostly from their growth locations in the bedding and the general environment of the cow. Some of them were classified by Kirk (2010) as follows:

Contagious Agents	**Environmental Agents**
Streptococcus agalactiae	*Streptococcus uberis*
Staphylococcus aureus	*Streptococcus dysagalactiae*
Mycoplasma species	*Coagulase –ve staphylococci*
Brucella species	*Coliforms* such as *Escherichia coli,*
Klebsiella.species	

(Kirk 2010)

2.3 Transmission Of Mastitis

Mastitis is often transmitted by contact with the milking machine and through contaminated milkers' hands and materials such as wash cloths. Infection of each

mammary gland occurs via the teat canal, the infection originating from two main sources; the infected udder and the environment. Entry via wounds such as a cut is also common (Kirk 2010). In dairy cattle, the important infections are those that persist readily in the udder, especially *Streptococcus agalactiae* and *Staphylococcus aureus*. Bacteria which are normal inhabitants of the environment such as *E. coli Pseudomonas* sp., cause mastitis much less frequently but, when they do, the disease is much more resistant to control by improved hygiene measures.

Blood *et. al.* (2006) identified two important groups of factors that are important in determining the ability of the bacterium or fungus to set up infection in the mammary tissue; first are bacterial characteristics which include the ability of the organism to survive in the cows' immediate environment(its resistance to environmental influences including cleaning and disinfection procedures), its ability to colonize the teat duct, its ability to adhere to mammary epithelium and set up a mastitic reaction and lastly its resistance to antibiotic therapy.

The second group of factors are known as transmission mechanisms and they depend on the amount of infection in the environment including infected quarters, efficiency of milking men, milking machines, including high milking speed, and especially hygiene in the milking parlor and the susceptibility of the cow (this is related to stage of lactation-first 2 months most susceptible, age of cow- older more than four lactations more susceptible, the level of inherited resistance, lesions on teat skin especially the orifice, immunological, including leucocyte, status of each mammary gland, including prior infection.

2.4 Prevalence of Mastitis Worldwide

In most countries surveys of the incidence of mastitis, irrespective of cause, show comparable figures of about 40% morbidity amongst dairy cows and an udder quarter infection rate of about 25%. A major survey of dairy herds in Britain revealed an udder quarter infection rate, in terms of positive cell count, of 27%, but the actual quarter infection rate, as indicated by infection with a significant pathogen, of only 9.6% (Blood *et al.* 2006)).

Mastitis is one of the most common and costly diseases of dairy cattle (Rodernberg, 2012). The annual reports for the four years 2008-2011 from the regional Veterinary Investigation Laboratory (VIL) Eldoret indicates confirmed cases of mastitis are 77.5% of all diagnosed diseases of large animals reported (VIL – Eldoret Annual Reports for 2008 –2011).

The same report ranks the bacterial species found to commonly cause mastitis in the region to be *Staphylococcus* (21%), *Streptococcus* (17%), *Klebsiella species* (14%), *Escherichia coli* (8%), *Corynebacterium species*(5%), *Enterobacter species* (3%) *and Candida species* (0.9%) in that descending order.

2.5 Effect of Mastitis on Milk Composition

Mastitis may cause a decline in potassium and lactoferrin. It also results in decreased casein, the major protein in milk. Because most calcium in milk is associated with casein, the disruption of casein synthesis contributes to lowered calcium content in milk. The milk protein continues to undergo further deterioration during processing and storage. Milk from cows with mastitis also has a higher somatic cell count. Generally the higher the somatic cell counts, the lower the milk quality (Jones and

Bailey, 2010). These changes in chemical composition of milk affect its processing quality.

2.6 Economic Losses Due to Mastitis

In terms of economic loss mastitis is undoubtedly the most important disease which the dairy industry has to contend with. The loss is caused by the reduction in milk production from affected quarters, by discarding of rejected milk and less so through death of the cow. Also there is the danger that the bacterial contamination of the milk from affected cows may render it unsuitable for human consumption or interfere with manufacturing process, or in rare cases, provide a mechanism of spread of disease to humans. Tuberculosis, Streptococcal sore throat and brucellosis may be spread in this way. Most estimates show that on the average an infected quarter suffers a 30% reduction in productivity and an affected cow is estimated to lose 15% of its production. Other losses include loss due to increased culling rates and the cost of treatment. It is suggested that total economic losses caused by mastitis are composed of the following items:-

Item of loss	**Percent of Total**
Value of milk production lost	70%
Value of cows lost by premature culling	14%
Value of milk discarded or downgraded	7%
Treatment and veterinary expenses	8%

(Blood *et. al*. 2006 and Kirk, 2010)

2.7 Mastitis Prevention In Dairy Cattle

Testing for mastitis before milking (fore-milking) is recommended by veterinarians around the world as the first step in ruling out mastitis in cows. Furthermore, it is a mandatory requirement in many countries. It also forms an important and integral part of any comprehensive hygienic milking routine. In addition to identifying mastitis it stimulates oxytocin release and assists the milk let-down reflex. It also helps remove bacteria from the teat canal (Kirk 2010).

Contagious mastitis can be effectively controlled through a thorough program of teat dipping and dry cow antibiotic treatment. Teats must be dipped in germicide after each milking (this decreases the incidence of the disease). Each quarter must be treated with dry cow therapy at the end of lactation to decrease the prevalence of the disease. Cows with contagious mastitis must be milked last or a separate milking claws (parlor) used. The milking parlor should be flushed with hot water or disinfectant after milking infected cows (this is called back flushing).

Individual cloth/paper towels should be used to wash/dry teats. Milkers should have clean hands and wear latex gloves. New additions to the herd should have their milk cultured and persistently infected cows should be culled. Teat lesions should be minimized (from chapping, frost bite, stepped on teats, lacerations or machine damage). Heifers should be given dry cow antibiotic treatment during gestation if *Staphylococcus aureus* is a problem in the heifers (Oliver *et al.*, 2011).

Environmental mastitis is more difficult to control than contagious mastitis because many of the organisms are resistant to germicides in teat dip and antibiotics in dry cow therapy. The key to control is identification of the source and removal (bedding, ponds and mud). Udders can be dipped to minimize the amount of manure clinging to the glands. Only clean dry teats should be milked. Teats should be pre-dipped with

germicide before milking. Cows should be kept standing after milking by offering them feed. Sterile single dose infusion products should be used and sterile infusion techniques (alcohol swab) should be used. The milking parlor should be kept clean. The teat dip should be kept clean at all times. Pipelines/water heater may need to be replaced in cases of *Pseudomonas* contamination (Jones and Bailey, 2010; Kirk and Sudhan, 2010).

2.8 Clinical Pathology Picture And Diagnostic Procedures In Mastitis Infections

2.8.0 General Symptoms

In the diagnosis and control of mastitis, laboratory procedures are of value in the examination of milk samples for cells, bacteria and chemical changes and for testing for sensitivity of bacteria to specific drugs. Field tests are based on physical and chemical changes in the milk.

These tests are indirect and detect only the presence of inflammatory changes, they are of value only as screening tests and may need to be supplemented by bacteriological examination for determination of the causative organism and if necessary, its sensitivity to antibiotics and chemotherapeutic agents (Blood *et al.*, 2006).

The physical tests carried out on milk in a mastitis examination are limited to the cell count and its immediate development, the bulk milk cell count. Indirect tests are also limited almost entirely to tests such as the California Mastitis Tests (CMT) and the white side test which are dependent on the cell count. Other indirect tests include the chloride content and electrical conductivity and the test for bovine serum albumin.

2.8.1. The use of a strip cup

This is an instrument recommended by veterinarians for use at milking to test the foremilk for mastitis as the first step. A little of the foremilk is squirted into the cup, swirled around as it is carefully observed visually for abnormalities such as the presence of blood clots, flakes, discoloration or abnormal smell that might indicate the presence of mastitis. This fore-milking also helps remove bacteria from the teat canal.

2.8.2 Bacteriological culture of milk

Culturing of milk is the standard method of examination for mastitis. Individual quarter samples are preferred because the cost of treatment requires that the least possible number of quarters be treated. In a mastitis control program the costs of bacteriological culture in the laboratory can be greatly reduced by screening the cows with an indirect tests first and then culturing the positive reactors. It is usually accompanied by sensitivity tests for antibiotics and chemotherapeutics (Blood *et a*l. , 2006).

2.8.3 Somatic cell counts (SCC) of milk

The California Mastitis Test (CMT) is based on the somatic cell count of milk. Somatic cell counts (SCC) are now used as a way of measuring milk quality. The SCC levels in the national dairy herd in the UK has declined steadily since the 1970s and are now well below 200,000 cells/ml, both in bulk milk tanks and in average individual cow milk in milk recorded herds. The maximum legal limit for saleable milk is 400,000 cells/ml in that country.

The somatic cells consist mainly of immune cells that enter the milk compartment of the udder. Only a minority of these cells are dead cells from the udder tissues. The

older the animal gets, the more somatic cells it tends to have in its milk. Similarly SCC levels are higher immediately after calving and towards the end of each lactation (Waller *et al.*, 2011).

When bacteria do enter the udder, the number of immune cells increases rapidly, as the immune system attempts to overcome the infection. Once the infection has been cleared, the SCC level gradually drops to normal. This can sometimes take weeks. However, in cases of chronic infection, where the bacteria persist in the udder, the SCC levels can remain high throughout the lactation. High SCC levels in milk cause deterioration of the milk quality. It has been shown that levels above 500,000 cells/ml decrease cheese yields and affect yoghurt making. The shelf life of milk is also affected but at a higher level of SCC.

Consistently high SCC levels in a herd are usually a sign of high levels of sub clinical mastitis. Most cases of sub clinical mastitis are caused by contagious mastitis bacteria (*Staphylococcus aureus*, or *Streptococcus agalactiae*), even though *Streptococcus uberis* is also considered to increasingly cause chronic mastitis as well (Waller et al. 2011).

2.8.4 Changes in electrical resistance of milk due to mastitis

The development of clinical or subclinical mastitis in the udder of a cow is accompanied by a rise in the level of salt in the milk, which immediately lowers its electrical resistance. The Draminski Mastitis Detector was developed by Draminski in 1989 as a result of this relationship. It is a highly sensitive electronic instrument designed to measure very small changes in milk electrical resistance very accurately. Readings above 300 units indicates that the milk sample is of high quality and is healthy. Readings between 300 units and 250 units show progressively increasing

incidence of subclinical infection as readings decrease. Readings below 250 units is an indication of a rapid increase in the severity of infection as subclinical mastitis progresses to clinical states. This is typified by somatic cells present rising from less than one million to many millions.

2.9 Use Of Antimicrobial Agents In Treating Mastitis And Antimicrobial Resistant Mastitis (ARM).

2.9.1 Treatment

Special bacterial types of mastitis require specific treatment. However the mainstay of treatment is the use of antibiotics or sulfonamides administered either parenterally or as intra-mammary infusions through the teat canal. The degree of response obtained depends particularly on the type of causative agent, the speed with which treatment is commenced and other factors such as the route of drug administration and on whether there is systemic involvement or not. Parenteral treatment is advisable in all cases of mastitis in which there is a marked systemic reaction, to control or prevent the development of septicemia or bacteraemia and to assist in the treatment of the infection in the gland. Parenteral treatment is also advised when the gland is badly swollen and intra-mammary antibiotics are unlikely to diffuse properly.

Because of convenience, udder infusions are the preferred method of treatment. Strict hygiene is necessary during treatment with disposable intra-mammary tubes to avoid the introduction of bacteria, fungi and yeasts into the treated quarter. After an intra-mammary infusion, emptying of the gland and thus losing the antibiotic or other drugs should be avoided for as long as possible by treating immediately after milking preferably in the evening (Schwarz *et a*l., 2010).

Treatment of dry cows is very good for chronic cases, particularly those caused by *Staphylococcus aureus*. Treatment at this stage is also a good prophylaxis against infection during the next lactation. The material is infused at the last milking and allowed to remain permanently in each quarter of the udder (Wang and Zhang, 2012).

2.9.2 Antimicrobial Resistance and Mastitis Pathogens.

Every use of an antimicrobial agent results in a selective pressure under which both pathogenic and non-pathogenic commensal bacteria can develop and/or acquire resistance to the respective antimicrobial agent and in some cases, also to certain other antimicrobial drugs. While antimicrobials are used for a number of reasons in dairy animals including lameness, respiratory diseases, reproductive tract disorders, and diarrhea, the most common reason for antimicrobial use on dairy farms is mastitis (National Animal Health Monitoring System (NAHMS), 2007).

Types of antimicrobial resistance

The term “antimicrobial resistance” describes a gradually variable non – susceptibility of bacteria to antimicrobial agents.

The level of non – susceptibility is measurable as the Minimum Inhibitory Concentration (MIC) and depends on;

i. The antimicrobial agent tested.
ii. The bacteria tested.
iii. The resistance mechanisms present in these bacteria.

Generally two types of resistance mechanisms can be differentiated according to Schwarz *et. al.,* (2006) and Call *et. al*., (2008);

a) Intrinsic resistance

This is a species or genus – specific resistance property of bacteria. It can be based on the absence or inaccessibility of the target site of the antimicrobial agent, the expression of a species – specific drug – inactivating enzyme or metabolic autotrophy. Examples of intrinsic resistance are resistance of the cell wall free *Mycoplasma species* to all antimicrobial agents that interfere with cell wall synthesis such as penicillins and cephalosporins, penicillin resistance of *Bordetella bronchiseptica* due to the specie – specific B- *Lactamase bor* – 1 gene or intrinsic resistance to sulfonamides and trimethoprim among enteroccoci and *lactobacilli* which can use exogenous folates.

b) Acquired Resistance.

This is a strain – specific resistance property of bacteria. It can be based on resistance – mediating mutations either in the genes that code for the targets of antimicrobial agents or its regulators. Other mutations leading to resistance are at specific positions in 16 S or 23 S rRNA, which are important to the binding of antimicrobial agents to the ribosome and the subsequent inhibition of protein biosynthesis. Mutations occur spontaneously in a bacterial population. Such mutated bacteria may have a selective advantage and survive anti-microbial therapy. More often acquired resistance is due to the acquisition of resistance genes.

Genetic background of antimicrobial resistance

The mechanisms to antimicrobial resistance specified by acquired resistance genes falls into three major categories

a) Enzymatic Inactivation

This may be due to resistance genes coding for enzymes that directly degrade the antimicrobial agents. Examples are β- lactamases that target the β-lactam ring of penicillins and cephalosporins or hydrolases that target the lactone ring of macrolides. However, resistance genes can also code for enzymes that transfer adenyl, acetyl or phosphoryl groups to the antimicrobial agent and thereby abolish its antimicrobial activity. Examples of this type of enzymatic inactivation are acetyl-transferases conferring chloramphenicol resistance or acetyl-, phenyl – or phosphoryl-transferases conferring amino-glycoside resistance. The genes for inactivating enzymes are often located on mobile genetic elements such as plasmids transposons or gene cassettes.

b) Decreased intracellular Drug accumulation

May be due to the reduced influx of or increased efflux of antimicrobial agents in or out of the bacterial cells. In Gram negative bacteria, the outer membrane represents a permeability barrier to antimicrobial agents. Reduced influx can be due to changes in the charge of the lipo-polysaccharides of the outer membrane. In addition, loss or down-regulation of outer membrane proteins, which act as an entry to the bacterial cell result in reduced influx. In contrast, increased efflux of antimicrobial agents from the bacterial cell is usually an active energy dependent process. There exist specific exporters, which differ in structure and function, but can only export specific classes of antimicrobial agents such as tetracyclines, macrolides and phenicols. Moreover most bacteria posses genes for so-called “multi drug transporters” which can export a wide variety of toxic compound from the bacterial cell wall.

c) Alterations at the cellular target sites of antimicrobial agent

These may occur in different ways. Resistance to macrolides, lincosamides and streptogramin B antibiotics often results from the methylation of their ribosomal binding site. This methylation prohibits the binding of these antimicrobial agents to their cellular target site. Tetracycline resistance may be due to the activity of ribosome protective proteins which bind to the ribosome, do not inhibit protein synthesis but prevent tetracycline from the binding to the ribosome (Table 1).

Table 1: Examples of target sites and mechanisms of resistance to some antimicrobial agents used in mastitis therapy.

	Class of antimicrobial agent	**Target site**	**Main resistance mechanism(s) known among mastitis pathogens**
1	Aminoglycosides	Protein biosynthesis	Enzymatic inactivation Target site mutation
2	β-Lactams (Penicillins, Cephalosporins)	Cell wall synthesis	Enzymatic inactivation Target replacement
3	Fluoroquinolones (norfloxacin, ciprofloxacin, ofloxacin)	DNA replication	Target site mutation Active efflux Target protection Decreased uptake
4	Lincosamides (Lincomycin)	Protein biosynthesis	Target site modification Enzymatic inactivation Active efflux
5	Macrolides	Protein biosynthesis	Target site modification Enzymatic inactivation Active efflux
6	Novobiacin	DNA Replication	Active efflux
7	Sulphanomides	Folate metabolism	Target replacement
8	Tetracyclines	Protein biosynthesis	Active efflux Target site protection
9	Trimethoprim	Folate metabolism	Target replacement Over – expression of sensitive target

Source: Schwarz *et. al.* 2006; Schwarz *et. al*. 2010.

CHAPTER THREE

MATERIALS AND METHODS

3.1 The Study Area And Location Of The Study Site

The study was carried out in Turbo and Soy sub-counties of Uasin Gishu County in Kenya. With reference to the National and UGD Maps, the region lies between latitudes of 0^0 03'S and 0^o 55'N and longitudes of 34^0 50'E and 35^0 37'W. To the North is Trans-Nzoia county, to the East, is Elgeyo Marakwet county, to the South, Baringo and Nandi counties and lastly to the west lies Kakamega county (Figure1).

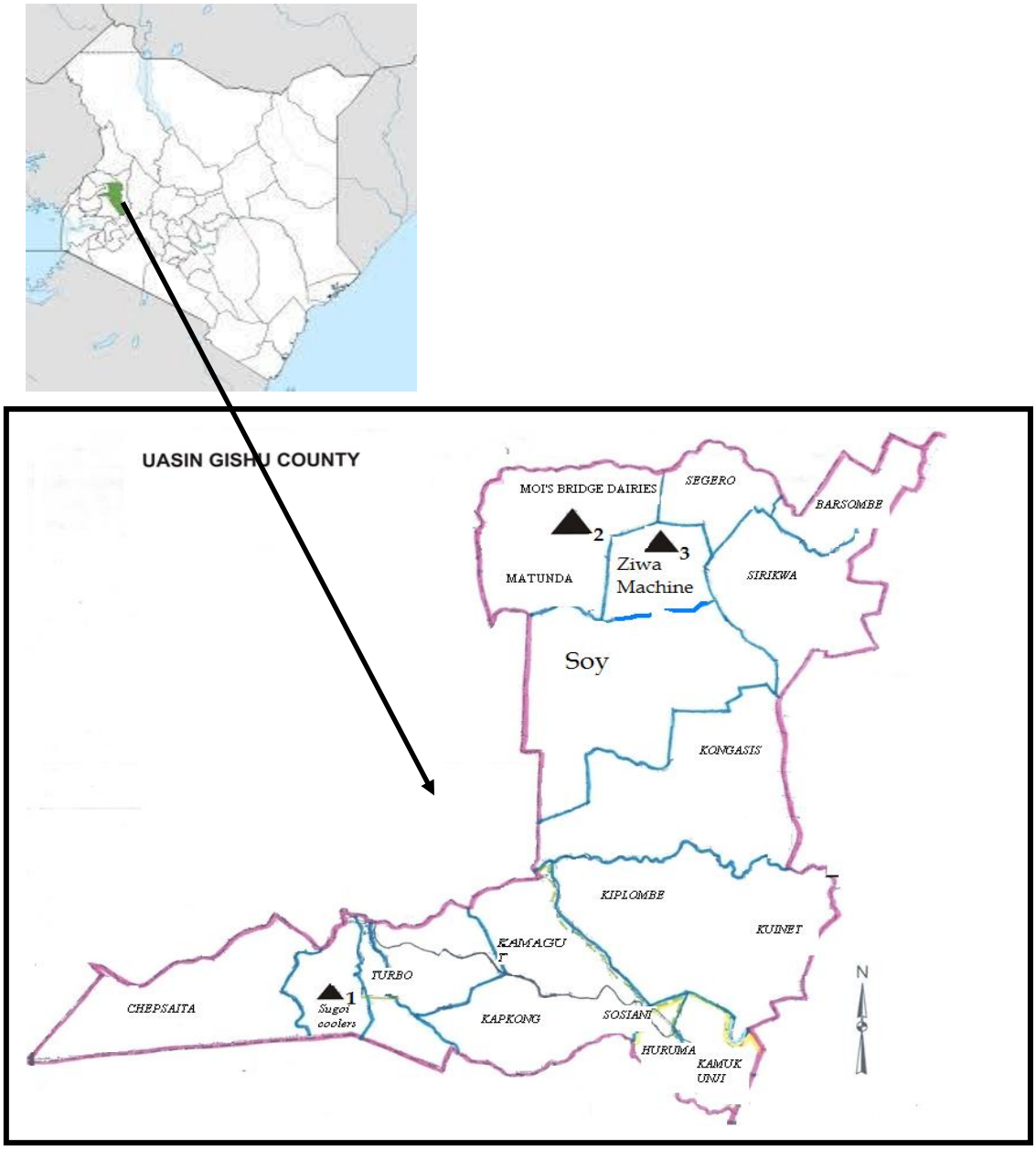
The sub-counties are located in the highlands of Kenya with an altitude of about 1200 feet above sea level. This is a high potential area being agro-ecological zones 2 and 3 with arable soils where mixed farming is practiced (Jaetzold and Schmidt 1983)

Dairy cattle are extensively kept as well as crop farming; mainly maize and wheat growing. The rainfall pattern is bimodal occurring between the months of February and November with two distinct peaks in May and August. The rainfall is reliable and evenly distributed with an annual average of up to 980 mm. Temperatures range from 9^0C to 26^0C. Humidity is moderate averaging around 60%. The average area of the two sub counties is approximately 1428 square kilometers. The majority of farmers in the area have cultural attachment to cattle and almost every household keeps some livestock particularly dairy cattle.

Milk is a very important food to the people in the study area and is also a source of ready income from its sales. Three study sites were purposively selected as study sites based on the density of dairy farmers. The indication for this was the presence of a centre for milk collection, cooling and bulk transporting to processors. Sugoi centre is a milk bulking and cooling plant (an International Fund for Agricultural Development (I.F.A.D). funded dairy commercialization unit with an estimated dairy

cattle population of 1,350 cows in milk at any one time; Ziwa Sirikwa is another milk bulking and cooling plant (a Bill and Melinda Gates funded project via the East African Dairy Development (E.A.D.D.) Project) with an estimated dairy cattle population of 16,875 cows. Moisbridge dairies, with a cattle population estimated to be approximately 20,000 was the third collection centre selected for study. It covers Moisbridge and Matunda locations, Kaplelai, Cherangani and parts of Trans -Nzoia county.

3.2: MAP OF STUDY AREA



Scale: 1cm – 20Km

KEY

- 1 - Study site 1 (Sugoi)
- 2 - Study site 2 (Moisbridge)
- 3 - Study Site (Ziwa Machine)
- County Boundary
- Sub County Boundary
- Ward Boundary

Figure 1: Map of the study area and sites (Source : Google Maps: 2015)

3.3 Consent and Collaboration

Permission to carry out the research was obtained the University of Eldoret administration, The Kenya Dairy Board- Eldoret, the Regional Veterinary Investigation Laboratory (VIL) in Eldoret, The Eldoret West Livestock Production Officer (D.L.P.O.), the District Veterinary Officer (D.V.O.), the management of the three milk bulking and cooling plants and all the farmers whose cows were sampled and screened. They all collaborated with us in the research.

3.4 Sample Size

We screened a total of one hundred and fifty cows, fifty per site, distributed across the three study sites for both clinical and sub-clinical mastitis. All the lactating animals in each farm visited were screened. Data regarding the breed, parity/age, stage of lactation, and the average daily milk yield of each cow sampled was taken at the same time. A questionnaire was administered to forty respondent farmers regarding mastitis testing and control measures.

3.5 Experimental Design

3.5.1 Experiment One: To Determine the Prevalence of Mastitis Through On-Farm Survey Sampling

The study covered the three study blocks/sites; i.e. Sugoi, Ziwa Machine and Moisbridge dairy blocks. The survey was based on a Randomized Block Design (RBD). All the lactating cows from randomly chosen dairy farms in each block were screened for mastitis. At least 15 ml of milk from each individual quarter of every cow sampled was squirted into the Draminski Electronic Mastitis Detector and the readings recorded. The electrical resistance readings of the respective milk samples

were taken and interpreted on the spot. Any milk from a quarter with a reading below 300 units was considered a positive reaction. The positive samples were taken to the V.I.L- Eldoret for bacterial culture and in vitro antimicrobial sensitivity testing. Any cow whose milk (using a strip cup) and udder showed visible changes (clots or colour changes) was recorded as having clinical mastitis. Negative reactors at farm level formed statistics for calculation of prevalence of mastitis among the sampled lactating dairy cows. The prevalence of mastitis was computed and expressed as a percentage of the number of positive reactors (infected cows) divided by the total number of cows that were screened in all the three study sites as follows;

$$\textbf{Prevalence} = \frac{\text{Number of cows whose milk showed reading <300units}}{\text{Total number of cows screened}}$$

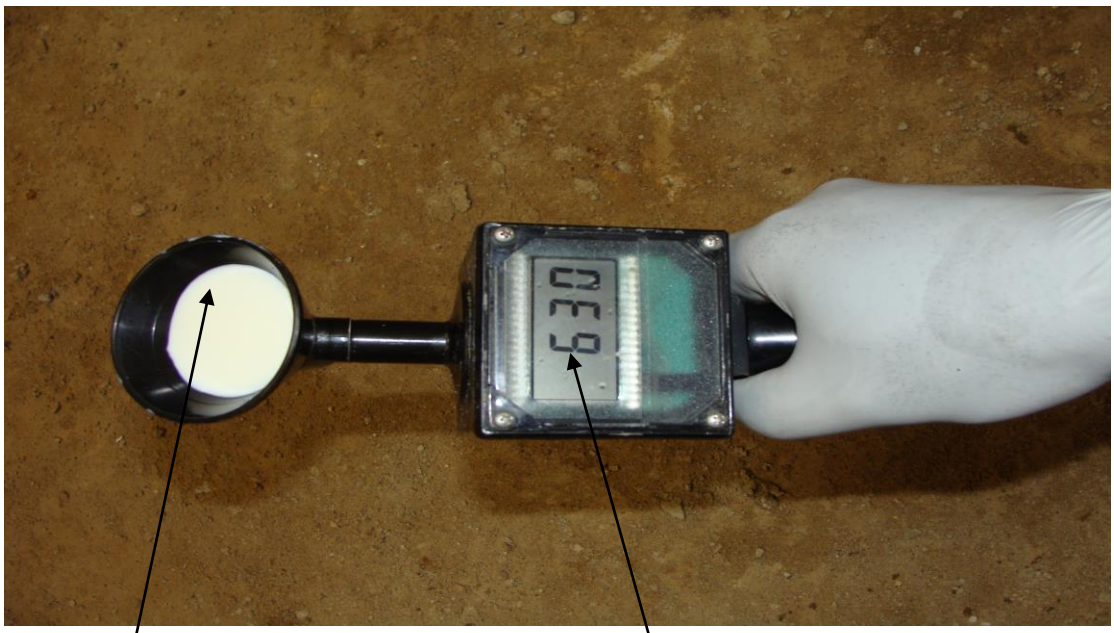
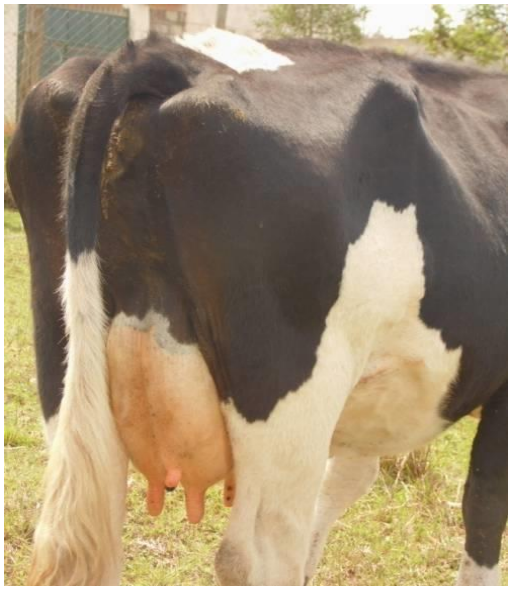


Figure 2: The Draminski Mastitis Detector showing a reading from milk obtained from one udder quarter

The Design was conceptualized as a Randomized Block Design of the three sites (Blocks) x two tests x 50 cows.

Table 2: Design of experiment one

Block/Sites	Test Reactors/Treatment Positive Reactors	Negative Reactions
	T_1	T_2
B1	C_1 C_2 C_3 . . . C_{50}	C_1 C_2 C_3 . . . C_{50}
B_2	C_1 C_2 C_3 . . . C_{50}	C_1 C_2 C_3 . . . C_{50}
B_3	C_1 C_2 C_3 . . . C_{50}	C_1 C_2 C_3 . . . C_{50}



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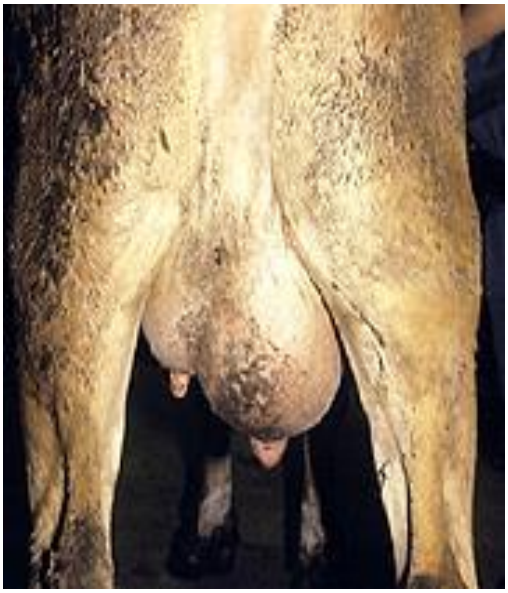


B

Figure 3: A cow with a normal udder (A) and a cow whose right hindquarter is inflamed due to mastitis (B)
(Source : Author, 2015)

3.5.2 Experiment Two: An Aassessment of the Extent to Which the Farmers Carry Out Routine Mastitis Testing and Other Control Measure at Milking.

This was done through administration of a questionnaire (Appendix I) to farmers during the time of screening for mastitis in the field. A total of forty respondents were interviewed. The specific aims of the respective questions were as follows;

1. To assess the extent to which the farmers test the fore-milk for mastitis at milking. This was obtained from questionnaire number 1.

Figure 4: A Strip Cup used to test the first foremilk for mastitis before milking

2. Pre-dipping is a procedure in which the teat is thoroughly covered with a suitable germicide (teat dip) before milking in order to prevent new infections especially by environmental bacteria. This is done for thirty seconds and during this period, any bacterium that might be present interacts with the germicide in the teat dip and is killed. This important practice was assessed by question number 4.
3. Post dipping on the other hand is one of the most important steps in controlling new infections from contagious bacteria. It is recommended that the entire teat up to the base of the udder is covered for maximum protection and that this is done routinely. During milking, the teat end sphincter is

opening and closing about 60 times per minute. At the end of milking, the muscles in the sphincter are fatigued and this leaves the sphincter open for a period of time. The sphincter recovers and closes tightly in 30-45 minutes post milking and until that happens, the mammary gland is at high risk for new infection especially if the teat end is placed on bedding or in manure. This was tested by question number 5.

4. Provision of fresh feed to the cows after milking encourages them to remain standing to eat while the sphincter closes thus reducing the risk of infection (Kirk 2010). Question 6 was designed to test the extent to which farmers carried out this important routine practice.

3.5.3 Experiment Three: To Assess The Influence Of Breed, Parity, Stage Of Lactation And Average Daily Milk Yield (ADMY) On The Incidence Of Mastitis

The goal was to investigate the existence of association between the breed, age/ parity, stage of lactation or Average Daily Milk Yield (ADMY) and the incidence of mastitis among the sampled dairy cows. This was achieved by way of the questionnaires and from analysis of farm records. The data was collected during the farm visits and screening for mastitis. The results were recorded as follows:

Table 3: Experiment three design:

Phenotypic and Genotypic determinants of mastitis

Block/Study site	Cow No.	Draminski reading	Clinical or Subclinical mastitis	Breed	Parity /Age	Stage of lactation	Average Daily Milk Yield (ADMY)
Sugoi	1 . . 50						
Mois Bridge	1 . . 50						
Ziwa Machine	1 . . 50						

Figure 5: Some lactating cows screened for mastitis

(Source: Author, 2015)

3.5.4: Experiment Four: Identification Of The Genera Of Microbes That Caused Mastitis In The Cows.

Laboratory culture of milk samples obtained from positive reactors was done to ascertain the types of microorganisms causing the particular mastitis. The procedure used is described by Silva *et al.* (2010) and by Carter (1998). It involved the careful streaking to inoculate each sample in blood agar (to ascertain their hemolytic characteristics) and McConkey media (a differential medium that differentiates lactose fermenting from non-lactose fermenting microbes) and incubating at 37^0C for 18-24 hours to determine bacterial growth and culture morphology. Gram staining was then done to the isolated microorganisms so as to categorize them into Gram +ve or Gram -ve .

Different genera of bacteria were then identified by their culture morphology and Gram reaction as shown by the examples below:

a) Gram + cocci ; *Staphylococcus sp* and *Streptococcus sp*

b) Gram + rods ; *Corynebacterium sp*

c) Gram – rods ; *Escherichia sp*, *Klebsiella sp* and *Pseudomonas sp*.

d) Gram + ovoid ; *Candida sp*

Table 4: Gram reaction of the isolated microbes

Study area	Genus	Gram reaction	
		Gram +	Gram -
B1-Sample No. 1……N			
B2-Sample No 1……..N			
B3-Sample No.1……..N			

3.5.5: Experiment Five

Detection Of The Presence Of Antimicrobial Resistant Mastitis Causing Organisms.

This was done using the agar diffusion method as described by Silva *et al* . (2010).

It is based on the determination of diameters of growth inhibition zone around a paper disc that is impregnated with a defined amount of antimicrobial agent.

The microbial inoculums were evenly spread on a blood agar plate for growth. Nine different types of antimicrobial discs were then applied and the agar incubated for a period of 12 hours at 37^{o}C. During this time period, the antimicrobial agent diffused from the disc into the agar and suppressed the growth of the bacteria depending on the susceptibility level of the corresponding bacteria. After this incubation period, the

zone diameter around each disc was measured in millimeters and compared with the zone diameter break points given in the respective AST manual. The antimicrobial discs that were used are shown in Table 5.

Table 5: Types and strengths of antimicrobial discs used in culture and sensitivity tests

Disc Type	Strength of the active ingredient per disc	Class of antimicrobial
Ampicillin (AMP)	25 µg	B-lactam antibiotic
Nalidixic acid (NA)	25 mg	Quinolone
Tetracycline (TE)	25 µg	Tetracycline
Co-trimoxazole (COT)	25 µg	Potentiated sulphonamide
Streptomycin (S)	10 µg	Aminoglycoside
Kanamycin (K)	30 µg	Aminoglycoside
Gentamicin (GEN)	10 µg	Aminoglycoside
Sulfamethoxazole (SX)	200 µg	Sulphonamide
Chloramphenicol (C)	30 µg	Chloramphenicol

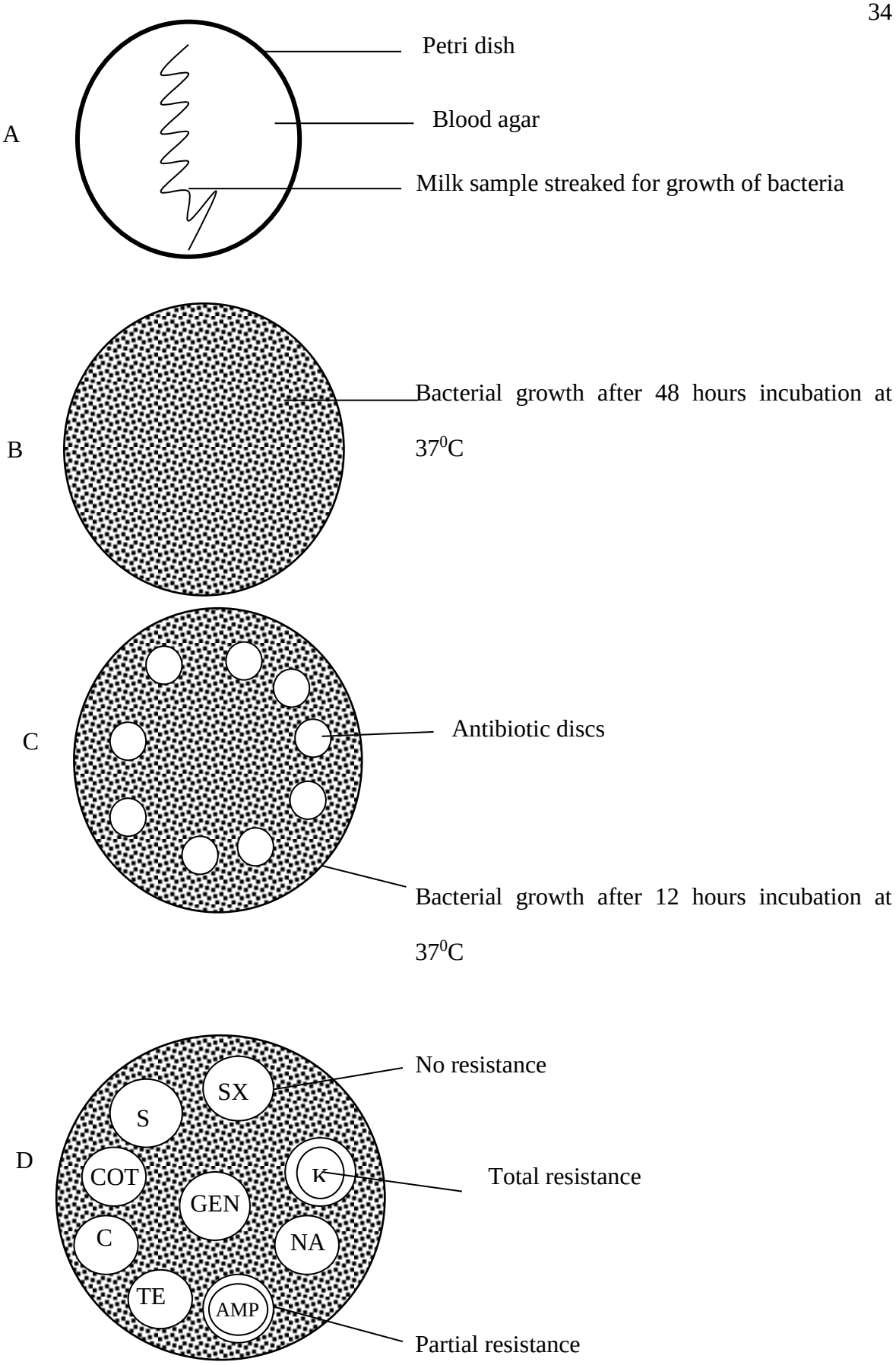


Figure 6: Sketch illustrating the use of petri dishes for microbial in vitro culture and sensitivity/resistance tests

The presence of bacterial growth around a disc after 48 hours incubation indicated bacterial resistance to that antimicrobial as shown in the discs (Fig 6). A clear area around a disc after the same period indicated sensitivity of the bacteria to the antimicrobial present in the disc. For example the figure 6 D above would indicate that the bacteria are resistant to streptomycin (S), gentamicin (GEN), sulfamethoxazole (SX) and chloramphenicol (C) while they are partially sensitive to ampicillin (AMP) and totally resistant to kanamycin (K). The diameters of the circular zone or bacterial growth clearance were measured and compared with published standards to determine susceptibility or resistance.

The effect of the various classes of antibacterial agents on the types of bacteria (Gram+ or Gram-) was also observed and recorded as follows;

Table 6: Results of *in vitro* culture and antimicrobial resistance tests

Class of antimicrobial agent	**Effects on bacteria**	
	Resistant	**Sensitive**
Penicillins		
Tetracyclines		
Sulphonamides		
Aminoglycosides		
Macrolides		
Chloramphenicol		

3.6 Statistical Data Analysis

All relevant data were subjected to descriptive statistics and analysis of variance (ANOVA) where appropriate. The SPSS, the statistical package for social scientists, was used to work out percentages, arithmetic means, standard deviation and coefficients of variation. Where ANOVA was used the means were separated and tested for significance at $p < 0.05$. Also proportions of those cases with antimicrobial resistant mastitis (ARM) organisms versus those with microorganisms sensitive to the available antimicrobials was worked out.

The prevalence of mastitis infection was expressed as a percentage of the ratio of those infected cattle versus those that are none infected using PROC GLM after data collection.

Graphical histograms tables and pie chart presentations were used to illustrate the influence of breed, parity, stage of lactation and average daily milk yield on the incidence of mastitis.

CHAPTER FOUR

RESULTS

4.1: Experiment One Results: Prevalence of Mastitis in the Study Area

Table 7 and Figure 7 show the prevalence of mastitis at the total sampled cow population level was 50.7% (76/150). Out of these, clinical mastitis was 24.7% (37/150, sub-clinical mastitis was 17.3% (26/150) and cows with both clinical and sub-clinical mastitis were 8.7 % (13/150). The udder quarter prevalence was 21.8 % (131/600). Out of this 11.5% (69/600) were clinical mastitis while 10.3% (62/600) were subclinical mastitis (Table 8). Of the 150 lactating cows sampled, Moisbridge had the highest incidence of mastitis (both clinical and subclinical) at 43.4% (33/76) followed by Sugoi at 28.9% (22/76) and lastly Ziwa Machine area 27.6% (21/76). However, there were no statistically significant differences in prevalence between the three study areas. The prevalence of clinical mastitis in Sugoi and Moisbridge was higher than that of subclinical mastitis. In Ziwa the opposite was true**.**

Table 7: Prevalence of the three forms of mastitis in the study areas.

Study site	No. of cows sampled	Clinical mastitis	Subclinical mastitis	Mixed infections (both clinical and sub-clinical)	Total positive reactors
SUGOI	50	10	6	6	22
MOISBRIDGE	50	18	10	5	33
ZIWA MACHINE	50	9	10	2	21
TOTAL	150	37	26	13	76
Percent prevalence among cows	100	24.7	17.3	8.7	50.7

The overall percent prevalence within the sites/ blocks and among the cows is 50.7%.

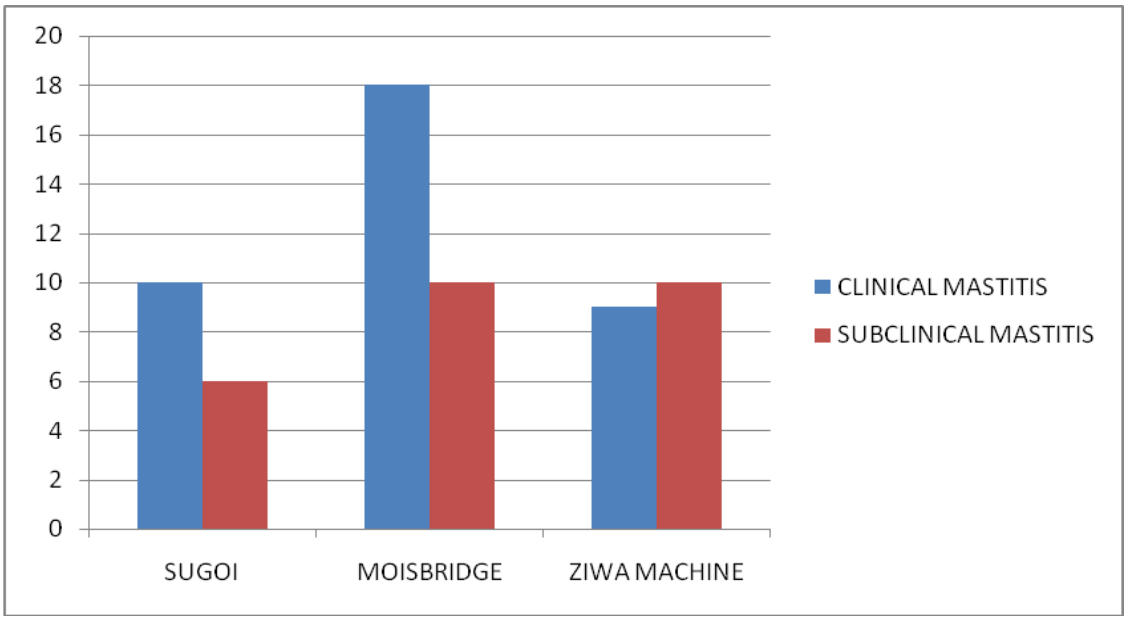


Figure 7: A comparison of the prevalence of clinical and subclinical mastitis within the sites

Table 8: The prevalence of mastitis by udder quarters

Quarter prevalence of mastitis	Out of 600 quarters	% Prevalence
Clinical mastitis	69/600	11.5
Subclinical mastitis	62/600	10.3
Total infected	131/600	21.8

4.2: Experiment Two Results: Routine Testing of the foremilk and Teat Dipping by the Farmers at Milking as Mastitis Control Measures.

Only 12.5% (5/40) of the farmers interviewed carried out routine testing for mastitis at milking. The rest (87.5%) neither performed nor had knowledge of the advantages of testing (screening) (Table 9). All of those who carried out routine testing used a strip cup while none was found to use either the Draminski mastitis detector or the California Mastitis Test (Table 10). Only 25% (10/40) of the farmers practiced dipping of teats (pre or post) at milking as a control measure of mastitis on their farm (Table 11). The practice of giving fresh feeds to the cows immediately after milking was practiced by only 25% of the farmers (Table 12). The rest fed the cows thirty minutes and after. This is too late if it is to encourage the cows to stay standing for 30-40 minutes post milking as the teat sphincters close up naturally after cessation of milking.

Table 9: Percent of farmers who routinely test the foremilk for mastitis

Farmers who carry out routine mastitis testing at milking	Number	Percentage
Yes	5	12.5
No	35	87.5
Total	40	100

Table 10: Methods used by the farmers that practiced pretesting of foremilk

Method of testing of fore milk for mastitis	Number using the method	Percentage
Strip cup	5	100
California Mastitis Test	0	0
Draminski Mastitis Detector	0	0
Total	5	100

Table 11: Results of routine teat dipping as mastitis control measure

Farmers who routinely carry out pre- and post- and milking dipping of teats as mastitis control measures	Number	Percentage
Yes	10	25
No	30	75
Total	40	100

Table 12: Time taken by the farmers to offer fresh feed to cows after milking

Time taken to commence feeding of the cow after milking	Number of respondent farmers	Percentage
Immediately after milking	10	25.0
30 -60 minutes	7	17.5
61-120 minutes	8	20.0
After 120 minutes	15	37.5
Total	40	100

4.3: Experiment Three Results: Association Between Phenotypic and Genotypic Characteristics of Cow and Incidence of Mastitis

4.3.1: Effect of Breed on Incidence of Mastitis

There is a clear association between the occurrence of mastitis and the breed of cow. Friesians (63.2%) had the highest incidence followed, in decreasing order, by Ayrshires (21.1%), Guernseys (9.2%), Friesian crosses (3.9%) and Jerseys (2.6%) (Table 13). However a different picture emerges when we take into consideration the sample size (N) of each breed sampled. The order becomes Jerseys (100%) followed in decreasing order by Guernseys (70%), Friesians (59.3%), Ayrshires (47.1%) and lastly Friesian crosses (37.5%) as shown in Figure 8.

Table 13: Effect of breed on the incidence of mastitis

Breed	Sample size (N)	Number of cases positive for mastitis	Cases as % of total No. of cows sampled of that breed	As a % of all cows with mastitis(N= 76)
Friesian	81	48	59.3	63.0
Ayrshire	34	16	47.1	21.0
Guernsey	10	7	70	9.2
Friesian crosses	8	3	37.5	3.9
Jerseys	2	2	100	2.6

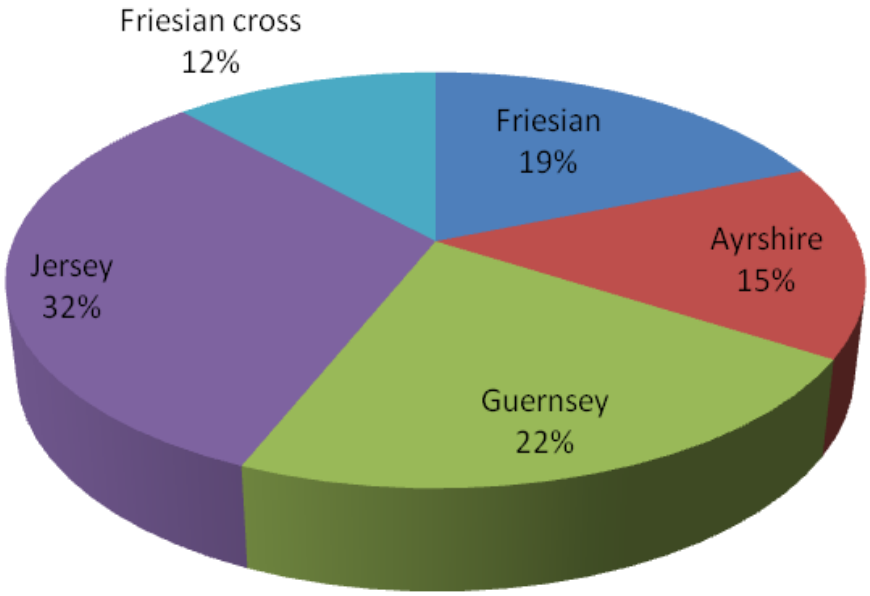


Figure 8: Effect of breed on incidence of mastitis

4.3.2: Effect of Parity of Cow on Incidence of Mastitis

Table 14 indicates a relationship between the occurrence of mastitis and parity of the cows sampled. There is a steady increase in percent prevalence of mastitis as parity increases from parity 1 at 29.2% all the way to the parity 10 at 100%.

Table 14: Effect of parity on the incidences of mastitis

Parity number	Sample size (N)	Number of cases positive for mastitis	Cases as a % of cows of that parity sampled
1	24	7	29.2
2	36	14	38.9
3	24	10	41.2
4	25	12	48
5	10	8	80
6	7	4	57.1
7	8	6	75.0
8	7	5	71.4
9	3	2	66.7
10	6	6	100

4.3.3: Effect of Stage of Lactation on Incidence of Mastitis.

The results, Table 15, show a clear relationship between the stage of lactation and the incidences of mastitis. Stage I (the first 2 months post calving) and stage III (the 5th month and above) show higher incidences than stage II (months 3 and 4). However when the sample size (N) of each category was considered, stage I had the highest at 80.6% followed by stage III at 53.1% and lastly stage II at 32.3%.

Table 15: Effect of stage of lactation on incidence of mastitis

Stage of lactation	Sample (N)	Number of cases	CASES as a % of all cows of that stage of lactation sampled	Cases as a % of total cows with mastitis
I(First 2 months)	36	29	80.6 (29/36)	38.2 (29/76)
II(Next 2 months)	65	20	32.3 (21/65)	27.6 (21/76)
III(5 months and more)	49	26	53.1 (26/49)	34.2 (26/76)
Total	150	76	-	-

4.3.4: Effect of Average Daily Milk Yield (ADMY) on Incidence of Mastitis.

There was a general increase in incidence of mastitis with increase in ADMY of the cows (Table 16 and Figure 9). The order of increasing incidence with variation in ADMY of the lactating cows sampled is; ADMY of ≤10 litres (36.7%), ADMY of 11-15 litres (56.3%), ADMY of 16-20 litres (38.6%), ADMY of 21-25 litres (66.7%), and ADMY of ≥ 26 litres (100%).

Table 16: Effect of Average Daily Milk Yield (ADMY) on incidence of mastitis

Level	Range of (ADMY)in Kg	Sample size (N)	No. of cases positive for mastitis	Cases as a % of cows of that range of ADMY sampled
1	≤10	30	11	36.7
2	11-15	64	36	56.3
3	16-20	44	17	38.6
4	21-25	6	4	66.7
5	≥26	6	6	100

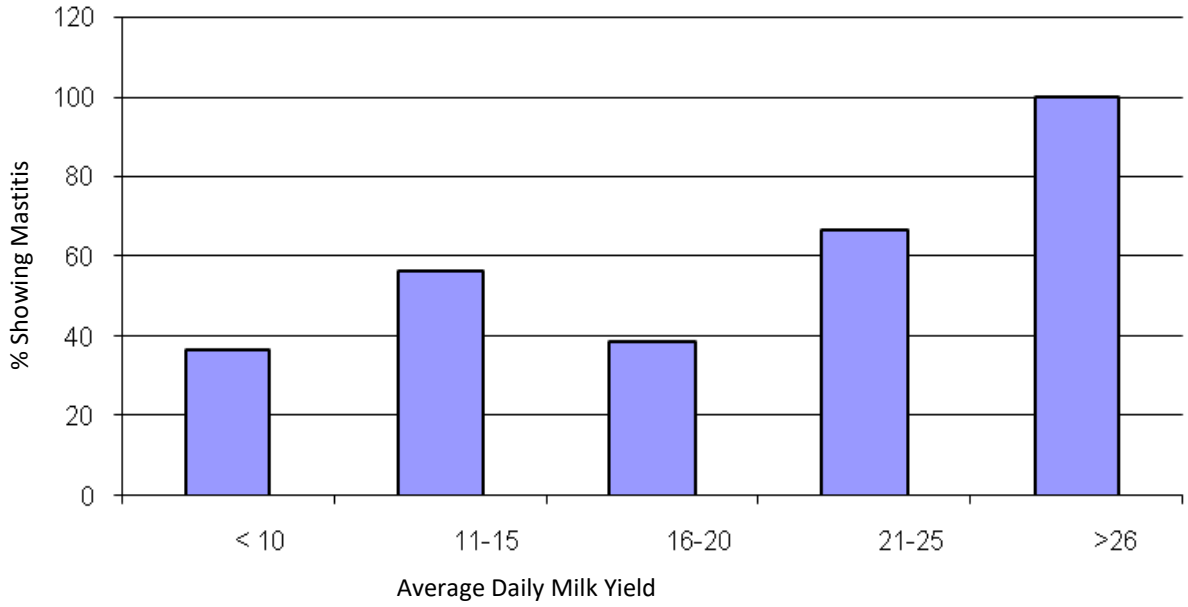


Figure 9: Incidence of mastitis as influenced by the range of Average Daily Milk Yield of cow

4.4: Experiment Four Results:

Identification of the Genera of Mastitis Causing Microorganisms Isolated from Laboratory Culture of Milk Samples

Out of the 76 samples tested, 72 cultures had isolates while 4 cultures had none (Table 17). Of the 72 cultures with isolates 66 (91.7%) grew one type of bacterium or yeast while 6 (8.3%) grew mixed infections. Six genera of bacteria and 1 of yeast (*Candida*) were isolated and identified. The microbe genera identified in decreasing order of prevalence were *Staphylococcus* 24 (31.6%), *Escherichia* 17 (22.4%), *Klebsiella* 14(18.4%), *Streptococcus* 13(17.1%), *Corynebacterium* 2 (2.6%), *Pseudomonas* 1(1.3%) and *Candida* 1(1.3%) as shown in Figure 10.

Table 17: Types of mastitis causing microorganisms isolated and identified in milk samples from different study areas

Microorganism genus	Study site: Sugoi	Moisbridge	Ziwa machine	Total samples of that isolate	Prevalence %	Order of ranking in frequency
Staphylococcus sp.	6	12	6	24	31.6	1
Escherichia sp.	5	7	5	17	22.4	2
Klebsiella sp.	3	7	4	14	18.4	3
Streptococcus sp.	4	6	3	13	17.1	4
Corynebacterium sp.	1	1	0	2	2.6	5
Pseudomonas sp	0	0	1	1	1.3	6
Candida sp.	1	0	0	1	1.3	6
No isolate	3	1	0	4	5.3	
Total	23	34	19	76		

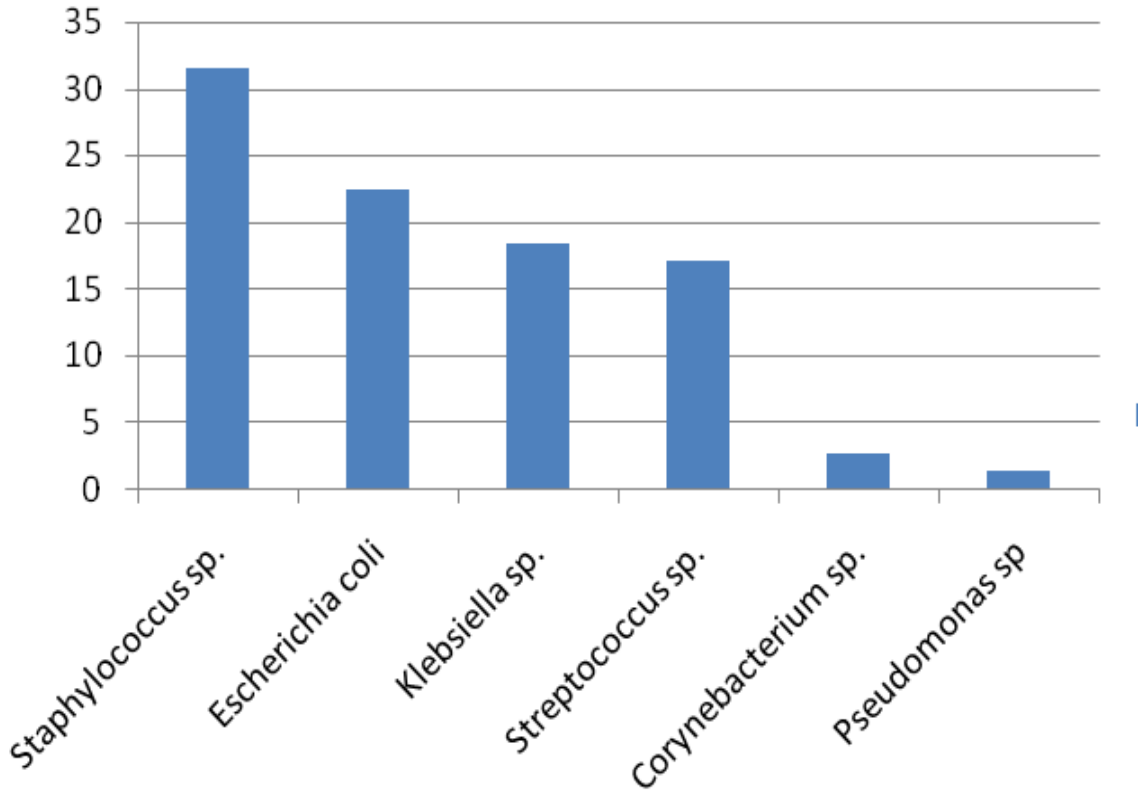


Figure 10: Prevalence as percentage of the identified microbes

4.5: Experiment Five Results: Antimicrobial Resistance By The Isolated Bacteria

The increasing order of *in vitro* resistance to the antimicrobials by the microbe samples was gentamicin (2.2%; 3 samples), kanamycin (2.8%; 8 samples), streptomycin (5.1%; 22 samples), tetracycline (11.2%; 39 samples), ampicillin (15.2%; 48 samples), nalidixic acid (15.9%; 52 samples), chloramphenicol (16.6%; 54 samples), sulphamethoxazole (17.3%; 58 samples) and cotrimoxazole (17.3%; 58 samples) (Tables 18 and 19).

The three aminoglycosides (gentamicin, kanamycin and streptomycin) as a group had the least number of samples resistant to them with a combined total of 33 (mean of 9.8 %). They were followed in increasing order of resistance by the tetracyclines 39 (11.3%), the penicillins 48 (15.2%), the quinolones 53 (15.9%), the chloramphenicols 54 (16.6%) and lastly the sulphonamides 116 (34.6%) (Table 18 and table 19).

Only two samples of *E. coli* and one of *Streptococcus sp*. were found to be resistant to gentamicin (Table 18).

The effectiveness of the sulphonamides (combined total of 1.2% for sulphamethoxazole and cotrimoxazole) as a group was found to be very low.

The percentage of resistance of the isolates to the antimicrobials agents tested across all the types of antimicrobials was found to be *Pseudomonas* sp. (20% mean), *Corynebacterium* sp. (14.3%), *Staphylococcus* sp (12.5%), *Klebsiella* sp. (12.4%), *Streptococcus* sp (11.1%) and *E. coli* (11.1%) as indicated in Table 19.

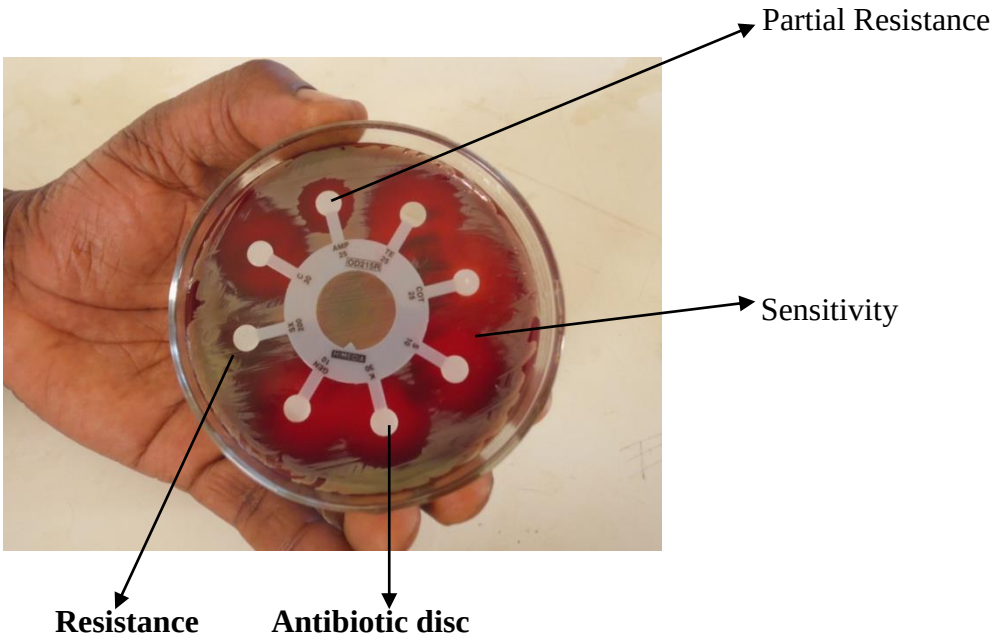


Figure 11: Results of culture showing resistance and sensitivity tests in blood agar

Table 18: Resistance of the isolated genera to antimicrobial agents

Type of microorganism isolated	Number of microbe samples resistant to the individual antimicrobial types									Total No. of samples showing cross resistance	Mean cross resistance
	AMP	NA	TE	C	SX	COT	S	K	GEN		
Staphylococcus sp	11	18	7	16	18	18	5	2	0	95	11.87
E. coli	16	12	15	15	17	17	6	2	2	102	11.33
Klebsiella sp	13	12	11	13	13	13	8	2	0	85	10.62
Streptococcus. Sp	5	7	5	7	7	7	2	2	1	43	4.78
Corynebacterium. Sp	2	2	1	2	2	2	1	0	0	12	1.71
Pseudomonas sp	1	1	0	1	1	1	0	0	0	5	1.00
Total	48	52	39	54	58	58	22	8	3	342	

Table 19: Percentage of samples resistant to individual antimicrobials

Type of micro-organism isolated	**AMP**	**NA**	**TE**	**C**	**SX**	**COT**	**S**	**K**	**GEN**	Mean % Resistance by microbes to all the antimicrobials
Staphylococcus sp.	11.6	18.9	7.4	16.8	18.9	18.9	5.3	2.1	0	12.5
Escherichia sp.	15.7	11.8	14.7	14.7	16.7	16.7	5.9	2	2	11.1
Klebsiella sp.	15.3	11.8	14.1	15.3	15.3	15.3	9.4	2.4	0	12.4
Streptococcus Sp.	11.6	16.3	11.6	16.3	16.3	16.3	4.7	4.7	2.3	11.1
Corynebacterium Sp.	16.7	16.7	8.3	16.7	16.7	16.7	8.3	0	0	14.3
Pseudomonas sp.	20	20	0	20	20	20	0	0	0	20
Mean % cross resistance to each antimicrobial agent	15.2	15.9	11.2	16.6	17.3	17.3	5.1	2.8	2.2	

NB: The means indicate the overall resistance to individual antimicrobials across bacterial genera.

An analysis of variance (ANOVA) of this data and comparison of the means of the antimicrobials is given in Table 20 below.

Table 20: The means of resistance to antimicrobials across micro-organisms

Antimicrobial	Mean
Ampicillin	15.15a
Nalidixic acid	15.92a
Tetracycline	9.35b
Chloramphenicol	16.63a
Sulphamethoxazole	17.32a
Cotrimoxazole	17.32a
Streptomycin	5.60b
Kanamycin	1.87c
Gentamicin	0.72c

S.E.M. ± 3.10
Means not sharing the same letter are significantly different ($p < 0.05$).

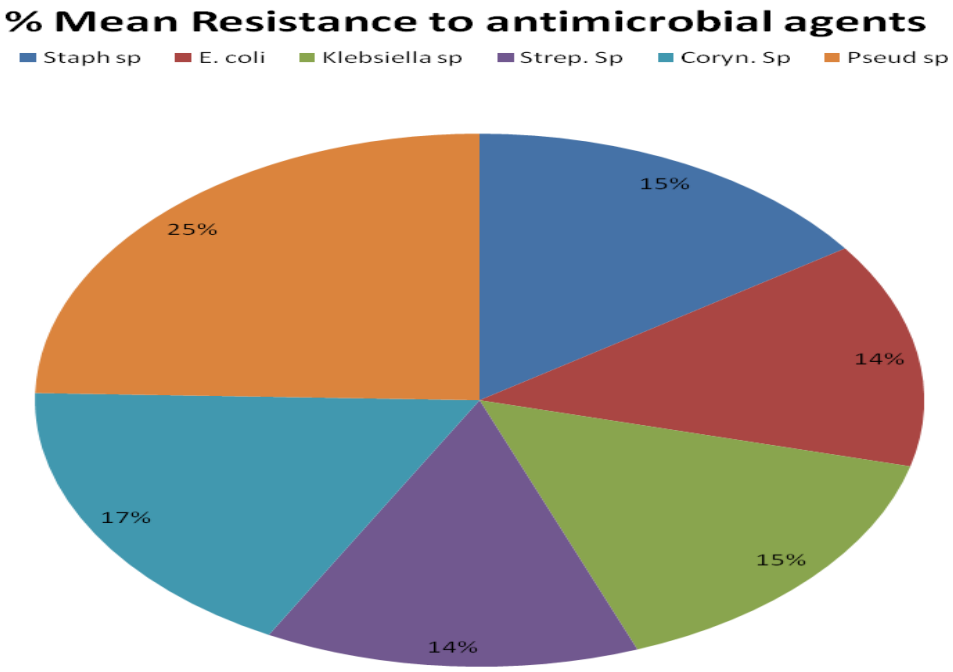


Figure 12: Mean % Resistance by microbes to antimicrobials

Table 21: Number of microorganism samples resistant to each antimicrobial group

	Group 1	Group2	Group3	Group4	Group5	Group6	Summary as mean number resistance across groups
*Staphylococcu*s Sp	11	18	7	16	36	7	15.83
Escherichia sp.	16	13	15	15	34	10	17.16
Klebsiella sp.	13	12	11	13	26	10	14.16
Streptococcus sp.	5	7	5	7	14	5	7.16
Corynebacterium Sp.	2	2	1	2	4	1	2.00
*Pseudomona*s sp.	1	1	0	1	2	0	0.83
Mean resistance to each antimicrobial group	8.00	8.83	6.50	9.00	19.33	5.50	

KEY: Group 1 = Penicillins (Ampicillin)

Group 2= Quinolones (Nalidixic acid)

Group 3= Tetracyclines (Tetracycline)

Group 4= Chloramphenicol (Chloramphenicol)

Group 5= Sulphonamides (sulphamethoxazole and cotrimoxazole)

Group6 = Aminoglycosides (streptomycin, kanamycin and gentamicin)

The order of least resistance is 5.50 (aminoglycosides, 6.50 (tetracyclines), 8.00 (penicillins), 8.83 (quinolones), 9.00 (chloramphenicol), 19.33 (sulphonamides)

Table 22: Summary of % resistance to each antimicrobial group by the isolated microorganisms

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Mean resistance of each bacterial specie across antimicrobial groups
Staphylococcus Sp	11.6	18.7	7.4	16.8	37.9	7.4	16.63
E.coli	15.7	11.8	14.7	14.7	33.3	9.8	16.67
Klebsiella sp	15.3	11.8	14.7	15.3	30.6	11.8	16.58
Streptococcus Sp	11.6	16.3	11.6	16.3	32.6	11.6	16.67
Corynebacterium Sp	16.7	16.7	8.3	16.7	33.3	8.3	16.67
Pseudomonas sp	20	20	0	20	40	0	16.67
Mean % resistance within each group	15.2	15.9	11.3	16.6	34.6	9.8	

KEY: Group 1 = Penicillins
Group 2 = Quinolones
Group 3 = Tetracyclines
Group 4 = Chloramphenicol
Group 5 = Sulphonamides
Group 6 = Aminoglycosides

NB: The means indicate the overall resistance to the group of antimicrobial by the micro-organisms.

An analysis of variance (ANOVA) of this data and comparison of the means of the antimicrobial group is given below in Table 23.

Table 23: The means of resistance to antimicrobial groups by the micro-organisms

Antimicrobial group	Means
Group 1 (Penicillins)	15.15b
Group 2 (Quinolones)	15.88b
Group 3 (Tetracyclines)	9.45c
Group 4 (Chloramphenicol)	16.63b
Group 5 (Sulphonamides)	34.55a
Group 6 (Aminoglycosides)	8.15c

S.E.M. ± 4.18

Means not sharing the same letter are significantly different ($p < 0.05$).

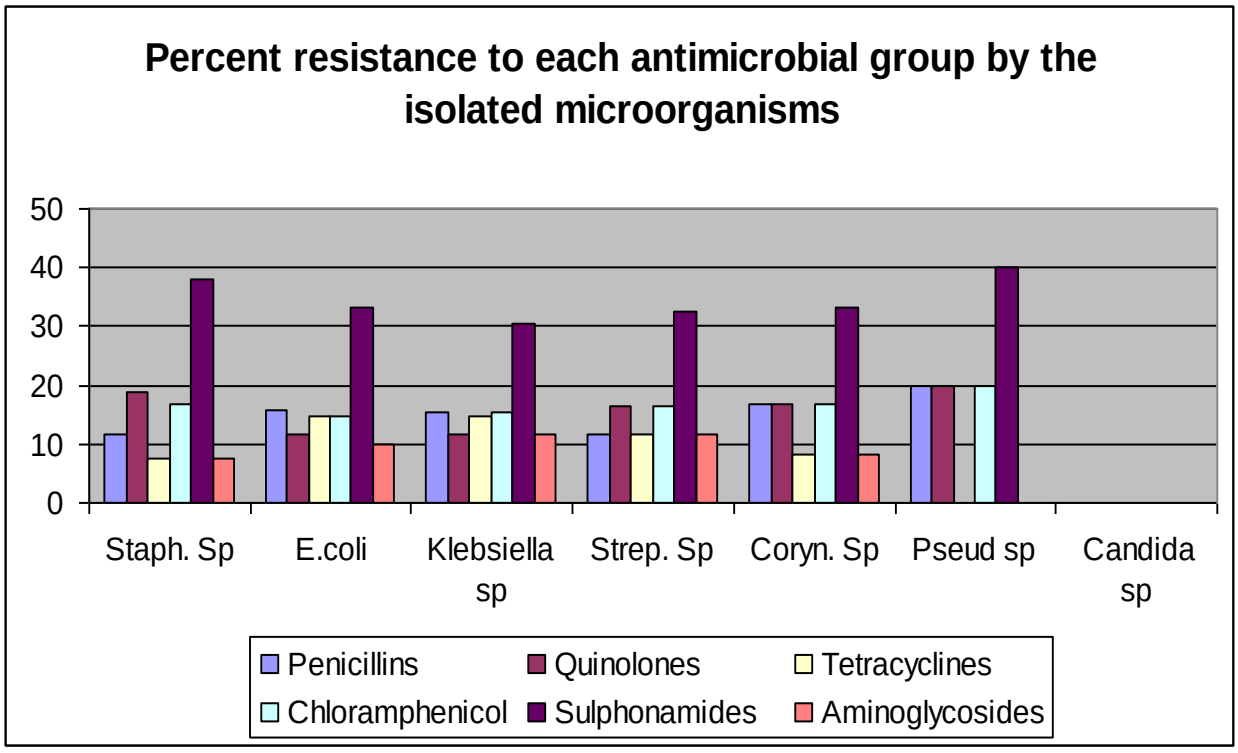
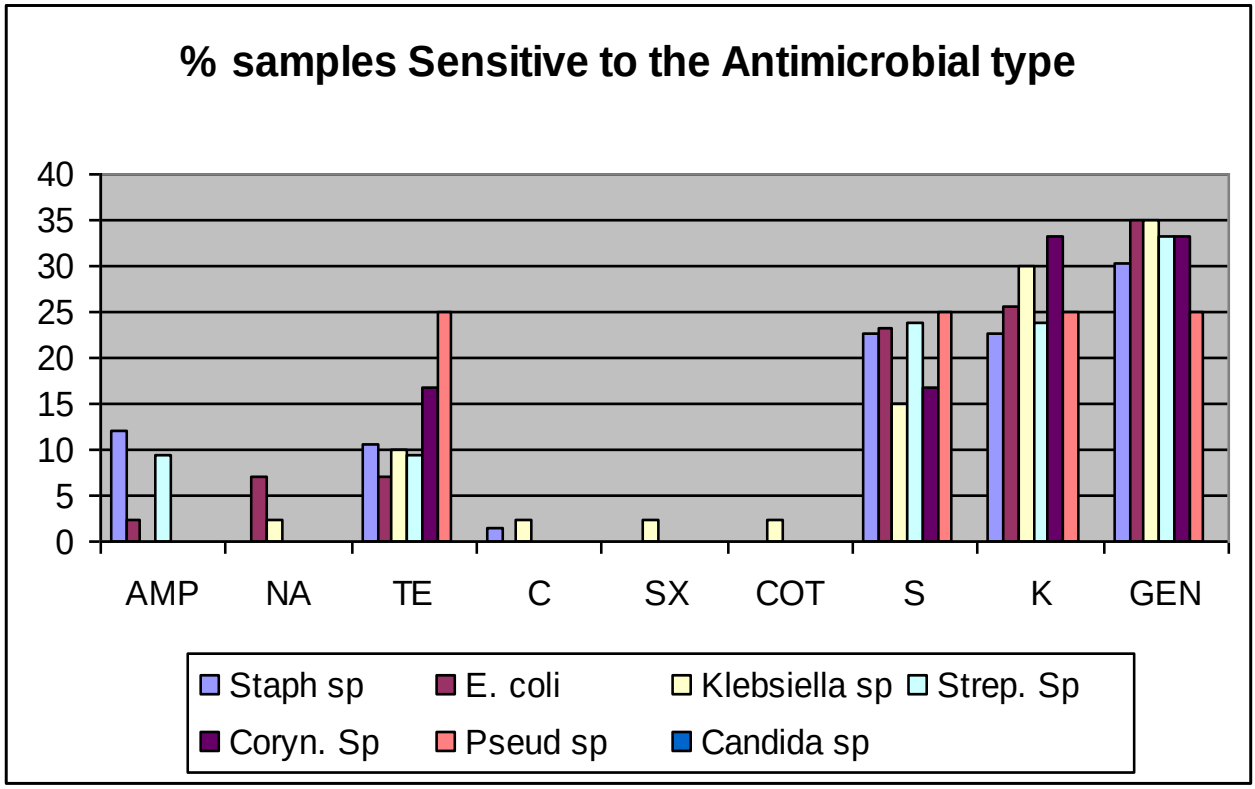


Figure 13: Percent resistance of the isolated microorganism genera to different antimicrobial groups



KEY: AMP = Ampicillin
NA = Nalidixic acid
TE = Tetracycline
C = Chloramphenicol
SX = Sulfamethoxazole
COT= Cotrimoxazole
S = Streptomycin
K = Kanamycin
GEN= Gentamicin

Figure 14: Percentage of samples sensitive to various antimicrobial agents

CHAPTER FIVE

DISCUSSION

The combined mean prevalence of both clinical and subclinical mastitis in the three study sites was 50.67% with an udder quarter infection rate of 21.8%. This is lower than 68.8% reported by Bishi *et.al*. (2003) on prevalence of mastitis around Addis Ababa in Ethiopia and 82.9% found by Ondiek *et. al*. (2013) at Tatton farm of Egerton University, Njoro in Kenya. According to Blood *et. al*. (2006), a major survey of dairy herds in the United Kingdom showed a mastitis prevalence of about 40%, which is lower than the findings, and a quarter infection rate of 27% which was higher than ours of 21.8%. The overall udder quarter prevalence of clinical mastitis and subclinical mastitis was 11.5% and 10.3% respectively.

However the udder quarter prevalence of subclinical mastitis in all the three areas which averaged 10.3% is high and is, therefore, an area of concern as it portends a silent reduction in milk yield from the affected cows, and it could expose healthy animals to contagious pathogens, by acting as a reservoir within the herd, which may then progress to become clinical mastitis. Worse still, subclinical mastitis can progress further to chronic infection that is unresponsive to antibiotic treatment (Hortet and Seegers, (1998). Barlow *et. al.* (2009) found that approximately 25-30% of cows with chronic cases of subclinical mastitis may exhibit clinical symptoms that require antibiotic treatment and withholding of milk with loss of income to the farmer. Hence there is a need for improvement of the detection and management of subclinical mastitis on the dairy farms.

There was widespread ignorance about the advantages of pre- testing for mastitis at milking (fore-milking) as only 12.5% of the farmers interviewed practiced it and all of them used a strip cup as the tool of choice. This rate is quite low and it means the

majority have no idea about the detection of subclinical mastitis and the silent economic losses it can cause to their dairy enterprises. They therefore only wait for acceptance or rejection of their milk after routine quality tests at the point of sale. By then there is not much the farmer can do to change things and his milk may be rejected for poor marketability, due to either clinical or subclinical mastitis, with subsequent loss of income.

Pre-dipping and post-dipping of the teats in a suitable germicide as a mastitis control measure was found to be practiced by only 25% of the respondent farmers. This is quite low because these two practices are crucial mastitis control strategies. The majority of farmers (75%) were found to offer fresh feed to their cows rather too late after milking (later than 30 minutes post milking) for any help in controlling the entry of environmental pathogens. In fact most of them were ignorant about it as a management strategy in the control of mastitis. This is very low because feeding immediately after milking encourages the cows to remain in a standing position and hence reduces the likelihood of disease causing microbes gaining entry into the open teat canal and predisposing the cows to mastitis (Kirk, 2010).

A comparison between breeds showed Friesian (63.3%) having the highest incidence, followed by Ayrshire (21.1%), Guernsey (9.2%), Friesian crosses (3.9%) and Jerseys (2.6%) in descending order. However when the sample size (N) of each breed tested was taken into consideration, a different picture emerged and it was concluded that there was no clear influence of breed on the incidence of mastitis.

The parity of cow was found to influence incidence of both clinical and sub-clinical mastitis (Table 15 and Figure 7). There was a steady rise in the cases of mastitis as the parity increased. At parity 1 there were 29.2% cases of mastitis while at parity ten the cases were 100%. At the midpoint of the range there were 68%. The reason for this is

that as the cow ages, the udder epithelium becomes more prone to chronic mastitis and the risk of clinical or subclinical mastitis rises. This is because the older cow will have been milked more and hence exposed to environmental pathogens that cause subclinical disease. They are also more likely to have damaged teats and larger udders, than younger cows, with higher chances of physical injury especially in early lactation when the udder often contacts the ground allowing entry of bacteria into teats and colonization of the udder (Nanita *et.al.* 2015).

The results indicated an association between the stage of lactation and the occurrence of mastitis. Taking the sample size of each of the three categories of stages of lactation into consideration, Stage I (the first two months post calving) had the highest prevalence of mastitis at 80.6% followed by stage III (five months and above post calving) at 53.1% and lastly stage II (months three and four post calving) at 32.3% Table 16, fig 8). This is consistent with what Blood *et al*. (2006) reports that the first two months of lactation shows the greatest susceptibility of the udder to infections. It also coincides with the period when the cow's milk production tends to peak post calving. This increased milk production probably exerts pressure and stress on the udder making it more prone to entry and colonization by disease causing pathogens. Stage I is also the stage when the milk is richest in protein and fats that will encourage bacterial growth.

The Average Daily Milk Yield (ADMY) influenced incidence of mastitis with mastitis cases increasing with increase in ADMY of cow. At the lowest ADMY (≤10 litres per day it was 36.7% and at the highest ADMY (≥26 litres a day) it was 100%. The high milk yield is a stress factor that increases the cow's susceptibility to transmission/ entry of pathogens into the udder to set up a mastitic state according to Blood et al., (2006).

Several mastitis causing pathogens were isolated and identified up to the genus level. The order of prevalence of the pathogens so isolated was *Staphylococcus sp* (31.6%), *Escherichia sp* (22.4%), *Klebsiella sp* (18.4%), *Streptococcus sp* (17.1%), *Corynebacterium sp* (2.6%), *Pseudomonas sp* (1.3%), *Candida sp* (1.3%). The prevalence of *Staphylococcus sp* in this study was similar (37.6%) to the findings of a study by Odongo *et al*.(2013) conducted around Kabete area of Kiambu County. It is however much lower than the 58.8% realized by Ondiek *et. al*. (2013) in Njoro.

In all the three different studies *Staphylococcus* species was the most prevalent cause of mastitis. The prevalence of *E. coli* (22.4%) and *Klebsiella sp* (18.4%) on the other hand is much higher compared to 17.2% and 9.7% of Ondieki *et. al*. (2013) respectively probably suggesting a lower effort on farm hygiene in the area of study given that these two coliforms are environmental pathogens.

The three coliforms; *Escherichia sp, Klebsiella sp* and *Pseudomonas sp*, had a combined total prevalence of 42.1%. This is quite high and suggests a high incidence of poor hygiene in and around the milking parlors since they are environmental agents that cause mastitis.

Staphylococcus sp were high at 31.6% in prevalence. This is indicative of a high rate of spread of mastitis by contact since all *Staphylococcus sp* (except coagulase –ve *Staphylococci*) are contagious agents. These results further confirm the outcome of experiment 2; that a low percentage of farmers in the areas studied practice pre-dipping and post-dipping of teat during milking as measures to control mastitis pathogens that are of environmental or contact nature.

In this study *Candida sp* at 1.3% was much lower than the findings of Odongo *et al.* (2013) in which it was 6.3%. This suggests that there has not been overuse of antibiotics in the treatment of mastitis among these farms, compared to Kabete farms,

since *Candida sp*, being fungal, tends to be an opportunistic pathogen especially where broad-spectrum antibiotics have been used for long periods of time or where there is immunosuppression of an individual animal for whatever reason. (Bozena, *et. al*., (2012) and Krukowski, (2001).

There were different degrees of resistance to the various antimicrobials by the isolated microbes. In general the three aminoglycosides were the antimicrobials to which the majority of the isolated pathogens had the least resistance with gentamicin having the least at 2.2% followed by kanamycin at 2.8% and streptomycin at 5.1% (Table 16). There were only two *Escherichia sp* and one *Streptococcus sp* sample isolates that were resistant to gentamicin. This is very encouraging since gentamicin is a last line drug for the treatment of mastitis and especially that caused by coliforms.

The other aminoglycosides also need to be used with caution to avoid development of resistance to them by microbes. The widespread resistance to the two sulphonamides (cotrimoxazole 17.3% and sulphamethoxazole 17.3%) at the other extreme is reason for worry since it suggests a possible long term or indiscriminate use of antimicrobial preparations containing them as the active ingredients in the areas studied allowing the pathogens to develop resistance to them. Hence there is need for caution in their use to avoid further development of resistance or their use without success in treating mastitis. An Analysis of Variance (ANOVA) shows that there is no significant difference ($p < 0.05$) between ampicillin, nalidixic acid, chloramphenicol, sulphamethoxazole and cotrimoxazole in terms of resistance to them across the bacterial genera. The same is true for tetracycline and streptomycin and forf kanamycin and gentamicin. In terms of total resistance by the microbes, *Pseudomonas sp* was the highest at 20%. The two coliforms *Klebsiella sp* and *Escherichia sp.* are Gram - bacteria that, are now largely resistant to the

sulphonamides, penicillins and tetracyclines. They are quite sensitive to the aminoglycosides especially to gentamicin and kanamycin both of which have preparations available in our market. Ondiek *et. al*. (2013) listed the best three drugs, in decreasing order of effectiveness at treating mastitis among dairy cows at Tatton farm of Njoro, as Augmentin® (a combination of amoxicillin and clavulanic acid), gentamicin and cotrimoxazole. In our study the order was found to be gentamicin, kanamycin and streptomycin all aminoglycosides. Cotrimoxazole was the least effective. However this study did not investigate Augmentin®.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

The study identified the following factors as important contributors to the prevalence of mastitis in the areas studied;

1. A high overall prevalence of mastitis (50.7%) among the dairy farms and failure to detect and recognize subclinical mastitis cases that then act as reservoirs for mastitis- causing bacteria.
2. A low frequency of routine testing for mastitis at milking among the farmers and lack of awareness about the advantages of pre and post dipping of teats in suitable germicides at milking as control measures.
3. Majority of the farmers (75%) offered fresh feeds to the milked cows thirty minutes and after. This is too late to help keep the animals standing as they feed and as the open teat sphincters close within 30-45 minutes post milking to help control entry of pathogens into the udder through the teat canal.
4. Lack of clear culling policy that keep the average age of the dairy cow in the herds young. The highest mastitis risk groups are cows between parity 5 to parity 10.
5. High milk yielding cows (21 liters and above) especially during the first 2 months post calving had the highest risk of developing mastitis of the three categories.
6. Widespread resistance by some of the microbes to some antimicrobials such as sulphamethoxazole (17.3%) and co-trimoxazole (17.3) that are among the most commonly used around here to treat mastitis.

6.1 Recommendations

The following measures were therefore recommended to alleviate the problem:

1. Regular routine testing for mastitis at milking by all the farmers to detect subclinical mastitis using a strip cup or by such electronic devices as the Draminski subclinical mastitis detector.
2. Prompt and vigilant treatment of any mastitis cases found using antimicrobials to which there has not been much resistance by the microbes such as gentamicin (2.2% resistance), kanamycin (2.8%) and streptomycin (5.1%) to avoid subclinical maturing to clinical mastitis and to minimize development of resistance by the microbes to the antimicrobials used in mastitis treatment.
3. Avoidance of routine use of the sulphonamide based antimicrobials especially cotrimoxazole and sulphamethoxozole to treat mastitis in the areas unless culture and sensitivity tests have proved them useful in each case.
4. Improvement of hygiene of the farm environment especially at the milking parlor to minimize the presence of mastitis causing agents that are environmental in origin such as *Escherichia sp* .and *Klebsiella sp*.
5. The encouragement of the use of routine pre-dipping of teats in suitable germicides at milking to reduce spread of new infections particularly from environmental bacteria such as *Escherichia sp., Klebsiella sp* and *Pseudomonas sp*.
6. Encouragement of routine post dipping of teats in suitable germicides after milking to control new infections from contagious bacteria such as Staphylococcus sp.

7. Encouragement of the practice of offering fresh feeds to the cows immediately, and within fifteen minutes, after the end of milking to keep the cows standing as they feed as long as possible so as to reduce entry of bacteria through the open teat canal into the udder tissue.
8. The adoption of an order of milking that puts the younger cows and the mastitis-free cows ahead of the older ones and those with clinical or subclinical mastitis.
9. Putting in place a culling policy that keeps the average cow in the herds as young as possible; parity 5 and below unless it is an excellent cow.
10. Taking special hygiene and mastitis control measures when handling the heavy milk producing cows (≥ 20 litres) especially during their first two months post calving.
11. Capacity building and education of all stakeholders including farmers, veterinarians, dairy professionals on how to avoid the risk factors above by testing of the foremilk regularly, by carrying out pre and post milking teat dipping, by knowing about antimicrobial resistant mastitis and by using the available antimicrobial agents to treat mastitis only after culture and sensitivity testing of the milk has been done.

6.3 Suggestions For Further Research

1. Research on the prevalence and identification of bacteria that cause clinical and subclinical mastitis up to species level and covering a wider area of study.
2. More studies on antibiotic resistance by mastitis etiological agents; identifying them up to the specie level and involving more antimicrobial agents.
3. An assessment of the economic impact of subclinical mastitis among dairy herds in the area.
4. Studies on the mechanisms of antimicrobial resistance by the microbes and other risk factors associated with the development of mastitis among dairy cattle in the area.

REFERENCES

Barlow J.W., L.J. White, R.N. Zadoks, Y.H. Schukken., 2009. A mathematical model demonstrating indirect and overall effects of lactation therapy targeting subclinical mastitis in dairy herds. *Prev. Vet*. 90:31-42.

Bishi., Degos, K., and Tareke,F.(2003). *Bovine mastitis in selected areas of southern Ethiopia by Tropical animal health and production,* Netherlands: Kluwer Academic Publishers.

Blood, D. C., Rodostits, O. M., and Henderson, J. A. (2006). *Veterinary Medicine,* U.K: Language Book Society.

Bozena Dworecka-Kaszak, Alicja Krutkiewicz, Daniel Szopa, Miroslaw Kleczkowski and Malgorzafa Bieganska: High Prevalence of *Candida* Yeast in Milk Samples from Cows Suffering from Mastitis in Poland. *Scientific World Journal*. Apr 2012:196347.

Call, D. R., Davis, M. A., and Sawant, A. A. (2008). "Antimicrobial resistance in beef and dairy cattle production." *Anim Health Res Rev*, 9(2), 159-167.

Carter, G. R. (1998). "Diagnostic Procedures in Veterinary Microbiology", in *C. T. Charles,* (Ed). Springfield, Illinois, U.S.A.

Downham, K. D., and Christie, G. J., (1946). "Preliminary report on the treatment of mastitis in dairy cows with penicillin." *Veterinary Record*, 58, 475.

Draminski, J. (1989).

Edwards, S. J. (1968). "Infections and resistance of bovine mammary gland." *Veterinary Annual Journal*, 17, 21.

Fell, L.R. (1964). Machine milking and mastitis. *A Review of Dairy Science*, 26: 551-569.

GOK. (2008-2011). "VIL – Eldoret Annual Reports.

Harmon, R. J. (1994). "Physiology of mastitis and factors affecting somatic cell counts." *J. Dairy Sci*, 77, 2103-2112.

Hortet, P. and H. Seegers, (1998). Calculated milk production losses associated with elevated somatic cell counts in dairy cows: review and critical discussion. *Vet. Res. 29:497-510.*

Jaetzold, R. J., and Schmidt, H. (1983). *Farm Management Handbook of Kenya:* Natural Conditions and Farm Management Information, Nairobi; Kenya: Ministry of Agriculture.

Jones, B. M., and Bailey, T. L. (2010). *Understanding the Basic of Mastitis,* U.S.A.: Virginia Cooperative Extension.

Kirk, H. J., and Sudhan, N. A. (2010). "Mastitis- An Important Production Disease of Dairy Animals."

Kirk, J. J. (2010). "A Practical Look at Environmental Mastitis", *Mastitis in Dairy Cows.* Virginia, U.S.A.: MacDonald Campus of McGill University.

Krukowski H. Mycotic mastitis in cows. *Medycyna Weterynaryjana*. 2001; 57(1): 18-20.

Nanita Blomquist, Alberta Ag-Info Centre, Alberta Agricultural and Rural Development. May, 2015. *Ag-info centre*.

National Mastitis Council (1996). *Current Concepts of Bovine mastitis, 4th edition,* Arlington National V.A.

National Animal Health Monitoring System (NAHMS). (2007).

Neave, D. R. (1959) and Fell, L. R. (1964). "Multi-factorial Etiology of Bovine Mastitis".

Odongo, M. O., Ndungu, T. N., Mulei, C. M., and Macharia, N. J. (2013). "Prevalence of microbial causes of bovine mastitis in the Kabete County and its environs (2001-2010)." *The Kenya Veterinarian VOL. V.*

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." *Food borne pathogens and diseases*, 8(3), 337-355.

Ondiek, J.O., Ogore, P.B., Shakala, E.K., Kaburu, G.M. Prevalence of bovine mastitis, its therapeutics and control in Tatton Agricultural Park, Egerton University Njoro District of Kenya. *Basic Research Journal of Agricultural Sciences and Review* ISSN 2315-6880 Vol. 2(1), pp. 15-20 Jan. 2013.

Petersen, M. E. (1938). *Association between Mastitis and pathogenic bacteria*: Imperial Bureau of Animal Health. U.K.

Rodernberg, J. (2012). *Mastitis Preventions for Dairy cattle: Environmental Control*, Woodstock, Ontario-Canada: Ministry of Agriculture, Food and Rural Affairs.

Schwarz, S., Aarestrup F.M. (2006). *Antimicrobial resistance in bacteria of animal origin*, Washington DC, USA: ASM press. pp 187-206 (ISBN 1-5558-306-2).

Schwarz, S., Monecke, S., and Ehricht, R. (2010). *Journal of Antimicrobial chemotherapy*. 2010: Apr, 65(4): 619-24. Characterization of methicillin-resistant *Staphylococcus aureu*s ST398 from cases of bovine mastitis.

Silva, J., Caraviello, D., Rodrigues, A., and Ruegg, P. (2010). "Use of petrifilm Tm for mastitis diagnosis." *Journal of Dairy Science*, 88(8), 3000-3008.

VIL Eldoret 2008-2011. Annual Report.

Waller, K. P., Aspan, A., Nyman, A., Persson, Y., and Andersson, U. G. (2011). "CNS species and antimicrobial resistance in clinical and subclinical bovine mastitis." *Veterinary microbiology*, 152(1), 112-116.

Wang, X., and Zhang, W. J. (2012). "Methicillin- Resistant *Staphylococcus aureus S*T9 from a case of bovine mastitis." *Journal of Antimicrobial Chemotherapy*, 67, 1287-1289.

APPENDICES

APPENDIX I: Questionnaire On Mastitis Testing And Control Measures.

Instructions to respondents

Kindly respond to the following questions by putting a tick (√) in the box against your appropriate choice.

Your identity is strictly confidential and do not write your name on the questionnaire sheets.

1. Do you routinely test for mastitis at milking?
   Yes ☐ No ☐

2. If yes, what method do you use?
   a) A strip cup
   b) Draminski mastitis detector
   c) The California Mastitis Test
   d) By boiling of the milk.
3. Do you know why it should be done?
   Yes ☐ No ☐

4. Do you routinely carry out pre-dipping of teats in a suitable germicide before milking?
   Yes ☐ No ☐

5. Do you routinely carry out post dipping of teats in a suitable germicide after milking?
   Yes ☐ No ☐
6. How soon after the end of the milking operation do you offer fresh feeds to your cows?
   a) Immediately after milking
   b) (30-60) minutes
   c) (61 – 120) minutes
   d) After 120 minutes

APPENDIX II: Summary of Antimicrobial Sensitivities

Sensitivity of the isolated microorganism species to antimicrobial agents

Table 24: Percentage sensitivity of the isolated microorganism species to antimicrobial agents

Percentage of number of samples sensitive to the antimicrobial type									
Type of microorganism isolated	AMP	NA	TE	C	SX	COT	S	K	GEN
Staphylococcus sp.	12.1	0	10.6	1.5	0	0	22.7	22.7	30.3
Escherichia sp.	2.3	7	7	0	0	0	23.3	25.6	34.9
Klebsiella sp.		2.5	10	2.5	2.5	2.5	15	30	35
Streptococcus Sp.	9.5	0	9.5	0	0	0	23.8	23.8	33.3
Corynebacterium Sp.	0	0	16.7	0	0	0	16.7	33.3	33.3
Pseudomonas sp.	0	0	25	0	0	0	25	25	25

Table 25: Summary table of number of samples sensitivity to each antimicrobial group

	Group1	Group2	Group3	Group4	Group5	Group6	Total
Staphylococcus Sp.	8	0	7	1	0	50	66
Escherichia sp.	1	3	3	0	0	36	43
*Klebsiell*a sp.	0	1	4	1	2	32	40
Streptococcus Sp.	2	0	2	0	0	17	21
Corynebacterium Sp.	0	0	1	0	0	5	6
Pseudomonas sp.	0	0	1	0	0	3	4
Total	**11**	**4**	**18**	**2**	**2**	**143**	**180**

KEY: Group 1 = Penicillins
Group 2 = Quinolones
Group 3 = Tetracyclines
Group 4 = Chloramphenicol
Group 5 = Sulphonamides
Group 6 = Aminoglycosides

Table 26: Summary table of percent sensitivity to each antimicrobial group

	Group1	Group2	Group3	Group4	Group5	Group6	Total
Staphylococcus sp.	12.1	0	10.6	1.5	0	75.8	
Escherichia sp.	2.3	7	7	0	0	83.7	
Klebsiella sp.	0	2.5	10	2.5	5	80	
Streptococcus Sp.	9.5	0	9.5	0	0	81	
Corynebactrium sp.	0	0	16.7	0	0	83.3	
Pseudomonas sp.	0	0	25	0	0	75	

KEY: Group 1 = Penicillins
Group 2 = Quinolones
Group 3 = Tetracyclines
Group 4 = Chloramphenicol
Group 5 = Sulphonamides
Group 6 = Aminoglycosides

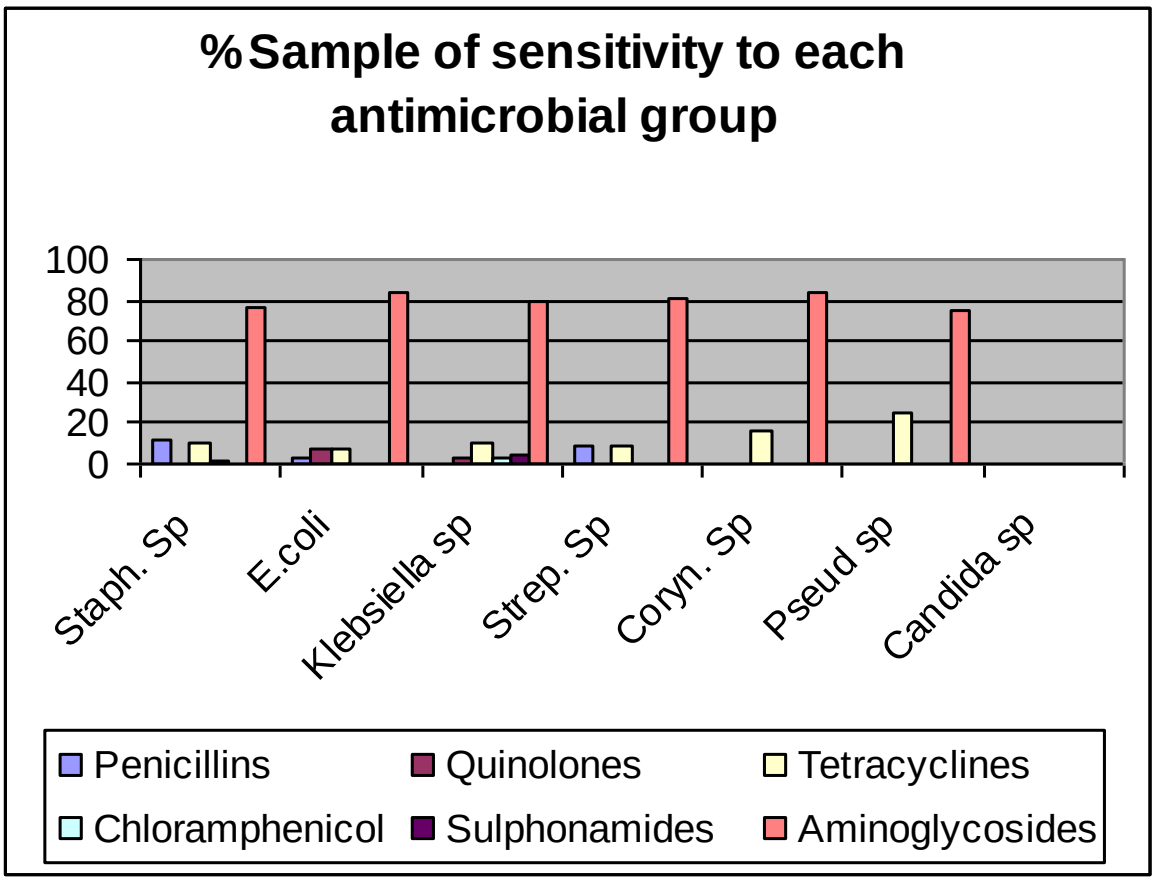


Figure 15: Percent sample sensitivity to various antimicrobial groups