INVESTIGATION OF BOVINE MASTITIS AND PATHOGENS SENSITIVITY TO ANTIMICROBIALS IN NORTH RIFT AND WESTERN PARTS OF KENYA FROM 2004 TO 2013.

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DECLARATION

This project is my original work and has never been presented for award of a degree in any university.

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DEDICATION

To the almighty God for his provision and favors, strength, patience and enduring spirit throughout my studies and for his never ending love.

To my parent (Madam Benter Ogonji) for her never ending love, encouragement, relentless moral and financial support ever since. I'm forever grateful and will always be indebted to you.

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LIST OF ABBREVIATIONS

C.M.T	California mastitis test
DHIA	Dairy Herd Improvement Association.
D.V.O	District Veterinary Officer
E. coli	*Escherichia coli*
FAO	Food Agricultural Organization
S.C.C	Somatic cell count
Spp.	Species

ABSTRACT

The purpose of this study was to determine the occurrence of bovine mastitis cases in the North Rift and parts of Western Kenya and sensitivity of mastitis causing organisms to antimicrobials for a period of ten (10) years. This was a retrospective study whereby data was obtained by going through record books at the Regional Veterinary Investigation Laboratory (RVIL) Eldoret starting from the year 2004 to 2013. From the records, a total of 2,869 cases of bovine mastitis had been reported to the RVIL Eldoret from the 2004 to 2013, out of this, confirmed cases were 2,225 and the rest had tested negative for any mastitis causing organisms. Antimicrobial Sensitivity was determined for 1,930 bacterial isolate starting from the year 2007 to 2013 since records of the earlier 3 years were not available. Sensitivity tests were done only for cases which had tested positive for bacterial isolates. Isolates selected were those giving pure cultures and also mixed culture colonies hence the reason for some tests showing partial sensitivity due to one of the isolate being sensitive while the other being resistant to the same antimicrobial used. Of the isolated bacteria, *Staphylococci* had a prevalence of 33.17%, *Streptococcus* 21.53%, *Klebsiella* 21.53%, *Escherichia coli* 15.24%, *Corynebacteria* 5.24% and the other bacteria had prevalence of less than 1% each. Of the twelve (12) antibacterial compounds used in sensitivity tests during this period, bacteria were most sensitive to Gentamicin at 90.41% followed by Kanamycin at 67.22% and were least sensitive to Sulphamethoxazole and Nalidixic acid at 2.95% and 7.50% respectively. From this study, it was established that bovine mastitis cases in the study area are fairly high and there is also high antimicrobial resistance. Therefore, there is need for producers' education and sensitization program for control of bovine mastitis in North Rift region and parts of Western Kenya.

CHAPTER 1: INTRODUCTION

Mastitis is the inflammation of the mammary glands caused by either trauma, chemical irritation or infection of the mammary glands by over 140 types of microorganisms, either singly or a combination of them (Dodd and Neave, 1970; Natzke, 1981; Radostits *et al.,* 1994). *Staphylococcus* and *Streptococcus* species are the most frequent mastitis causing organisms with *Staphylococcus* being the most notorious (Lauerman *et al.,* 1973; Mbise *et al.,* 1985; Odongo *et al.,* 1989; Anakalo *et al*., 2004). Coliform mastitis is also common but usually associated with cow's environmental hygiene (Eberhart *et al.,* 1979; Blood *et al*., 1983). Nutrition, hygiene, genetics and environmental conditions influence the host ability to respond to mastitis challenges (Wilson *et al*., 1997)

Mastitis can be said to be either subclinical or clinical in nature. Clinical signs range from mild to per acute. Subclinical mastitis only manifests as presence of pathogenic microorganisms in the milk and a corresponding elevation of somatic cell count (SCC) and only diagnosed with screening test like California Mastitis Test (CMT) or laboratory procedures (National mastitis council, 1987). Contagious mastitis is caused by mainly *Streptococcus* species and *Staphylococcus aureus*. Environmental mastitis is due to *E. coli*, *Streptococcus dysgalactiae* and *S. uberis*. Minor pathogens include *Corynebacterium bovis* and *Staphylococcus epidermis* (Tyler *et al*., 1998).

Bovine mastitis is considered to be the most costly disease in the dairy industry. Losses are due to reduced milk production and quality, early replacement costs due to culled and dead cows, discarded milk, extra labor, veterinary drugs and services (Fetrow, 2000). From previous studies, it has been noted that bovine mastitis is a problem in Kenya dairy herds (Laurman *et al.,* 1973; Munene *et al*., 1987; Omore *et al*., 1996; Anakalo *et al*., 2004).

In earlier times, dairy industry in Kenya was mainly occupied by large scale farmers and was intended for urban and export markets (Muriuki *et al*., 2003; Ngigi, 2004). The subdivision of land led to the emergence of small scale dairy farming as a means of live hood (Ngigi, 2004). By the year 2006, smallholder dairy units were approximately 666,000 and had about 2.5million dairy cattle and the number has increased (Owen *et al*., 2005; Lanyasunya *et al.,* 2006). Small scale farmers raise dairy cows under very intense production system and this has made it easy for the spread of both infectious and environmental disease like bovine mastitis and at the same time making it difficult for its control (Owen *et al*., 2005). However, small scale dairy production contribute 80% of the total milk produced and marketed in Kenya (Wakhungu, 2001; Muriuki *et al*., 2003; Owen *et al*., 2005). In comparison to the smallholder dairy units, the medium and large scale dairy farmers are better managed (Gachiuri *et al*., 1998; Wakhungu, 2001). These farms are owned by individuals, private firms or public institutions such as Agricultural Development Corporation (ADC) (Ojongo *et al.,* 2001; Wakhungu, 2001) and have more milk production per individual cow and overall milk yield per herd. They contribute 20% of the total milk marketed in Kenya (Muriuki *et al*., 2003) and provide the industry with quality milk.

Mastitis is the single most common disease syndrome in dairy cows accounting for 38% of all morbidity. On an annual basis, 3 of every 10 dairy cows have clinically apparent inflammation of the mammary gland and of the affected cattle, 7% are culled and 1% die as a result of the disease (Tyler *et al.*, 1998). Therefore, there is need to assess the current status of bovine mastitis, its clinical prevalence and causative agents amongst this sector.

This study aimed at obtaining information from the RVIL Eldoret within milk producing region in the North Rift and Western parts of Kenya to enable assessment of the level of mastitis and pathogens resistance to antimicrobials in this area.

1.1 General objective of the study

The objective of this study was to carry out a retrospective study and investigate on cases of bovine mastitis in the North Rift and Western parts of Kenya from 2004 to 2013.

1.2 Specific objectives of the study

- To determine the occurrence of bovine mastitis in North Rift and Western parts of Kenya between the year 2004 to 2013.
- To determine the bacteria causing bovine mastitis and their sensitivity to antimicrobials in North Rift and Western parts of Kenya.

1.3 Justification

Dairy farming is a food manufacturing industry producing everyday raw milk which is important to food industry and milk is an important food too. Therefore, there is a greater need to know how to manage dairy farming on a daily basis for animal health and wellbeing, public health, environmental health and its financial wellbeing. Dairy farming being a business, farmers do suffer high economic losses in terms of milk rejection, veterinary costs and high costs of drugs. More than 25% of all disease related economic losses of dairy cattle can be directly attributed to mastitis thus the need for a good mastitis control program. This can only be realized by evaluating on prevalence of mastitis, know which causative organisms are the most prevalent and which is (are) the most efficient drug(s) for treatment of bovine mastitis.

CHAPTER 2: LITERATURE REVIEW

2.1 Mastitis (General)

Mastitis is the inflammation of mammary glands. Mastitis may results from introduction of the microorganisms through the teat sphincter. It is most often transmitted by contact with contaminated milking machines, contaminated hands or contaminated materials. The clinical course of the diseases varies with the ability of bacteria to colonize and thrive in the mammary gland secretions, their inherent virulence and type, magnitude and duration of the host response to the bacterial invasion (Tyler *et al*., 1998; Jones *et al*., 2010). Resulting inflammation of the mammary gland is indicated by a wide variety of clinical signs.

Mastitis has a negative nutritional effect since it can cause a decline in potassium and lactoferrin. It also results in decreased casein the major protein in milk. Disruption of casein synthesis contributes to lowered calcium in milk since most calcium in milk is associated with casein (Jones *et al*., 2010). Milk from cows with mastitis also has high somatic cell count which lowers milk quality.

Mastitis can be divided into two broad categories that is; contagious mastitis and environmental mastitis on the basis of the source of infectious agent (Tyler *et al*., 1998). Contagious mastitis has the infected mammary gland quarter as its primary reservoir. Most common bacteria for this category include: *Streptococcus agalactiae, Corynebacterium bovis, Staphylococcus aureus* and *Mycoplasma* spp. Most important mode of transmission of these organisms involves the Cow-to–cow transfer of bacteria laden milk (Tyler *et al*., 1998; Keefe, 1997; Odongo *et al*., 1989).

Primary environmental pathogens include (2) two types of bacteria: coliform bacteria and species of *Streptococci* other than *Strep. agalactiae*. The primary source of environmental pathogens is the surrounding in which a cow lives. Housed cows are at a greater risk for environmental

mastitis than cows on pasture (Hogan *et al.,* 1987). Warmer environmental temperatures favor growth of pathogens and can result to environmental mastitis. Environmental conditions that can increase exposure to environmental mastitis include: overcrowding; poor ventilation; inadequate manure removal; dirty maternity stalls or calving areas and general lack of farm cleanliness and sanitation (Hogan *et al.,* 1987).

2.2 General classification of mastitis

Mastitis can be classified as either subclinical mastitis, where few, if any, symptoms are present in most cases with no apparent change in milk or clinical mastitis, where animals show signs of sickness accompanied by abnormal milk and caused by microbial infection involving *S. aureus, S. agalactiae, S. dysgalactiae, S. uberis,* Coliform organisms, *Pseudomonas spp.* and *Mycoplasma* spp. which cause serious problem for the dairy industry (Tyler *et al*., 1998). In Kenya, subclinical mastitis has a higher prevalence than clinical mastitis (Ngatia, 1988; Omore *et al*., 1996; Ondieki *et al*., 2013). A finding which conforms to what was found in this study.

2.2.1Subclinical mastitis

Subclinical mastitis occurs when the mammary gland is infected and the number of leukocyte (somatic cell count) is increased. The milk will appear grossly normal with no visible sign of inflammation on the mammary gland. Subclinical mastitis is detected by routine tests like strip cup, California mastitis test (CMT), Somatic cell count (SCC) or routine culturing of all quarters (Ndirangu *et al.,* 2013). With time, subclinical mastitis usually results in fibrosis of mammary tissue, firmness and large gland and decreased milk production. This is mostly caused by *Streptococcus agalactiae* and *Staphylococcus aureus* (Dahoo *et al*., 1982; Omore et *al.,* 1989; Keefe, 1997; Tyler *et al.,* 1998).

2.2.2Clinical mastitis

Clinical mastitis is characterized by grossly abnormal milk and evidence of varying degrees of mammary gland inflammation. The milk can vary from having some few clots (gadget) to serum with clamps of fibrin in the secretion. Clinical mastitis can further be classified into acute mastitis, acute gangrenous mastitis and chronic active mastitis (Mbise *et al*., 1985; Tyler *et al*., 1998; Gonzalez *et al*., 1989)

2.2.2.1Acute mastitis

Acute clinical mastitis is often characterized by a swollen and painful gland that may be edematous or very hard impairing mobility .There may be slight or severe systemic signs with sudden onset (Mbise *et al.,* 1985; Tyler *et al*., 1998; Gonzalez *et al*., 1989)

Anorexia, depression and elevated rectal temperature are often associated with this clinical event. Severe toxic cases may have low serum calcium and paraplegia resembling milk fever. Associated hypocalcaemia is largely nonresponsive to parental calcium administration .Acute mastitis can be new infection or escalation of chronic infections. The secretions may contain clots of milk and can be watery, serous or purulent. Isolated organisms are usually gram negative bacteria, *S. dysgalactiae* and *S. uberis* (Eberhart *et al*., 1979; Waage *et al*., 1999).

2.2.2.2Acute gangrenous mastitis

Acute gangrenous mastitis is usually not a common event. Patient exhibits anorexia, dehydration, depression, fever and signs of toxemia .Etiologic agent is primarily *Staphylococcus* with involvement of one to all quarters. Infection caused by *Clostridium, Staphylococcus* and Coliforms sometimes results into gangrenous mastitis (McDonald, 1997).The gland is red, swollen and warm early in the disease .However within a few hours the teat becomes cold with watery and sanguineous secretions. The gland then exhibit an area of sharply delineated blue

discoloration .Sloughing of this area occurs within 10-14 days and is followed by secondary bacterial infection; necrosis and continued sloughing off of the glandular tissue .Organism mostly associated with gangrenous mastitis are *Clostridium perfringens* and *Staphylococcus aureus* (McDonald, 1997; Tyler *et al*., 1998).

2.2.2.3Chronic active mastitis

Patient may exhibit no clinical signs for prolonged intervals. Somatic cell count (SCCs) are generally chronically elevated and mammary secretions periodically contain flakes, clots or shreds of fibrin .In the chronic type of mastitis, there is prolonged destruction of mammary glands alveoli and ducts with replacement by scar tissue resulting in decreased milk production. Bacteria incriminated in this type of mastitis are Coliforms, *S. agalactiae, S. aureus* and *Salmonella dublin* (Keefe, 1997; Tyler *et al*., 1998; Smith *et al*., 1989).

2.3 Prevalence of mastitis pathogens in Kenya and pathogens sensitivity to antimicrobials

Bovine mastitis studies done in Kenya to determine prevalence of mastitis pathogens and their antimicrobial sensitivity in different parts of the country have been of great value to dairy industry. A study carried out in Kabete region of Kiambu County established the prevalence of *Staphylococcus* spp. to be 31.7%, *E coli* (17.2%), *Streptococcus* spp.(10.3%), *Klebsiella spp.* (9.7), *Pseudomonas aeroginosa* (7.6%), *Bacillus* spp.(4.8%), *Arcanobacterium* (*Corynebacterium*) *pyogenes* (4.1%) and yeast(*Candinda albicas*) (6.3%) causing mycotic mastitis in the region (Odongo *et al*.,2012).

In another study, *Corynebacterium* had a prevalence of 45%, *Staphylococcus* (30%), *Streptococcus* (22.5%) and *Pseudomonas* (2.65%) (Muthee *et al.,* 2005). Pathogens were most sensitive to Ampi-clox and Cefaclor at 98% both and less sensitive to Sulphamethoxazole and

Cotrimoxazole both at 2%. *Pseudomonas* was sensitive to gentamicin, norfloxacin and tetracycline only (Muthee *et al*., 2005).

2.4 Mastitis control principles

This includes; use of post milking disinfectant teat dips since they are superior to spray application because they provide optimal coverage and contact on the teat skin (Tyler *et al.,* 1998). Predips (Barrier dips), predipping is often employed as a measure of contagious mastitis control and as a means to meet the guideline of the Pasteurized Milk Ordinance (PMO) that require milking a clean dry presanitized mammary gland (Ruegg and Dohoo,1997; Berry *et al*.,1997). Intramammary dry cow antibiotic therapy also controls contagions mastitis pathogens. Preparation used contains high doses of slow release antibiotic which is highly effective against *Streptococcus* spp. and *Staphylococcus* spp. (Guterbock *et al*., 1993). Dry cow therapy has no effect against gram negative bacteria or *Mycoplasma* spp. infection (Jasper, 1981). All in all the best control for contagious mastitis is to break the chain of transmission for the infectious agent either by eliminating the source of infection through treatment or by isolation and strict hygiene during the time of milking (Berry *et al.,* 1997;Tyler *et al*.,1998;Saran, 1995). The National Mastitis Council (http: //WWW. nmconline.org) recommends a time proven five-point control program to solves this disease problem; Milking machines maintenance, teat dipping, early treatment of clinical cases, dry cow therapy and culling of cows with chronic mastitis.

Control of environmental mastitis is more problematic since it does not respond to contagious mastitis control programs. Reducing exposure of the teat ends to environmental pathogens and maximizing resistance of the cow to intramammary infections are the critical strategies to be employed for control of environmental mastitis caused by environmental mastitis pathogens (Radostits *et al*., 1994; Tyler *et al.,* 1998). Organisms causing environmental mastitis do not

require presence of an infected quarter to perpetuate within a herd. Fecal material or fomites may all harbor and nature bacterial population capable of causing disease if introduced into the mammary gland .Gram-negative coliform bacteria responsible are; *Escherichia coli, Klebsiella spp., Enterobacter spp., Pseudomonas* and *Proteus* (Eberhart *et al*., 1979; Tyler *et al*., 1998; Saran, 1995).

2.5 Milking hygiene and milking practices

Contagious mastitis infection are spread between cows at the time of milking hence particular attention and focus must be placed on how cows are handled at the time of milking .No equipment should be carried from cow to cow without prior disinfection (Radostits *et al*., 1994; Saran, 1995). Milking machines should be properly functioning to avoid reverse milk flows. Excessive water use in premilking preparation of the mammary glands mobilizes skin contaminant bacteria increasing contamination of teat ends during milking. Additionally water sources or low water delivery systems can become colonized by bacterial like *Serratia* spp. (Radostits *et al*., 1994; Saran, 1995; Tyler *et al*., 1998)

2.6 Feeding strategies

Feeding also forms an important component of environmental mastitis control program. Providing fresh palatable feedstuff after each milking increase the interval that cow remains standing thus allowing for proper closure of the teat canal hence enhancing teat end hygiene (Radostits *et al.,* 1994; McDonald, 1997; Tyler *et al*., 1998). Proper nutrition is critical for the cow to develop and maintain optimal immune function and disease resistance .In particular, Vitamin E and selenium deficiencies have been associated with incidence of clinical gram-negative mastitis (Smith *et al*., 1997; Erskine *et al*., 1997).When environmental mastitis is present in a herd, all the cows should receive balanced diet including micronutrient

supplementation. This also includes those that are dry or non-lactating. Blood selenium and Vitamin E concentrations can be measured during late gestation and early lactation (Weiss *et al*., 1997; Erskine *et al*., 1997).

2.7 Microbiological techniques for diagnosing bovine mastitis

2.7.1Routine culture of mammary gland secretions

Routine culture of mammary gland secretions is necessary because prevalence of subclinical mastitis in dairy herds is usually high and has significant impact on productivity and profitability of the herd. Determining the etiology of individual animal or herd outbreak is important for efficient management strategy to be developed to reduce number of new mastitis cases and to improve prognosis of the patient (Sears *et al*., 1993). It is important to initiate a program of routine milk culturing when one or more of the following benchmarks occurs: Bulk tank SCCs are >250,000, Dairy herd improvement association (DHIA)-SCCs reveals that >15 % of lactating cows in the herd have a linear score (LS) >4.5, new clinical cases in the herd are >2% per month and acutely ill cows in the herd are >1% per year.

2.7.2 Sample collection, transport and processing.

The results of milk culture are no better than the sample; and the sample is no better than the manner in which it is collected transported and processed.

2.7.3 Sample collection and transport

Samples are best collected just before milking since foremilk usually contains more mastitis pathogens than milk taken during milking. Milk is collected in a sterile glass or plastic tube of about 15ml capacity with a tight fitting cap. Udder and teats are washed and dried. Each quarter is sampled separately or one can take milk from all quarters to obtain single (composite) sample. When collecting milk, one holds the tube close to a horizontal plane and the cap is held with its

inside facing downwards. Do not allow the teat to come in to contact with the container. Fill the container approximately half full then seal. Sample should be transported to the laboratory as quick as possible and at the right temperatures (Tyler *et al*., 1998).

2.7.4 Routine culture of clinical mastitis

Culture all quarters with clinical mastitis .Sample should be taken before therapy is given so as to determine the true cause(s) of clinical mastitis in a herd (Tyler *et al*., 1998). Routine culture of all clinical cases also provides an opportunity for antibiotic susceptibility testing to guide future treatment recommendation for the herd.

Approximately 25%-30% of clinical sample will show negative culture results .It is probable that many of these come from cows with Coliform infections that have already been controlled by the animal own defense since coliform infections are of short duration (Eberhart *et al.,* 1979; Cebra *et al*., 1996)

Herd test culture is indicated most often when *S. agalactiae, S. aureus, Mycoplasma bovis* or some other contagious pathogen is known to be a problem in the herd (McDonald, 1977). Objective is to identify infected cows for segregation and or treatment (e.g. *S. agalactiae*). Here the task of sample collection, culturing and record keeping is substantially reduced by collection of composite sample since each cow is handled as a unit.

CHAPTER 3. MATERIAL AND METHODS

3.1 Study area

The study was carried out at Regional Veterinary Investigation Laboratory (RVIL) Eldoret which serves mostly North Rift in the former Rift valley province and major parts of former Western province .North Rift is made up of seven counties and western province has four counties hence the laboratory serves about 11 counties out of 47 counties of the total counties in the country.

The laboratory was chosen due to its accessibility and because it serves a large area hence giving an almost exact picture of mastitis prevalence in the region of North rift and Western Kenya. Also most of the population in North Rift practice dairy farming as a major income generating activity. The laboratory mostly receives cases and specimen from farmers /owners, VIL staff, DVO /field staff and surrounding institutions like G.K prisons. Private veterinarians do submit cases and specimens to the laboratory too.

However, most of the specimen and cases submitted to the laboratory are usually from Uasin Gishu County and this could be attributed to the county's proximity to the laboratory .Other cases /specimens are mostly from neighboring counties in the North Rift. Western region submit the least cases and specimen to the laboratory which could be due to distance and also few people keeping dairy cows in Western as compared to Rift valley province.

3.2 Study design

The data was collected from the records kept in the laboratory. After receiving milk samples of cases suspected to be of bovine mastitis, the samples are cultured to confirm for mastitis and to also determine which etiological agent is involved. Confirmation of mastitis at the laboratory is by culture of the milk sample on blood agar with 5% sheep blood and also on MacConkey agar. After 24 hours of incubation, plates are examined for growth of microorganisms. Bacteria are

then identified microscopically by gram staining, colonial morphology and by the arrangement of stained bacterial cells. Biochemical test mainly catalase test is done to differentiate gram positive cocci and rods and indole test done to differentiate gram negative cocci.

Sensitivity tests for 1,930 bacterial isolate were done which represented the year 2007 to 2013 for lack of sensitivity tests for the other earlier 3 years. Sensitivity tests were only for confirmed bovine mastitis cases. Occurrence of clinical bovine mastitis versus subclinical bovine mastitis was also analyzed by looking into the history of reported and confirmed cases.

Antibiotics used were; Ampicillin, Tetracycline, Cotrimoxazole, Streptomycin, Kanamycin Gentamicin, Sulphamethoxazole, Chloramphenicol, Nitrofurantoin, Nalidixic acid, Cefuroxime and Augmentin although the latter four were not much used

3.3 Data collection

This was done by a retrospective study of the samples submitted to the laboratory for culture and sensitivity test from cases suspected to be of mastitis. Records of cases of bovine mastitis were collected and proportion of each causative agent against total cases confirmed of mastitis for the 10 year period was determined. The case attributes recorded included; number of milk samples presented to the laboratory for culture and sensitivity, confirmed cases, number of each pathological agent isolated and the percentage of each drug sensitivity and resistance in the tests.

3.4 Data analysis

Data analysis was done by getting the percentage of the different attributes that were recorded when collecting data. The total number of samples was obtained by adding the numbers of all samples of mastitis submitted to the laboratory for each year in the ten year period under the study. Of the samples submitted those that tested positive for mastitis pathogens were recorded

as confirmed cases. Samples where there were no isolate were recorded as sterile cases or no isolate. The percentage for each isolated organism was obtained by getting the total number of times the organism was isolated and this figure was divided by the number of all confirmed cases of bovine mastitis then multiplied by 100 to get the percentage.

Taking for example X to be the number an organism was isolated for the ten year period, and Y to be the number of all confirmed bovine mastitis cases for the same period, then the percentage of X was calculated by the formula;

$$\text{Prevalence of } X = X/Y*100$$

The percentage of all isolated organism was calculated and recorded in table 4.1.

Sensitivity analysis was also done by getting sensitivity percentage of each antimicrobial used during the study period. Taking for example antibiotic X was used N times in the sensitivity test and it was sensitive n times. Then sensitivity percentage of antibiotic X was obtained by the formula;

$$X \text{ sensitivity} = n/N*100$$

Sensitivity of each antimicrobial used was then calculated and recorded in table 4.2. During sensitivity calculation, cases in which there was partial sensitivity to the antimicrobial were considered as resistant and their percent figures were included in calculation of resistance percentages. In table 4.2, the percentages of partial sensitivity (PS) are not shown since the figures are already included in percent calculation of resistance.

CHAPTER 4. RESULTS

A total of 2,869 cases were submitted to the RVIL Eldoret between the period 2004 and 2013. Out of this, confirmed bovine mastitis cases were 2,225 and the rest tested negative for bovine mastitis causing organisms hence were termed sterile cases. (Table 4.1). Confirmed cases represented 77.6% of the total cases reported to the laboratory which were suspected to be bovine mastitis. Negative (sterile) cases were 644(22.45%) of suspected bovine mastitis cases.

From the files records, it was noted that subclinical bovine mastitis had a higher prevalence as compared to the clinical form of bovine mastitis.

	Clinical mastitis	Subclinical mastitis	confirmed cases
	832	2,037	2,869
Prevalence	29.00%	71.00%	100%

Percentages of isolates were *Staphylococcus* 738(33.17%), *Streptococcus* spp. 479(21.53%) *Klebsiella* 478(21.48%) *E. coli* 339 (15.29%) *Corynebacteria* 119 (5.35%), *Pseudomonas* spp. 23(1.03%), *Proteus* spp. 13(0.58%), *Enterobacter* spp. 5(0.23%), *Pasteurella* spp. 2 (0.09%) and *Bacillus* spp. 1(0.05%). (Table 4.1). Fungal isolates were 28(1.26%) of the total confirmed cases.

Gentamicin sensitivity was 90.41%, Chloramphenicol (72.40%), Kanamycin (67.22%), Streptomycin (53.52%), Tetracycline (37.46%), Ampicillin (17.93%), Cotrimoxazole (12.12%) and Sulphamethoxazole (2.95%). (Table 4.2).Nitrofurantoine sensitivity was 10.00%, Nalidixic acid (7.50), Augmentin (48.57) and Cefuroxime (45.71) (Table 4.2).

Table 4.1: Occurrence of bovine mastitis in North Rift and Western parts of Kenya from 2004-2013. Data from RVIL Eldoret

Mastitis causing organisms	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	Total cases	Percentage%
Staphylococcus	16	25	69	48	55	76	79	135	140	95	738	33.17%
Streptococcus	10	17	27	50	25	45	35	120	74	76	479	21.53%
Klebsiella	6	5	30	46	47	77	72	62	49	84	478	21.48%
Escherichia coli	26	10	21	32	18	28	26	52	75	51	339	15.24%
Corynebacteria	8	1	16	22	11	17	5	22	11	6	119	5.24%
Pseudomonas spp.	0	0	0	0	0	4	1	9	7	2	23	1.034%
Proteus spp.	0	0	0	2	4	1	1	0	2	3	13	0.58%
Enterobacter spp.	1	2	2	0	0	0	0	0	0	0	5	0.23%
Pasteurella spp.	0	0	2	0	0	0	0	0	0	0	2	0.09%
Fungal(candida albicans)	0	0	1	0	0	4	2	8	6	7	28	1.26%
Bacillus spp.	-	-	-	-	-	-	-	-	1	-	1	0.05%
Sterile (no isolate)	9	15	26	69	55	98	129	110	77	56	644	22.45%
Confirmed cases	67	60	168	200	160	252	221	408	365	324	2,225	77.55%
Reported cases	76	75	194	269	215	350	350	518	442	380	2869	
%confirmed	88.16%	80.60%	86.60%	74.35%	74.42%	72.00%	63.14%	78.76%	82.58%	85.26%	77.55%	

Table 4.2: Pathogens sensitivity and resistance to antimicrobials in North Rift and Western parts of Kenya from 2007 to 2013

Antimicrobials	2007			2008			2009			2010			2011			2012			2013			%SENSITIVITY /RESISTANCE	
	S	R	PS	S	R	PS	S	R	PS	S	R	PS	S	R	PS	S	R	PS	S	R	PS	S	R
Gentamicin	177	16	7	140	15	5	230	14	8	204	14	3	359	17	32	323	18	24	312	10	2	90.41%	9.59%
Kanamycin	154	40	6	109	23	28	125	101	26	153	53	15	235	118	55	276	37	52	193	24	29	67.22%	32.78%
Streptomycin	18	34	4	36	60	64	121	117	14	134	60	27	164	173	71	246	39	80	237	31	56	53.52%	46.47%
Tetracycline	78	110	12	60	60	40	94	134	24	96	100	25	102	176	130	163	174	28	130	108	86	37.46%	62.54%
Ampicillin	54	133	13	14	124	22	25	202	27	79	126	16	49	276	83	68	200	97	57	180	87	17.93%	82.07%
Cotrimoxazole	24	171	5	18	111	31	14	22	17	46	154	21	68	314	26	31	246	88	33	221	70	12.12%	87.88%
Sulphamethoxazole	-	-	-	7	143	10	9	233	10	7	204	10	6	355	27	10	325	30	12	274	38	2.95%	97.05%
Chloramphenicol	138	61	1	126	22	12	138	99	15	181	32	8	303	73	32	260	58	47	234	41	25	72.40%	27.60%
Nalidixic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	46	28	7.50%	92.50%
Nitrofurantoine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	50	22	10.00%	90.00%
Augmentin	34	36	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	48.57%	51.43%
Cefuroxime	32	38	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	45.71%	54.29%

Key

S=Sensitivity R=Resistance PS=Partial sensitivity

CHAPTER 5.DISCUSSION

From the results, subclinical cases were the most common. This conforms to report by others in different parts of Kenya (Ngatia, 1988; Omore *et al.,* 1996; Ondieki *et al*., 2013). Clinical mastitis cases reported were few as compared to subclinical cases of bovine mastitis. Prevalence of subclinical mastitis is most prevalent in late lactation since this is the time farmers report more cases of mastitis, an observation made by Ayano *et al*. (2013). Determining clinical and subclinical cases was by reading through the history reported by farmers to the laboratory when reporting cases or when submitting milk samples.

In this study, high percentage of bacteria organisms isolated were *Staphylococcus* spp. at (33.17%), *Streptococcus* spp. (21.53%), *Klebsiella (*21.48%) and *Escherichia coli (*15.24%) which agrees with other studies (Lauerman *et al*., 1973; Omore *et al*., 1996; Barkema *et al*., 1999; Radostitis 2001, Erskine *et al.,* 2002; Gitau *et al.,* 2003; Haile, 2004; Anakalo *et al*., 2004). Other less prevalent isolates were *Enterobacter* spp.0.23%, *Pseudomonas* spp.1.03% and *Proteus* spp.0.58%. Another study carried out in Zanzibar also showed *Staphylococcus* and *Streptococcus* spp. to be the most prevalent in small holder dairy farmers (Gitau *et al*., 2003). A study carried out in Kabete area of Kiambu County showed the prevalence of *Staphylococcus* as 30%, Streptococcus 22.5% and *Pseudomonas* 2.65% (Muthee *et al.,* 2005). This is a finding which is similar to the finding of this study.

Percentages of isolates were *Staphytococcus* 738(33.17%), *Streptococcus* spp. 479 (21.53%) *Klebsiella* 478(21.48%) *E. coli* 339 (15.29%) *Corynebacteria* 119 (5.35%), *Pseudomonas* spp. 23(1.03%), *Proteus* spp. 13(0.58%), *Enterobacter* spp. 5(0.23%), *Pasteurella* spp.2 (0.09%) and *Bacillus* spp. 1(0.05%). Almost similar bacterial isolates have been reported by other authors (Gitau *et al*., 2012; Ondieki *et al*., 2013).

The fungal isolates were 28(1.26%) and all were resistant to antibiotic which is true for fungal infection. *Candida albicans* was the notorious fungus isolated at the laboratory. Almost similar findings have been reported by other authors (Odongo *et al*., 2012).

Drug resistance is a major concern in control of Bovine Mastitis. The increasing incidence of the use of non-efficacious and sub-therapeutic regimes of antimicrobial especially in developing countries by all type of para-veterinarians and quacks is a major concern (Gitau *et al*., 2003). Antibiotics resistance is one of the reasons for low efficacy of antibiotic therapy of mastitis. In Kenya, only a few studies have reported the antibiotic sensitivity rates of bacterial causing mastitis (Gitau *et al*., 2011). Successful mastitis treatment programs require prior *in vitro* antibiotic sensitivity testing so as to avoid indiscriminate use of antibiotics (Ndirangu *et al*., 2013).

Out of the 2,869 cases reported, the samples that were negative were 644 which represented 22.45% of all the cases of suspected bovine mastitis that were reported. Similar findings have been reported by Odongo *et al*. (2012). This could be due to administration of therapy before collecting milk samples for culture. Cases with no isolate could be suspected to be those of coliforms which are usually short lived and self-limiting hence by the time of collection and culture of samples most coliforms would have disappeared (Tyler *et al*., 1998). Sterile cases could also be due to misdiagnosis of mastitis.

In the antibiotic sensitivity tests, Gentamicin showed highest sensitivity at 90.41%. Chloramphenicol (72.40%), Kanamycin (67.22%) Streptomycin (53.52%) Tetracycline (37.46%). Ampicillin (17.93%). Cotrimoxazole (12.12%) and Sulphamethoxazole (2.95%). . Similar finding have been reported by others (Ondieki *et al*., 2013; Gitau *et al.*, 2011). The

reason for the resistance to most drugs could be due to extensive use and misuse for many years especially tetracycline and ampicillin. Gentamicin and kanamycin were introduced recently in Kenya thus resistance to the two drugs has not yet developed extensively. Despite chloramphenicol having 72.40% sensitivity, its use in food animal is prohibited by Food agricultural organization (FAO).

Culture and sensitivity is a must before initiating any mastitis therapy .This helps in avoiding misuse of drugs, also helps to combat drug resistance development by microbial agents and it's a sure way of confirming the exact mastitis causing organism affecting the individual cow in a herd or cows in the herd. It is no secret that losses arising from cases of bovine mastitis are significant and need to be established and cost/benefit analysis done on ensuing control and monitoring program.

Experience from the U.S, New Zealand and Europe has shown a return on investment of about 300% to 400% in an effective mastitis control program (Muthee *et al*., 2005).

CHAPTER 6: CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Bovine mastitis in North Rift and western Kenya as per the records from RVIL Eldoret was relatively high for the 10 year period under study. Subclinical form of the disease was high compared to the clinical form hence most of the cases go unnoticed by producers to enable them look for/ make early intervention before they suffer major economic losses together with reproductive losses of their animals .Effective control measures need to be instituted and such to include; improvement of milking hygiene and the milking parlor, teat disinfection, routine testing for mastitis and effective treatment of all mastitis cases.

Mastitis etiological agents found were *Staphylococcus* spp., *Streptococcus* spp. *Corynebacteria* and *Escherichia coli*. Hence maintenance of environmental hygiene can help to reduce intramammary infections with *Staphylococci* and *E. coli.*

Bacterial isolates were most sensitive to Gentamicin and kanamycin and these are the drugs most effective for mastitis therapy in the study area. However, antibiotic sensitivity testing is a pre-requisite before initiating any mastitis therapy.

6.2 Recommendation

1. Farmers /producers should be encouraged to be taking more samples to the diagnostic laboratory for the country to get the real picture of status of bovine mastitis and to determine the level of microbial sensitivity and resistance to antimicrobials being used in Kenya.

2. The government especially Ministry of Agriculture which deals with dairy industry, should take measures of educating farmers more so the small holder dairy farmers on different mastitis control strategies.

3. Producers to maintain environmental hygiene so as to reduce intra mammary infections.

4. Gentamicin and Kanamycin would be the recommended antimicrobials for mastitis therapy in the study area. However, antibiotic sensitivity testing is a must prior to initiating mastitis therapy.

5. Collection of the milk sample in cases suspected to be of bovine mastitis before initiating mastitis therapy. Samples should be collected in the correct manner, stored in the correct manner and transported to the laboratory without contaminating them for effective antibiotic sensitivity testing to be done.

6. Well-designed epidemiological study should be carried out to give a better and precise picture of antibacterial resistance to various mastitis pathogens in North rift and Western parts of Kenya and also in other areas of Kenya so as to establish best therapeutic regime.

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