# BIOCHEMICAL AND MOLECULAR IDENTIFICATION OF KEY PATHOGENS CAUSING MASTITIS IN DAIRY GOATS ON SMALLHOLDER FARMS IN KENYA

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## DECLARATION AND RECOMMENDATION

### Declaration

I declare that the work is original and has not been presented before.

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## DEDICATION

This thesis is dedicated to my dad, Johnston Shivairo Khamisi who is my mentor and the guiding light in my life.

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## ABSTRACT

Mastitis in dairy goats was investigated with the objective of establishing the effect of management and the identification of the key causative agents through common diagnostic procedures of somatic cell counts, bacteriological identification and, for the first time in goat mastitis, by use of Polymerase Chain Reaction. A cross-sectional survey, using a structured questionnaire was conducted in three agro-climatic regions of Coast, Nyanza and Rift Valley, with goat keeping clusters serving as sampling sub-units. The focus of the questions was on housing, feeding, labour, water sources, record keeping, socio-economic status of respondents and availability of extension service. California Mastitis Test (CMT) was done at the farm-level, Somatic Cell Counts (SCC) and bacterial isolation were done in the laboratory. Polymerase Chain Reaction (PCR) on the two key bacteria, *Staphylococcus aureus* and *Escherichia coli* were subsequently carried out on 16 randomly selected samples representative of the three dairy goat keeping agro-ecological zones. The management survey indicated that 56.9% of respondents were peasant farmers only 12% of respondents could afford hired labour. There was evidence of scarcity of quality water, with only 9% using water from rain catchment and the rest depending on wells, dams and rivers. There was no training package on dairy goats. The CMT scores for the two key organisms ranged between 2 and 3, making it a reliable test for udder infection. Somatic Cell Counts (SCC) was determined for 239 samples with scores ranging between 0.248 $10^6$ and 1.693 $10^6$ with a mean of 0.869 $10^6$. This study demonstrated significant SCC variations amongst the breeds, there was also significant variation in SCC scores for various locations. Other factors in the study were lactation length and parity all of which affected SCC scores. The bacterial isolation showed dominance of *Staphylococcus aureus* and *Escherichia coli* respectively. The two species of bacteria are significant indicators of the state of hygiene on the farms. The PCR identification of the two organisms showed that they were distributed in all three regions of study. There is a need for more intensive studies on the various diagnostic tools especially CMT, SCC, bacteriology and PCR to enable the development of quality standards in Kenya for goat milk which do not exist at the moment.

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| | |
|---|---|
| AEZ | Agro-Ecological Zone |
| BTC | Bulk Tank Count |
| CAE | Caprine Arthritis Encephalitis virus |
| CBOs | Community Based Organization |
| CM | Cytoplasmic Mass |
| CMT | California Mastitis Test. |
| CNS | Coagulase Negative Staphylococcus |
| DMC | Direct Microscopic Count (of bacteria). |
| DMCC | Direct Microscopic Cell Count (of leukocytes). |
| EC | Epithelial Cells |
| FAO | Food and Agricultural Organization |
| FSK | Farming Systems Kenya |
| GDS | German Development Service |
| GTZ | German Technical Assistance Agency |
| HPI | Heifer Project International |
| IMI | Intramammary Infection |
| ISLP | Integrated Small Livestock Project |
| MCFAs | Medium Chain Fatty Acids |
| NAHRS | National Animal Husbandry Research Station |
| NGOs | Non-Governmental Organization |
| PMNs | Polymorphonuclear cells |
| SCC | Somatic Cell Counts (counts of leukocytes). |
| SCM | Sub Clinical Mastitis |
| SPC | Standard Plate Count of bacteria. |
| SPC | Standard Plate Counts |
| SR-CRSP | Small Ruminants Collaborative Research Support Programme |
| UNDP | United Nations Development Programme |
| WMT | Wisconsin Mastitis Test. |

## DEFINITIONS OF TERMS

1. Somatic Cell - any body cell other than a germ cell (germ cells are ovum or sperm) used here in reference to leukocytes.
2. White blood cells (Leukocytes) - cells that contain a nucleus (have DNA) and cytoplasm and help protect the body from infection and disease.
3. Epithelial cells - these make up the membranous tissue covering most internal and external surfaces of the body and its organs.
4. Cytoplasmic particulate debris - debris particles from the protoplasm outside the nucleus of a cell.
5. Apocrine Secretion - type of glandular secretion in which the tip of secreting cell is released along with the milk – as in the case of goats. This type of milk secretion leads to cytoplasmic debris and epithelial cells which are about the same size as the white blood cells that appear in mastitis. They may therefore lead to a falsely high somatic cell count.
6. Merocrine secretion - a type of milk secretion in the honeycomb type, cells remain undamaged during secretion, only milk is released, as is in the case of cows.
7. Contagious Mastitis Pathogens - bacterial species which live primarily in the gland of the goat and are spread by the cluster or milkers hands.
8. Environmental Mastitis Pathogens - group of bacteria present in the environment of the goat at all times and under various circumstances may cause significant mastitis problem.

# CHAPTER ONE
# INTRODUCTION

## 1.0 General Introduction

Worldwide dairy goats contribute between two and two and half percent of the total milk consumed. In the past 20 years, there has been significant rise in goat production, with the low income countries leading, followed by intermediate income and high income countries respectively (Morand-Fehr *et al.,* 2004). Food and Agricultural Organization statistics (FAO, 2001) indicated that between 1980 and 1999 goat milk production worldwide increased by 55 percent, from 7720 metric tons to 12161 metric tons, while at the same time goat population rose by 58 percent, from 458 million to 710 million. It was pointed out that these increases were likely to be higher than the official statistics due to unreported home consumption of milk, especially in developing countries. One of the reasons advanced for the increases was increasing population and rise in demand for the goat as poor man's cow. Therefore, several reasons have been advanced for the rise in demand, like poor resource smallholder farmer who cannot afford a dairy cow would prefer a dairy goat; women and children benefit from nutrition and sales of extra milk for petty cash, and goats provide manure for kitchen gardens (Peacock, 1998).

Goat's milk has nutritional and health benefits to humans like food allergies caused by cow milk proteins which are not experienced in goat's milk. Goat milk promotes rapid weight gains, height, skeletal mineralization and blood components in children as compared to cow's milk (Sabbah *et al.,* 1997). Goat milk substitute for cow's milk brought about higher digestibility and absorption of iron and copper, thus preventing anaemia, (Barrionueso *et al.,* 2002). In these studies the utilization of fat and weight gain was improved, and levels of total cholesterol reduced on the goat milk diet. Thus goat milk was recommended as a useful alternative to cow milk for rehabilitation of malnourished children.

In developing countries dairy goat milk is generally home consumed, given to neighbours or sold within the villages, unlike cow milk which in many countries has an organized and regulated production and marketing (Agreste, 2001). In recent times commercial dairy goat farming in Kenya has increased significantly both in the high potential areas and in the arid and semi arid regions. These areas include; Mount Kenya region (Meru, Embu, Nyeri, Muranga, Kerugoya),

Rift Valley (Eldoret, Nakuru, Bomet, Naivasha), Western (Bungoma), Lower Eastern (Kitui, Mwingi), Nyanza (Siaya, Nyando, Migori) and in Coast (Kwale). Declining feed resources in the highly populated areas and unpredictable climatic changes have contributed to the shift by many farmers to small ruminants. During the past 25 years the dairy goat population in Kenya has increased rapidly from 6,000 40,000 (MOLD, 1995), 100,000 (Ahuya *et al.,* 2005), to a current estimate of 175,000 (MOLD, 2010). The rapid growth has been attributed to the involvement of Non-Governmental Organizations which came up with an innovative idea of targeting Community Based Organizations smallholder peasant farmers (Ahuya *et al.,* 2005).

The average dairy goat milk production per lactation in Kenya is estimated at 323 litres. Coast has an average production per lactation of 365 litres, Nyanza 322 litres and Rift Valley 281 litres. On average, a doe produces 2.5 litres of milk per day though it may produce between 4 and 8 litres, if well managed (organic farmer, 2011). The pure breeds produce higher than crossbreds and sustain milk production for a longer period of between 9 to 10 months (Ogola *et al.,* 2009).

The dairy goat value chain analysis indicate that goat milk has an annual demand of 331,200 Kg (35% in hospitals as healthy product, 34% among Somali community in Eastleigh, 13% in high class hotels and 18% in supermarkets) valued at KES 19.9m. An informal situational analysis at the Kenyan Coast (Malindi and Lamu) indicates existence of a large unexploited market for goat cheeses like Mozzarella. Increase in consumer demand for goat milk is due to its nutritive and physiological benefits (Attaie, *et al.,* 2000). This biomedical superiority is playing a major role in promoting the consumption of goats' milk in Kenya (Willet and Stampfer, 2003). However, the yield is too low to meet the demand.

The constraints experienced in Kenya by dairy goat farmers and feeds, the goats are fed on many types of fodder including napier grass, pasture grasses, sweet potato, vines and household vegetable wastes. Lack of adequate and proper breeding stock to upgrade local goats and high cost of pure-bred goats has led to low adoption of goat rearing in some parts of Kenya like Kericho, Murang'a, Kiambu and Nyandarua. Another constraint is the attitude by some consumers that goat's milk has a specific adour and taste that is not appealing like the cow's milk. Availability of credit facilities to support dairy goat farming is also cited as a constraint.

The lack of appropriate feeding regimes, management skills and breeding programme are the major constraints to improvement of dairy goat milk production (Ogola *et al.,* 2010). Among the major constraints in dairy goat farming is low milk production due to udder health. This is mainly due to mastitis as a result of aforementioned practices of dairy goat management.

Mastitis which is the inflammation of the mammary gland, characterised by physical, chemical and bacteriological changes in milk, and pathological changes in the udder tissue. The disease occurring at different levels of intensity can be defined as clinical, exhibiting symptoms such as milk clots, flakes, watery milk, discoloured secretions and slight swelling and tenderness and sub-clinical mastitis which is less obvious and only detectable by measures of cellular milk content, the somatic cells. Sub-clinical mastitis is important because it is said to be 15 to 40 times more prevalent than the clinical form, usually precedes the clinical form and is difficult to detect (Shearer & Harris, Jr., 2003).

About 90 organisms have been shown to be involved in goat mastitis, but 90 to 95% of all cases are caused by only four, *Staphylococcus aureus, Streptococcus agalactiae Streptococcus dysagalactiae,* and *Streptococcus uberis. Staphylococcus aureus* has been shown to be the most important pathogen in dairy goat mastitis (Mannasmith, 1981; Shearer & Harris Jr., 2003). In Kenya, the causal agents of dairy goat mastitis were hardly documented. More so, the management practices and how they influence udder health is not documented. Therefore, the general aspects of management of the dairy goat, with regard to disease control, especially mastitis, hygiene and housing are the major limiting factors in dairy goat. It is therefore important that as the sector grows the parameters of udder health for the purpose of monitoring and treatment were documented through this study. It was also important in this study to characterise the key mastitis causative pathogens for focused treatment, in varying agro-climatic zones, breeds and management systems. Additionally, the key causative isolates were identified through molecular techniques. This would open up the prospect of vaccine development to specific mastitis causing pathogens.

## 1.1 Statement of the Problem

Elimination of extreme poverty and hunger is a key component in achieving vision 2030. The dairy goat value chain analysis indicates that goat milk has an annual demand of 331,200 Kg. The demand for goat milk and products is increasing and there is therefore, a need to increase production. Constraints which the increase in goat milk yields include management practices like quality housing, milking hygiene and feeding. Information on how to tackle these constraints is scanty. Udder health is regarded as the most important of these constraints. Detection tests and quality standards applied to goat milk are those of the cow, not validated for goat milk. Therefore, improving milk yield in goats needs good husbandry practices which will reduce udder health. This study focused on how management practices influenced udder health and identification of key mastitis causing pathogens. PCR was used to further identify the key strains associated with mastitis in goats.

## 1.2 Objectives

The general objective is to improve the udder health of dairy goats for improved milk yields.

### 1.2.1 Specific Objectives

i. Determine the impact of management practices on udder health.
ii. Identify the mastitis causing pathogens in dairy goats based on breed and agro-climatic zones.
iii. Identify molecular biodiversity of key mastitis causing pathogens through PCR.

## 1.3 Hypothesis

i. Management practices do not have significant effect udder health of the dairy goat.
ii. The biochemical and molecular characteristics of key mastitis causing pathogens in dairy goats are known.

## 1.4 Justification

The dairy goat sector has the potential to improve nutrition, provide an economic activity for rural women and transform the peasant farming to commercial activity. Mastitis is regarded as a major constraint to dairy production. Considering the fact that the dairy goat programme in

Kenya is rapidly growing and involving smallholder, poor resource farmers who are generally semi-illiterate with regard to knowledge of goat husbandry, compounded by poor or unaffordable extension services, a study to characterize key organisms causing udder infection, especially sub-clinical mastitis in Kenya is necessary. It is important that as the dairy goat sector grows, the key mastitis causes pathogens, parameters of udder infection monitoring and treatment regimes be established.

### 1.5 Scope and Limitation of the Study

This was a survey conducted amongst smallholder dairy goat farmers on Heifer Project International Programme (HPI) located in Nyanza, Rift Valley and Coast province and the Farming Systems Kenya (FSK) programme located in Nakuru and Nyandarua districts.

Among the limitations was small numbers of lactating does in the various farm clusters to be studied without experience in dairy farming and poor record keeping, there was likely to be lack of information on the history of mastitis incidence and treatment.

Various breeds were involved in this study with the dominance of the Saanen amongst the HPI farmers and the Toggenburg amongst the FSK farmers, and the crosses.

The climatic variations amongst the regions i.e. Coast, Rift Valley and Nyanza paused a challenge in terms of management e.g. availability of water, hygienic conditions and the prevailing species of bacteria in each environment.

# CHAPTER TWO
# LITERATURE REVIEW

## 2.1 Dairy Goats in Kenya

In the past 25 years the dairy goat sector in Kenya has witnessed rapid population growth, from 6,000 (Stotz, 1983), 40,000 (MOLD, 1995), 100,000 (Ahuya, *et al.,* 2005), to current estimate of 175,000 (MOLD, 2010). The rapid growth has been attributed to the involvement of various NGOs. Dairy goats were first introduced in Kenya in the 1950s by the British settler farmers. The goats which originated from Europe were confined to the highlands of Kenya. From the settler farms the goats spread to the adjoining peasant farms, mostly through purchase of bucks, which were used to upgrade the indigenous goats.

In the 1970s and 1980s dairy goats were introduced on government farms and agricultural institution, such as Egerton College (now Egerton University), Wambugu Farmers Training Centre, and Embu Institute of Agriculture, from where animals were sold to farmers (Wanjohi, 1998). During the same period a joint Kenya Government, Food and Agricultural Organization (FAO) and United Nations Development Programme (UNDP) sheep and goats project was started at various centres such as the National Animal Husbandry Research Station (NAHRS), Naivasha, Ol'Joro and Marindas, amongst others. These centres were used to evaluate dairy goats through research. Early in the 1980s also saw the introduction of on-farm research on the dual-purposed goats project the Small Ruminants Collaborative Research Support Programme (SR-CRSP), Western Kenya.

In 1992, the Integrated Small Livestock Project (ISLP) was started jointly between Kenyan government and the German Technical Assistance Agency (the GTZ) in Nyeri, Muranga, Kirinyaga, Thika, Maragua and Embu districts. The project introduced the German Alpine bucks for upgrading the local goat breeds, with the intension to raise it up to 87.5 percent. Initially 102 bucks were imported, a total of 66 farmers groups with an average membership of 21 per group were formed. The initial membership of 1400 farmers rose to 5930 by the year 2004. GTZ and German Development Service (GDS) have continued to support the dairy goats industry. Currently, they are focused on supporting goat milk and cheese marketing.

In 1996, a British NGO, FARM-Africa, started a dairy goat project in Meru and Tharaka-Niithi districts of Eastern Province of Kenya. The project had about 589 farmers in 25 groups and their breed of choice has been Toggenburg (Gichohi, 1998; Ahuya *et al.,* 2005).
In 1999, the Heifer Project International (HPI) started a smallholder dairy goat project in various districts of Nyanza, Rift Valley, Central and Coast Provinces of Kenya. The Saanen imported from South Africa has been their breed of choice. The HPI project currently covers more districts than any other NGO.

In the early 2000s the Farming System of Kenya (FSK) initiated smallholder dairy goat farming in Nakuru and Nyandarua districts of the Rift Valley Provinces. They are currently supporting well over 500 farmers registered into Community Based Organization (CBOs). The Toggenburg has been of choice, sourced from the FARM-Africa project in Meru, although there has been growing interest in the German Alpine breed lately which has already been introduced in these districts. According to Ahuya *et al.,* (2005), it was not until the 1990s when a number of Non-Governmental Organization (NGOs) introduced smallholder dairy goat projects through the innovative idea of farmer participation that the present growth in population was realized.

### 2.1.1 Milk Production Process and Composition in Goats

The milk secretory unit of the mammary gland is the alveolus, a microscopic anatomical unit, lined with Epithelial Cells (EC), which synthesize and secrete milk compounds; fat, proteins and lactose. Epithelial Cells slough off as a moral physiological process in the mammary gland.
Anatomical features in goat mammary gland differ from those of the cow. Two teats compared to four; less obvious larger inside volume of the teat gland and gland cistern of goats which gives a collapsed and empty appearance after milking, and a faster milk let down of about one second in goats compared to one minute in the cows (Escobar, 2007), see Figures 1 and 2.
The process of milk secretion in the goats differs significantly from that of the cow. "Apocrine" milk secretion involves shedding of nucleated and non- nucleated Cytoplasmic Mass (CM) into milk. Non-nucleated particles which are non-leukocyte will be included in the total cell count. Milk secretion in the cow is by 'merocrine', a process in which Cytoplasmic Mass are not shed, (Escobar, 2002; Haelein, 2004), as illustrated in Figures 3 and 4.

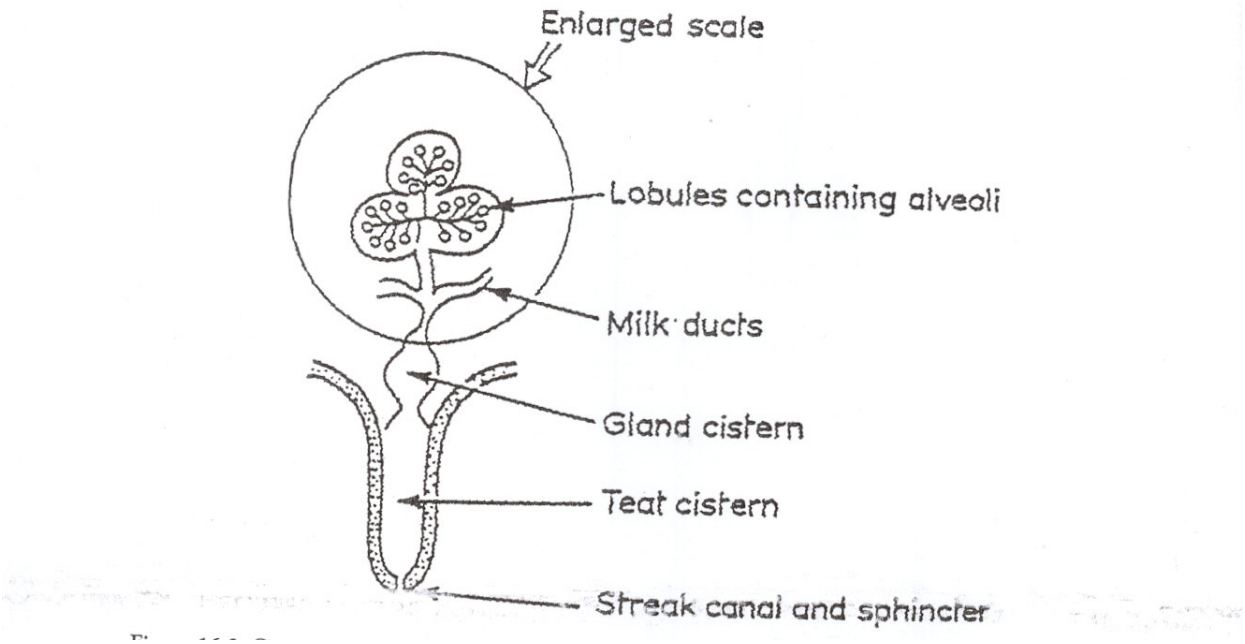


**Figure 1: Cross-section of an Udder at the Teat (Escobar, 2007)**

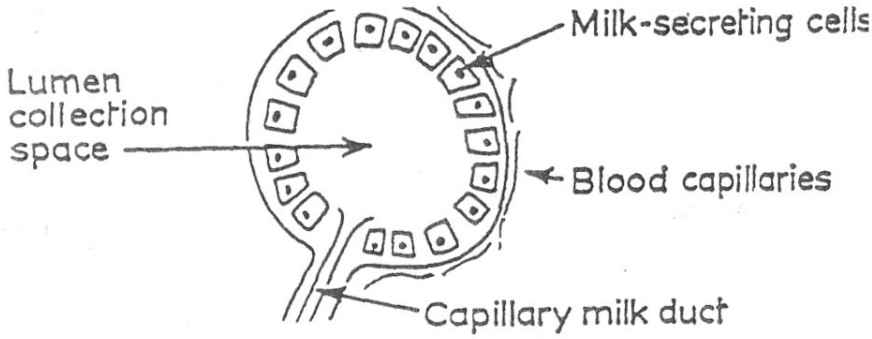


**Figure 2: Section of an Alveolus Cell (Escobar, 2007)**

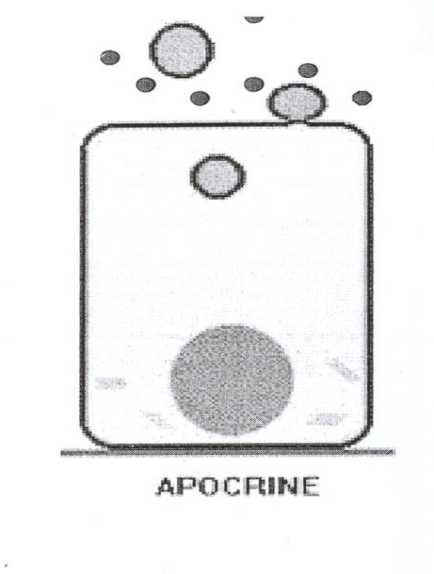


**Figure 3: Apocrine Secretion (Escobar, 2007)**
**Goat milk secretions in the high point of the cell and a portion of the cell, including plasma membrane is pinched off for secretion**

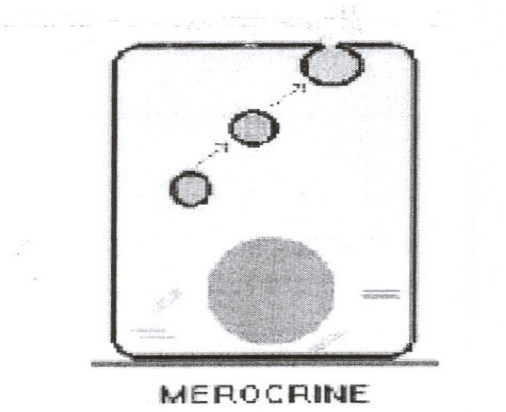


**Figure 4: Merocrine Secretion (Escobar, 2007)**
**Minute droplets form in the cells and accumulate in the high point, fusing with the upper most plasma membrane and are secreted into lumen of the gland by a cellular process of exocytosis**

In addition to the physiological differences in milk secretion, goat milk proteins have many significant differences in amino acid compositions from cow milk, especially in genetic polymorphism. The major protein in cow milk is alpha-s-l-casein. Goat milk differs genetically by having "null" or "high" types. Null types have shorter rennet coagulation time, less resistance to heat treatment, curd firmness is weaker, pH is higher, protein and mineral content lower, and cheese yields are less than in high types. This explains the significant differences in cow milk in digestibility by infants and patients (Haenlein, 2002).

The composition of goat milk fat may have more important differences in composition to that of the cow milk (Haenlein, 1992). Goat milk has 35% of the Medium Chain Fatty Acids (MCFAs) (C6 - C14) compared to the cow's 17%. Three FAs are named after the goat; Caproic (C6), Caprylic (C8), Capric (C10). Besides their unique flavour, these MCFAs have become of considerable interest in medicine due to their unique benefits in many metabolic human diseases (Babayan, 1981) Capric and Caprylic have been used for treatment in malabsorption syndromes, intestinal disorders, coronary diseases, premature infant nutrition, cystic fibrosis and gallstones, due to their abilities to provide energy and lowering / inhibiting or dissolving cholesterol (Haenlein, 2002).

### 2.1.2 Mastitis

Mastitis is a general term which refers to inflammation of the mammary gland. It is characterized by physical, chemical, usually bacteriological changes in milk, and pathological changes in the udder tissue. The disease occurs at different levels of intensity and can be defined as clinical (exhibiting symptoms) or sub clinical (not showing any symptoms). Clinical mastitis may vary greatly in severity during the course of the disease. Sub acute (mildly clinical) case may only exhibit minor alternations in the milk in the affected half udder, such as clots, flakes, or discoloured secretions, with slight swelling and tenderness in the affected half. Cases of acute mastitis are characterized by sudden onset, pain, heat swelling, redness and reduced as well as altered milk secretion from affected halves. The most consistently observed signs in clinical mastitis are clots, flakes or watery milk. Depending on upon severity and the causative agent, acute mastitis cases may have systemic involvement characterized by fever, depression, weakness. In its most severe form it can be fatal.

Sub-clinical mastitis is less obvious and only detectable by measures of the milk's cellular content, the somatic cells. This form of mastitis is important for the following reasons; it is said to be 15 to 40 times more prevalent than the clinical form, it usually precedes the clinical form, it is of long duration, it is difficult to detect, it reduces milk production and affects its quality, and it constitutes a reservoir of micro-organisms that lead to infection of other animals (Mannasmith, C.H., 1981; Shearer J. K. and Harris, Jr., 2003; Thiraptasakuu, 1999).

### 2.1.3 Common Mastitis Pathogens of Goats

Research has shown that more than 90 organisms are involved in goat mastitis, but 90 to 95% of all cases are caused by four organisms, *Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysagalactiae* and *Streptococcus uberis. Staphylococcus aureus* has been shown to be the most important pathogen in dairy goat mastitis (Mannasmith, 1981; Shearer J. K., and Harris Jr., 2003). There are two recognized categories of mastitis causing organism, namely; contagious mastitis pathogens, these live primarily in the mammary gland of the animal and on the teat skin. They are spread primarily by milkers' hands. This category includes *Staphylococcus aureus* and *Streptococcus agalactiae*.

*Mycoplasma* mastitis causing organisms are categorized as contagious because the reserviour for infection is other infected animals, usually spread from the respiratory system to the udder (Ruegg, 2002). A history of respiratory disease may precede mycoplasma mastitis outbreak, mycoplasma mastitis is more documented in the bovine due to *Mycoplasma bovis*, not much research work has been done in caprine mastitis. The environmental pathogens, consists of a large group of bacteria present in the environment of the goat at all times which, under various circumstances may cause a significant mastitis problem. These include, among others, Coagulase Negative *Staphylococcus* (CNS) sometimes just referred to as environmental *Staphs* bacteria, these are *Staphylococcus* that are not *Staphylococcus aureus*. Coagulase–negative refers to a laboratory test that differentiates this species of bacteria from the "coagulase – positive" *Staphylococcus aureus*. CNS live on the teat skin and can colonise the teat canal. Anything that decreases the patency of the teat sphincter can allow infection to occur.

Environmental *Streptococcus* refers to species other than *Streptococcus agalactia* that are isolated from caprine mastitis, and often referred to collectively as Strep non-ags. The most

common of these are *Streptococcus uberis* and *Streptococcus dysagalactia*. They are capable of living on the body of the animal including the vulva, hair, skin, muzzle and udder. Bedding types, especially straw, may play a role in determining numbers of organisms present in housing areas.

The other group of environmental pathogens is termed as "coliforms", which includes *Escherichia coli* and a somewhat similar bacteria group termed *Klebsiella. C*oliforms are present at all times. They are faecal bacteria also referred to as manure bacteria, so each time the animal defecates they are placed in the environment. They are capable of living in bedding, especially sawdust, shavings, when organic bedding materials are mixed with manure, urine, leaked milk, and then warmed up with body heat or warm weather, rapid growth of these bacteria can occur (Ingalls, 2003; Ruegg, 1997, 2002). Mastitis caused by these organisms is therefore due to unsanitary conditions. Some observations seem to indicate higher incidence of *Escherichia coli* when the ration has excess protein, high levels of nitrates in feed or water, or urea and Non-Protein Nitrogen.

Farnsworth & Sieber, (1979), one of the major differences between bovine and caprine mastitis appears to be the response to Coagulase Negative Staphylococcus (CNS), which seems to have little pathogenicity for cows, while in goats it is responsible for the chronic mastitis. Coliforms and *Pseudomonas* are ever present (Farnwoth & Sieber, 1979). *Escherichia coli* usually causes very acute mastitis, with the goat being very sick and toxic. However, the organism can be present in sub clinical form causing very high, both somatic cell and Standard Plate Counts (SPC) (Mannasmith, C. H., 1981). *Staphylococcus* bacteria have the ability to invade living tissue. Any physical damage, however, slight opens the door to *Staphylococcus* invasion. *Streptococcus* infection, on the other hand is generally associated with poor milk "let-down", "milk-out", or anything increasing residual milk. *Corynebactenium* mastitis is commonly associated with abscesses in the herds (Holiday, 1998).

### 2.1.4 Economic Importance of Mastitis

Decreased milk production and increased management costs are the direct economic effects of mastitis. The drop in revenues, non- marketable milk contaminated with antibiotics, decreased milk yields, possible laboratory costs, possible death of infected animals, udder damage, and interruption in breeding programme and culling, all constitute direct economic losses (Thirapatsakuu, 1989).

Indirect economic losses are, however, not easily noticeable. In severe clinical mastitis gross abnormalities are observable and the milk discarded, such milk does not enter the human food chain. In sub-clinical mastitis, however, this does not happen. Some harmful bacteria produce heat resistant enterotoxins which can cause serious food poisoning. Another public health concern is that if after antibiotic use, compliance with the proper withholding period is not observed, antibiotic residues in milk could lead to allergic reaction or lead to the development of antibiotic – resistant strains of bacteria. In USA, regulatory standards for bacterial counts in cow milk are set at less than 100,000 bacteria per ml in Bulk Tank Count (BTC), and the somatic cell counts is set at 750,000 cells per ml. Both bacteria and somatic cells can alter the quality of milk through enzymatic destruction of milk protein and fat. Production of quality milk therefore depends on control of mastitis (Wallace, 1998).

### 2.1.5 Control of Mastitis

The initial evaluation of mastitis situation in the herd should be determined by; interviewing the farmer, evaluating the state of health of the whole herd through physical examination, and evaluating the general herd management , including; housing, bedding, feeding, manure removal. Also evaluating milking procedures and hygiene, mastitis treatment records, good hygiene practices, provision of a stress-free atmosphere, especially around kidding. Dietary deficiencies such as Vitamin E & Vitamin A result in increased incidence of mastitis (Wallace, 1998).

Prompt identification and treatment of mastitis in goats affords the best opportunity for successful outcome when therapy is required. Far better is the prevention of mastitis through establishment of good husbandry practices, sanitation, sound milking procedures including post-milk teat - dipping, treatment during the non-lactating period, and culling of chronically infected does (Shearer J. K., and Harris Jr., 2003).

### 2.1.6 Mastitis Detection

The presence of individual cases of mastitis may be detected by using any of the several techniques.

**i. Somatic Cell Counts (SCC)**

Milk SCC has been the subject of published research reports since 1910. Somatic cell count is the most widely used indicator of udder health in cow, sheep and goat milk, but unfortunately SCC is difficult to interpret in goat milk. Compared to sheep and cows, SCC in goat milk is relatively high in healthy udder for reasons stated above. Therefore, standards established for SCC in cow milk are not appropriate for goats. However, according to Poultrel *et al.* (1997) elevated SCC is mainly in response to infection.

Somatic cells are composed of white blood cells and occasional sloughed epithelial cells. In normal milk of uninfected glands the cells include neutrophils (1 – 11%), macrophages (66 – 88%) lymphocytes (10 – 27%) and epithelial cells (0 – 7%). The macrophages provide surveillance in the uninfected gland. When bacteria invade and colonise the mammary gland, the macrophages respond by initiating inflammatory response that affects polymorphonuclear cells (PMNs) into milk to engulf and destroy bacteria. Mastitis is the most important factor influencing Somaic Cell production. Neutrophils form upto 90% of SCC in infected glands (Ruegg, 2002). Normal goat milk has a higher cell count than normal cow milk, in part caused by increased rate of sloughing of Epithelial Cells (EC), and the presence of Cytoplasmic Masses (CM) which occurs as a result of apocrine milk secretion process in goats (Shearer & Harris, 2003). Factors other than infection that tend to increase leukocyte members and increase CMT reactions include; positive reaction one to two weeks following treatment, very early (colostrum) and late lactation, teat injury, injury to udder, periods of estrous, and abnormal health, e.g. C.A.E (viral infection) leading to excessive sloughing thus raising ECs.(Marshall & Edmondson, 2007).

California Mastitis Test (CMT) is a common indirect method of measuring SCC in cows, but some authors claim it is unreliable for diagnosis of IMI in goats (Bergonorer *et al.,* 2003). Other studies, however, report that CMT may be useful for detection of healthy udders (Karzis *et al.,* 2007; Petzer *et al.,* 2008). The main advantages of CMT are that it is quick, cheap and simple "animal side" test. California Mastitis Test also referred to as Schalm Test was developed by Schalm and Noorland (1957) as a cow side test for detection of somatic cells in sub clinical

mastitis. The CMT is based on a reagent, triethanolanine (alkyl) sulphonatedestroying the membranes of somatic cells in milk and binding to the cellular DNA. This process results in an increase of the milk viscosity depending on the number of cells, thus allowing for rough estimate of somatic cells.

High levels of leukocyte count in milk strongly indicate presence of mastitis causing bacteria. The CMT reagent when added to milk reacts with the DNA part of the leukocytes nucleus forming agel. The greater the mastitis infection, the more leucocytes present and the more gel-like substance that forms. The reaction occurs on the paddle and is graded subjectively as - negative, trace, 1, 2, 3, for reliable results tests should be conducted just before milking, after stimulating milk let down, and discarding fore milk (Haenlein, 2003; Schaeren & Maurer, 2006).

**Tables 1: CMT Interpretation and Scoring in Goat Milk**

| **CMT Score** | **Reaction** | **Mean No. of Neutrophils / ml** |
|---|---|---|
| Negative - 0 | No reaction appears | Below 200,000 |
| Trace T- Trace | Slight slime, tends to disappear with continued swirling | 150,000 - 500,000 |
| Weak Positive - 1 | Distinct slime, without agel | 400,000 – 1,500,000 |
| Distinct Positive - 2 | Immediate gel formation, moves as mass during swirling | 800,000 – 500,000 |
| Strong Positive - 3 | Gel develops a convex surface and adheres to bottom of the cap | Over 5,000,000 |

Schalm, O. W., *et al*., (1971), Marshall, R. T., & Edmondson, J. E., (2007) classification.

**ii. The Direct Somatic Cell Counting can be done by use of Improved Neubauer Chamber**

Universal definition of a cell count threshold to distinguish between healthy and infected udder halves does not exist yet due to the influence of both physiological and pathological factors. It is however, agreed that status of infection represents the most important factor influencing milk SCC (Stuhr & Aulrich, 2010). The national conference on interstate milk shipment of USA in 1983 resolved on differential staining of goat milk samples for accurate SCC. This method

identifies only (nucleated cells) leukocytes, thus yielding a more accurate SCC. According to Heinlein (2003) valuable research done over the years led to the conclusion that the only officially acceptable method to confirm somatic cells in goat milk is the direct microscopic SCC, while electronic methods are unreliable.

**PCR as a Diagnostic Tool**

Mastitis, especially in the dairy cow has been documented to cause great economic loss all over the world. In a report by the National Mastitis Council (NMC, 1998) estimated losses per cow of upto 184 dollars were reported. It has been demonstrated that early detection of mastitis can enhance curing rates and reduce the recovery time for normal production to resume. Identification of the pathogens is not only important to determine effective antimicrobial treatment, but also to monitor and control the rate of infection at the farm level. Conventional microbial methods involving bacterial culture and subsequent biochemical tests are time consuming, usually lasting 48 hours to complete bacterial identification. In addition, false results can be obtained when mastitis causing bacteria are capable of intravascular survival (Phuektes, *et al.,* 2003). Application of DNA based assays might circumvent some of the drawbacks with conventional procedures. DNA based diagnostic assays target the unique genetic information of the bacterial genome (Fitzgerald J.R., & Musser, J.M., 2001; Martineal *et al.,* 1998; Meiri-Bendek *et al.,* 2002). Genome assays have increased sensitivity, specificity and efficiency, using the DNA Biochip arrays, also better in rapid identifying large number of pathogens simultaneously with definite confirmation within hours.

In a review by Taponen *et al.,* (2009) it was concluded that all common mastitis bacteria can occur in quantities in clinical mastitis samples that exhibit no growth in conventional culture. The Real-time PCR assay has been found to solve this puzzle. It has been known that no-growth occurs in 20 – 30% samples taken from quarters with clinical mastitis (Bradley *et al.,* 2007). According to Balsom (2010) using PCR testing to determine cause of mastitis infection could boost producer, confidence, compared with traditional bacterial culture. As a result of the above the (British) National Milk Record fully converted fully to use of PCR analysis since February 2010.

# CHAPTER THREE

# EFFECT OF MANAGEMENT PRACTICES ON UDDERHEALTH

## 3.1 Introduction

In the last 20 years the dairy goat population in Kenya has risen rapidly from an estimated 6,000 (Stotz, 1981) to 175,000 (MOLD, 2010). The rapid growth has been attributed to the involvement of Non-governmental Organizations which deal directly with Community Based Organizations of poor smallholder peasant farmers (Ahuya *et al.,* 2005). Among the constraints facing the sector are inadequate land set aside for folder production, for all year round feeding (Semenye, 1990), poor housing, inadequately trained and distributed extension services, poor breeding policy, poor health/disease management and marketing (ISLP, 1997).

## 3.2 Materials and Methods

A cross-sectional survey, using a structured questionnaire was conducted on dairy goat farms in three agro-climatic zones with dairy goat keeping clusters serving as sub-units for sampling. The focus of the questions were on the housing, feeding, labour, water sources, record keeping, socio-economics of respondent households, and availability of extension services. General linear model was used for statistical analysis.

### 3.2.1 Study Sites

Figure 5 is the map of the sampling sites. The three regions of study were Nyanza province which has the following districts; Homa Bay, Suba, Migori, Nyando and Siaya. In Rift Valley province the sites included Bomet, Nakuru, Nyandarua, and lastly Coast province study sites were Kwale and Taita/Taveta. Each cluster formed a separate population unit. Kwale County in the Coast region is in the lower agro-ecological zones 2 and 3; Homa bay, Suba, Migori, Nyando and Siaya all in Nyanza region are in low – medium potential agro-ecological zone 1 and 2. Bomet, Nakuru and Nyandarua are in agro-ecological zone 2. Agro-ecological zone refers to a land resource mapping defined in terms of climate, land form, soils and / or land cover, and having specific range of potentials and constraints for land use (Jaetzold & Schmidt, 1983). Agro-ecological zone 1 is humid, 2 is sub-humid, 3 semi-humid, 4 transitional, 5 semi-arid, 6 arid and 7 very arid. Zones refer to temperature belts i.e. belts having similar temperatures are put in one zone.

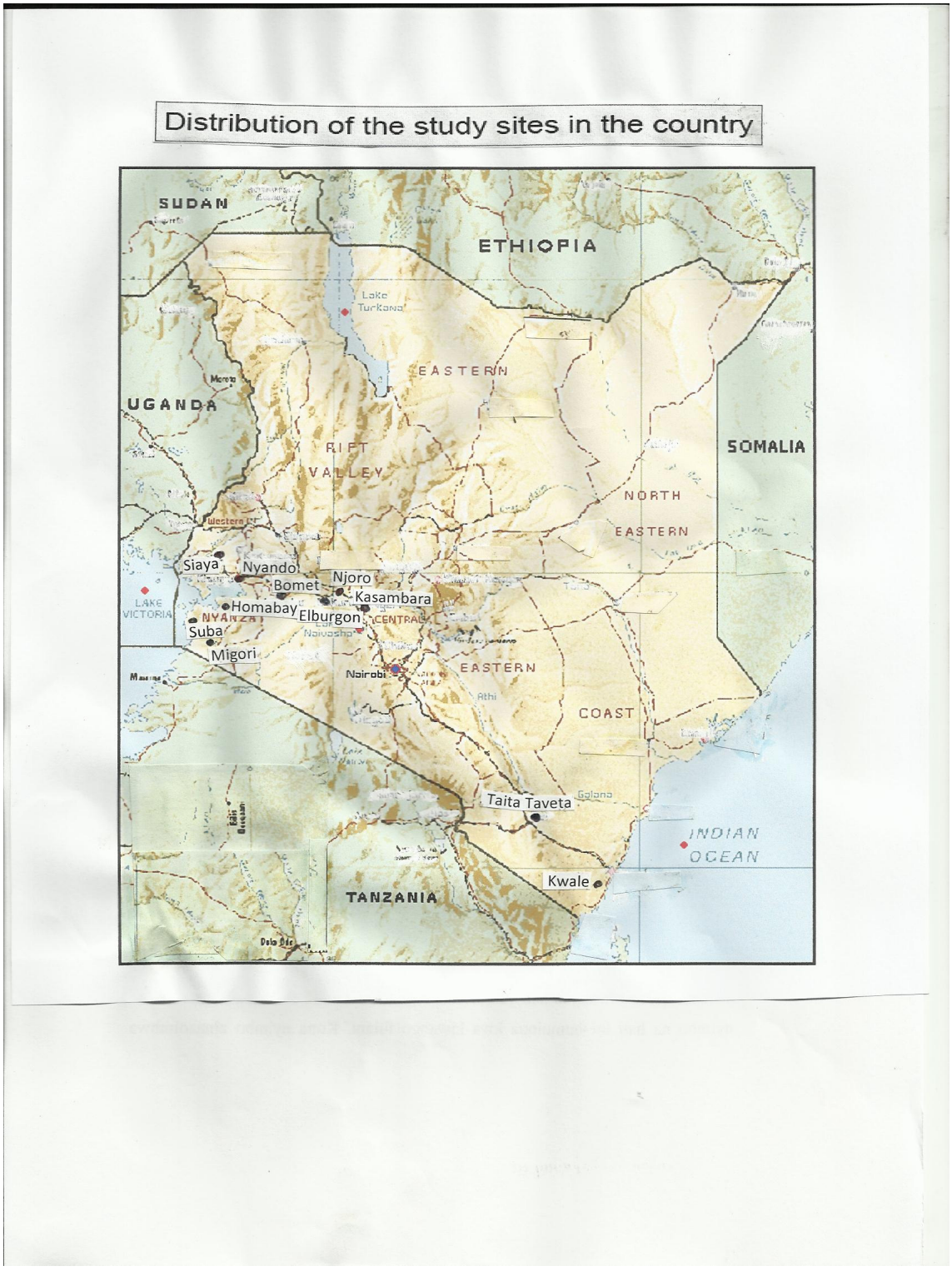


**Figure 5: Distribution of the Study Sites**

### 3.2.2 Experimental Design

The independent variables were breed, stage of lactation, parity, location and management practices which includes housing and milking hygiene. The dependent variables were CMT, SCC pure bacterial isolates. The response variables were CMT scores, SCC/ml and types of bacteria.

### 3.2.3 Animals

The population at risk of contracting mastitis were in the lactating does in each study cluster.
The sampling unit was the "half udder" of the lactating does.

### 3.2.4 Sampling Procedure

Two procedures used were clinical and farm inspection. Clinical inspection focused on udder health udder quality, teat shape, lesions, goats treated for mastitis, management of mastitis goats and culling. A clinical mastitis evaluation form was filled for each sampled doe. Farm inspection focused on management practices including housing types and conditions, housing for lactating and dry does, floor, ventilation, grazing / ban size, bedding materials. Milking procedure; wet / dry udder preparation, use of milking salve, post-dipping management of mastitis. This information was also recorded on clinical mastitis evaluation form.

## 3.3 Results

### 3.3.1 Demographic information

Baseline demographic data in Table 3 indicated the ratio of women to men who were registered as the goat owner was 4:1, 36% of the women were widows. In the respondent families 48.4% of the children were orphans.

**Table 2: Interviews and Sampling Schedule**

| Location | Sample Size of Farmers | Total Amount |
|---|---|---|
| Kwale | 11 | 18 |
| Taita/Taveta | 3 | 3 |
| Bomet | 9 | 17 |
| Homa Bay | 4 | 8 |
| Nyando | 20 | 24 |
| Migori | 8 | 7 |
| Suba | 5 | 7 |
| Siaya | 4 | 4 |
| **Total** | **60** | **88** |

**Table 3: Ratio of Women to Men in some Sampling Locations**

| Location | Women | Men | Total |
|---|---|---|---|
| Nyando | 70 | - | 70 |
| Bomet | 9 | 14 | 63 |
| Homa Bay | 410 | 121 | 531 |
| Suba | 80 | 44 | 124 |
| Migori | 131 | 30 | 161 |
| Kwale | 86 | 17 | 103 |
| **Total** | **826** | **226** | **1052** |

### 3.3.1 Sources of Labour

Figure 6 shows the sources of labour for goat care. Only 7 (12%) the interviewed families depended on hired labour, while 51 (88%) depended on members of their family for labour. Most families could not afford hired labour.

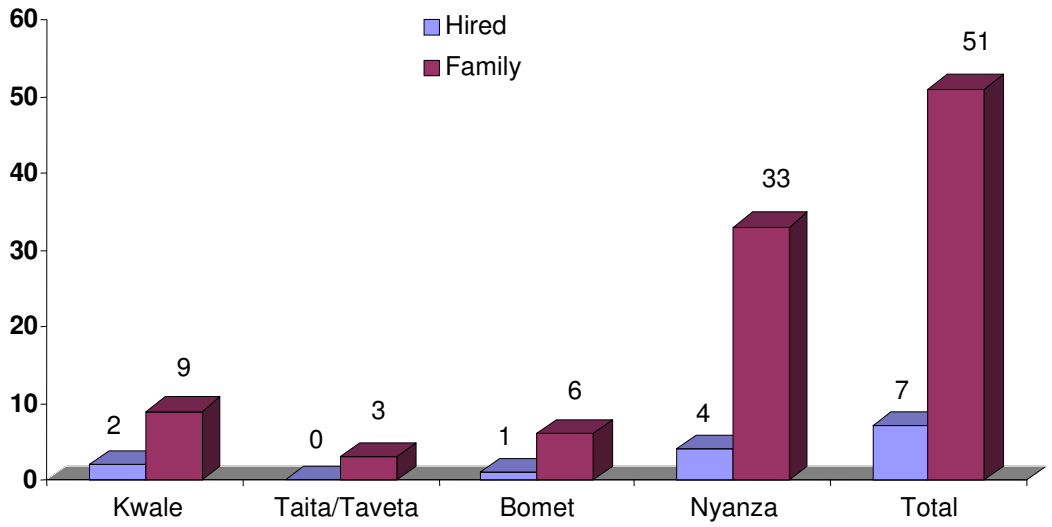


**Figure 6: Source of labour in smallholder dairy goat farming areas**

Table 4 indicates the age brackets of those who cared for the goats. 63% of the family members caring for goats fell in the age bracket of 31 to 50 years old, 32% were aged above 50 years, while only 5% were below the age of 30 years. Younger members of family were not keen to get involved in goat management.

**Table 4: Sources of Labour in Smallholder Dairy Goat Farming areas in Kenya**

| Age | Kwale | | T/Taveta | | Bomet | | Nyanza | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|
| group | Number | % | Number | % | Number | % | Number | % | Number | % |
| < 31 | 0 | 0 | 1 | 33 | 0 | 0 | 1 | 2 | 2 | 5 |
| 31 - 50 | 4 | 36 | 2 | 67 | 6 | 83 | 25 | 67 | 37 | 63 |
| > 50 | 7 | 64 | 0 | 0 | 1 | 17 | 11 | 26 | 19 | 32 |

### 3.3.2 Sources of Income

The goat keeping households 56.9% depended entirely on their farms for livelihoods, while 43.1% had supplementary sources of income, mostly from employed member of family.

In Kwale Coast region 90.9% and in Bomet Rift Valley 71.4% of the families depended entirely on farms. Fig. 7 shows these differences.

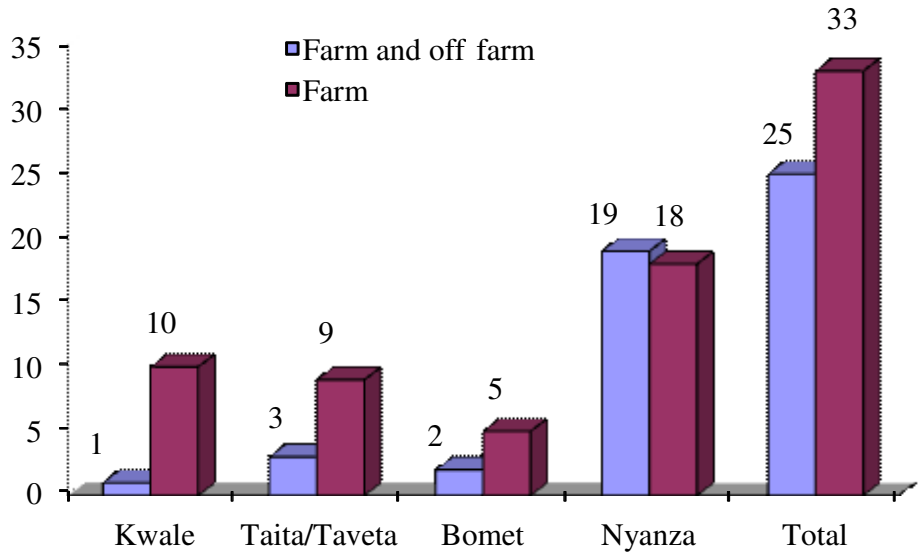


**Figure 7: Source of income among the smallholder dairy goat farmers in Kenya**

### 3.3.3 Reasons for Keeping Dairy Goat

Table 5 indicates in summary the reasons for keeping dairy goats, 74% of respondents kept the dairy goats for income, milk consumption and manure, 14% kept them for food and income, while 6% kept them for food, another 6% kept them for income, milk consumption, manure and breeding.

**Table 5: Reasons for Keeping Dairy Goat**

| Reasons | Number of Respondents | Percentage (%) |
|---|---|---|
| Food | 3 | 6 |
| Income and Food | 7 | 14 |
| Income, Food and Manure | 37 | 74 |
| Income , Food, Manure and Breeding | 3 | 6 |
| **Total** | **50** | **100** |

### 3.3.4 Milk Production

Table 6 shows 63.8% of the households had at least one lactating doe, 60.3% consumed all milk produced, 27.6% had surplus milk to sell, while 18% had to purchase additional milk to meet household needs.

**Table 6: Farmers whose goats were in production, the number who consumed their own milk and those who sold per cluster**

| Regions | Kwale | Taita | Bomet | Nyanza | Total | Percentage (%) |
|---|---|---|---|---|---|---|
| Farmers having goats in production | 8 | 2 | 4 | 23 | 37 | 63.8 |
| Farmers consuming goats milk | 8 | - | 4 | 23 | 35 | 60.3 |
| Farmers able to sell milk | 3 | - | 1 | 12 | 16 | 27.6 |
| Farmers who purchased additional milk | 2 | 3 | 1 | 5 | 11 | 18 |
| Farmers interviewed | 11 | 3 | 7 | 37 | 58 | |

Figure 8 summarizes the ranges of production levels in various locations ranging between half a litre 10 litres, with a mean of 2.15 litres 10.6% of the goats produced above 4 litres.

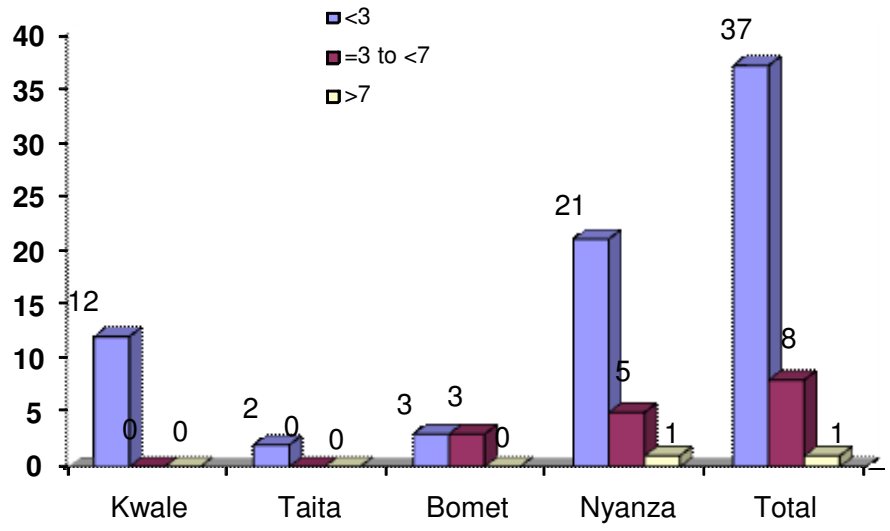


**Figure 8: Frequencies of goats in different milk yield range (kgs) in the smallholder dairy goat farms in Kenya**

### 3.3.5 Land Use Distribution

The distribution of family land in several sites indicated overall 55% allocated to crops, 35% to livestock while the homestead took 10%. An exception is Bomet, a traditionally livestock keeping community, with an average of 71.7% of family land set aside for livestock. At the extreme end, clusters in Nyanza Province had small pieces of family land averaging 3.7 acres out of which allocation for livestock ranged between 0.8 acres and zero, as shown in Table 7. In Oriang cluster, 5 out of 6 respondents had no land set aside for livestock, while in Ndiru cluster, it was not possible to estimate land for livestock use. In both cases communal land was used for livestock and hedge-rows were used for growing fodder trees and napier grass.

**Table 7: Land Allocation between Crops, Livestock and Homestead (acres)**

| Cluster/District | Land for Livestock | Land for Crops | Land for Homestead | Total |
|---|---|---|---|---|
| Kwale | 2.4 | 8 | 0.9 | 11.3 |
| Bomet | 6.1 | 1.8 | 0.6 | 8.5 |
| Serone | 30 | 40 | 8 | 78 |
| Andimo | 1.3 | 2 | 0.5 | 3.8 |
| Nyalienga | 1.2 | 3.8 | 0.3 | 5.3 |
| Imbo | 1.2 | 1.6 | 0.3 | 3.1 |
| Oriang | - | - | - | - |
| St. Monica | 0.4 | 1.2 | 0.5 | 2.1 |
| St. Mary's | 0.8 | 1.2 | 0.5 | 2.1 |
| Ndiru | - | 2.1 | 0.5 | 2.6 |
| Nyi Alego | 0.4 | 1.4 | 0.25 | 2.05 |
| **Mean** | **1.7 (35%)** | **2.7 (55%)** | **0.5(10%)** | **49** |

### 3.3.6 Sources of Water

All respondents (100%) regarded water as a critical and limiting resource due to the seasonality of all the sources, and the long distances covered to fetch it. None of the clusters had quality piped water. Only 9% could afford to buy or build a tank for roof catchment, which was regarded as quality water by all in Figure 9. All who used river, well, dam and lake sources, regarded

them as dirty and health risk for human and livestock use. Plate 9 indicates some of the common water sources.

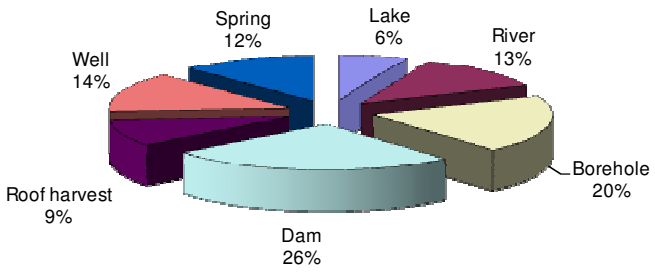


**Figure 9: Sources of water to animals in smallholder dairy goat farming areas in Kenya**

### 3.3.7 Sources of Feeds

In Figure 10, the various feed sources are indicated. Most households depended on their own farms for forage production, especially napier grass, which was the most popular bulk feed in all the clusters. Fodder trees were grown in most clusters as hedge-rows. Potato vines were equally popular in all clusters, especially in Nyi Alego, Siaya district where farmers acquired extra land for growing potatoes for human and livestock use. In Oriang and Ndiru clusters, there was little or no land allocated for livestock. The farmers in Oriang depended on the lake shore for wild grasses.

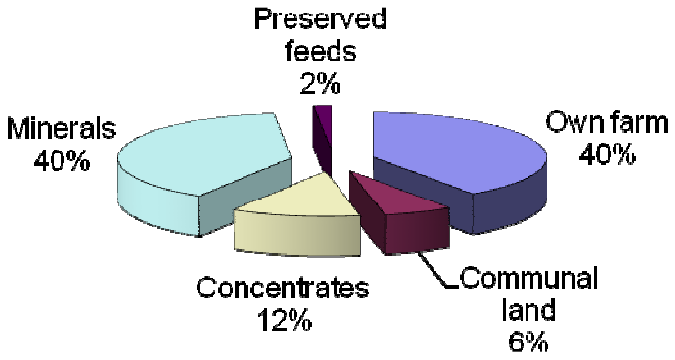


**Figure 10: Sources of feeds for animals in smallholder dairy goat farming areas in Kenya**

### 3.3.8 Goat Housing

All the respondents had houses or shelters for goats based on designs provided by the sponsoring NGO but they had many variations in quality. Significant variations were also, noted in the quality of the materials. While all (100%) had slatted floors, 16% used planks, 80% used sticks and barely 3%, especially in Kwale district used mud for the outer walls. Only 10% of the farmers (in Kwale) used the insect-proof mesh recommended for tsetse fly- infested Coastal strip and parts of Nyanza province.

Most houses had approximately apportioned areas for bucks, kids, does and milking space, with feeding troughs to the outside. Plate 8 indicates variations of the housing structures.

### 3.3.9 The Dairy Goat Breeds

In Figure 11, the overall distribution of the breeds shows that 54% of the goats on the farms were Saanen, imported from South Africa, German Alpine constituted 17%, Toggenburg were 10%, with 3% Anglo-Nubian. The 16% crosses represented undefined crosses.

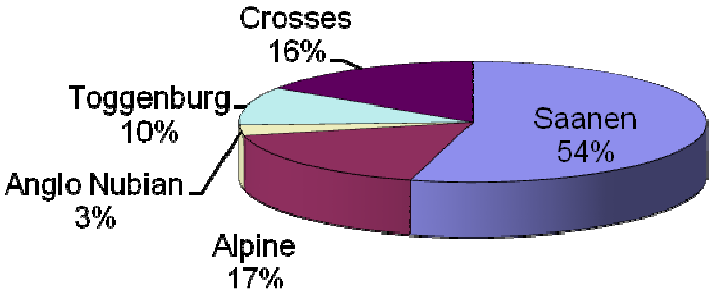


**Figure 11: Goat breed distribution in smallholder dairy goat farming areas in Kenya**

### 3.3.10 Source of Extension Advice

Table 8, summarizes the various sources of extension information, which included occasional visits from government extension agents (55%), group advice (60%), some form of residential training, (56%) and attendance at field days, (34%).

**Table 8: Sources of Extension Services**

| | Kwale | Taita | Bomet | Nyanza | Total | Percentage (%) |
|---|---|---|---|---|---|---|
| Extension | 5 | 1 | 6 | 20 | 32 | 55 |
| Group Advice | 8 | 2 | 7 | 18 | 35 | 60 |
| Field Day | 1 | 1 | 5 | 14 | 20 | 34 |
| Training | 7 | 1 | 7 | 18 | 33 | 56 |
| Respondents | 11 | 3 | 7 | 37 | 58 | |

### 3.3.11 Record Keeping

In Table 9, the types of records kept by farmers are shown. 96% had a form of animal identification, 60% had some milk records, 20% had health record, especially deworming and 10% had record for service and kidding.

**Table 9: Record Keeping**

| Record & Region | Identification of animals; names, tag | Milk yields | Service / kidding dates | Treatments |
|---|---|---|---|---|
| Kwale | 11 | 9 | 3 | 4 |
| Taita / Taveta | 3 | 0 | 1 | 1 |
| Bomet | 7 | 4 | 1 | 0 |
| Nyanza | 37 | 23 | 1 | 7 |
| **Total** | **58/60(96%)** | **36/60(60%)** | **6/60(10%)** | **12/60(20%)** |

### 3.4 Discussion

The demographic information indicated that the overall ratio of women to men was 4:1, with 36.8% of the women as widows. Out of the estimated 4545 children in the entire sample, 220(48.4%) were orphans. This data is consistent with the key objective of the various dairy goat supporting (funding) NGOs, to support poor families, especially women and children, for improved nutrition and income generation. This is supported by the fact that in 56.9% of the respondent households were peasant farmers wholly dependent on farming for livelihood. In Kwale 90.9%, while in Bomet 71.4% of households were peasant farmers without any other source of livelihood as shown in Figure 7.

The fact that 74% of those interviewed were female reflected one of the key criteria by the dairy goat supporting NGOs whose main intention was to support females due to the fact that majority of females tend to stay at home without employment opportunities. Nyanza had the highest number of widows and orphans. The fact that only 12% of the households interviewed used hired labour, while 88% depended on family labour, with age distribution of those caring for the goats falling between 31 and 50 years (63%) was an indication of low economic status of the farmers (Table 4).

It was noteworthy that only 9% respondents could afford to use roof-catchment water regarded as good quality, while the rest depended on other sources river, well, dam that were generally regarded as poor quality water. The quality of water is capable of impacting on the standards of hygiene, especially in case of mastitis in goats.

Standard house designs were availed to farmers. It was however, noted that the quality / standard varied depending on each ones ability to purchase the materials. Poor house standards impacted on manure contamination and therefore levels of hygiene, which inturn contributes towards levels of mastitis. Even though most respondents had received some form of training in goat keeping, or a visit from a government extension worker, a number of observations emerged from the interviews. There was no training package designed specifically for dairy goats, and extension service providers were poorly distributed and none had been trained specifically on dairy goat diseases. None of the farmers routinely practiced milking hygiene like the use of clean water, soap, drying towels. This is bound to have had a negative effect on mastitis management. The respondents indicated poor record keeping in key areas, like disease diagnosis / treatments, births/weights, weaning weights, daily milk yields and breeding records in general. None of the interviewees could remember ever carrying out treatments for mastitis, even though on clinical examination a number of goat udder halves had been damaged due to chronic mastitis.

# CHAPTER FOUR

# IDENTIFICATION OF MASTITIS CAUSING PATHOGENS

## 4.1 Introduction

The assessment of udder health in goats, as in the cow, has been based on detection of elevation of somatic cells, using California Mastitis Test (CMT). The CMT reagent reacts with the DNA material of somatic cells to form a gel. The CMT is graded subjectively as Negative (O) Trace, positive (+ve) (1, 2, 3). Best results are obtained when CMT is conducted just before milking after stimulation of let down and discarding fore milk. CMT is regarded as an indirect Somatic Cells measurement. A direct determination of somatic cells (Somatic Cell Count – SCC) can be done in several ways, e.g. by use of Improved Neubauer Chamber – for a total Leukocyte Count (Shearer & Harris Jrn, 2006).

Somatic Cell Counts in goats show a great variation, (e.g. during lactation) and can increase due to physiological factors like breed, parity, stage of lactation, estrus, hygienic standards. Goat milk SCC are, however, higher than cow's milk due to cytoplasmic particulate debris and epithelial cells. The debris and epithelial cells are about the same size and somatic cells / white blood cells (Haskell, S. R, 2005; Escobar E. N., 2007). Determination of bacteriological status is, however, regarded as the gold standard for mastitis detection (Stuhr & Aulrich, 2010).

## 4.2 Materials and Methods

### i. Field Test,

### California Mastitis Test (CMT)

California Mastitis Test procedure involved drawing foremilk from each half udder into separate cup of the CMT plastic paddle. The paddle is tilted to equalize milk quantities at about half teaspoon each. The CMT reagent was added at about half a teaspoon into each cup. The paddle was rotated to mix, and observed for changes in colour and gel formation within 10 + 0 20 seconds after mixing. Milk from a normal quarter remained liquid and flowed freely. In moderate reaction the gel formed was fragile and breaking into small masses or clamps. Milk samples that reacted strongly formed agelatinous mass that clung together as the paddle was rotated. CMT interpretation, based on Marshall & Edmondson (2007), where no reaction represented O, while trace reaction represented by the score 1, distinct positive reaction was represented by 2 score, while 3 score represented a strong positive.

**ii. Laboratory Tests**

**Somatic Cell Counts (SCC)**

Direct Somatic Cell Count was carried out using Improved Neubauer Chamber. Sample milk, prediluted 1:4 in 0.9% Sodium Chloride solution (Physiological Saline) was sucked up to the 0.5 mark of the leukocyte pipette (white bead). Tuck's solution (Merck) was added by sucking up to the 11 mark. This resulted in the dilution of 1:21. The mixture was stirred on a vibrator for 3 minutes. The first drops from the pipette after stirring were discarded and the rest used to fill both sides of the Neubauer Chamber. The chamber was examined under a light microscope (AO, American Optical, USA) and the numbers of particles (cells) counted in each of the four large corner squares at X10 magnification. To calculate the sum of the particles in the four corner squares X84X0.1, the product divided by 4 gives the total leucocytes per microlitre. To get the number of leukocytes per millitre, the figure obtained per microlitre is multiplied by $10^3$.

**Table 10: Interpretation of SCC from individual goat milk samples**

| SCC / ml of milk | Interpretation |
|---|---|
| Less than 1,000 | Healthy gland |
| 500,000 - 2,000 | Infection by weak pathogens |
| Over 1,500,000 | Signals infection |

(Shearer J. K.; Harris, B, 2006)

**Bacterial Isolation**

Milk was collected from each udder half of only lactating does for bacteriological analysis. From each half udder a milk sample of 0.03 ml was spread onto blood-agar plates containing 5% of washed sheep red blood cells onto MacConkey plates. Direct and enrichment cultures were incubated at $37^0$c for 12 hours. Selection of colonies from subcultures was done according to their predominance and homogeneity. All blood agar plates that showed no growth were re-examined 48 hrs and 72 hours of incubation while fast growing non-haemolytic colonies were sub-cultured on nutrient agar (oxoid).

Direct and enrichment cultures were incubated at $37^0$C for 12 hours. Growth of direct cultures was examined and sub-cultured. Enrichment broth cultures were sub-cultured by streaking on

5% sheep blood agar and incubated at $37^0$C for 24 hours. Selection of colonies from sub-cultures was done according to their predominance and homogeneity throughout the streak, type of haemolysis and the comparison of growth characteristics in both direct and indirect cultures. Special emphasis was given to the scrutiny for the slow growing and more fastidious colonies.
All blood agar plates that showed none or scarce growth were re-examined after 48 hours and 72 hours of incubation. Haemolytic colonies were sub-cultured onto blood agar whereas fast growing non-haemolytic colonies were sub-cultured on nutrient agar (Oxoid).

**Storage of Pure Isolates**

Pure colonies from the sub-cultures were harvested using a sterile cotton swab and suspended in sterile 0.25 molar sucrose solution in plastic vials with screw caps and stored at $-23^0$C in a deep freezer. The sucrose was prepared by taking 8.6g of sucrose and dissolving in 100ml distilled water. The solution was then dispensed at 0.5ml amounts in each vial and autoclaved at $121^0$C for 15 minutes. It was stored at $4^0$C until the time of use. When required, the organisms were removed from the freezer, thawed and loopful of them sub-cultured on blood agar. The remaining was stored again for future use.

**Identification of Colonies from Cultures**

Pure cultures from the subcultures were screened for identification based on colony morphology and haemolysis on blood agar. Gram stain, catalase and coagulase tests done according to Carter *et al.* (1990). Graim-positive, catalase positive cocci in clusters (Staphylococcus) were further differentiated from the other catalase positive cocci according to their ability to ferment glucose and mannitol.

**Catalase Test**

This was done according to Carter *et al.* (1990). A drop of physiological saline was placed on a clean glass slide using a wire loop, about 2 to 3 colonies of the test organisms were emulsified in the drop to make a thick suspension. A drop of rabbit plasma was added to the suspension and mixed gently. Clumping of organisms within 10 seconds was recorded as coagulase positive for *Staphylococcus aureus* and no clumping was recorded as coagulase negative.

**Biochemical Differentiation of *Staphylococcus spp.***

All *Staphylococcus spp.* that were positive in clumping factor test were further differentiated and confirmed as *Staphylococcus aureus* from the rest of the coagulase negative. *Staphylococci* according to their fermentation of carbohydrates especially mannitol and glucose. Organisms were inoculated into the sugar medium as described below in the case of strephtococcal biochemical differentiation. They were incubated at $37^0$C for 12 hours. Change of colour from red to yellow indicated positive fermentation.

**Fermentation of Carbohydrates**

Casein Triptic Agar (CTA) (BBL) was used as the basic medium and with added carbohydrates, fermentation reactions of fastidious microorganisms were tested. CTA consists of L-cystine, pancreatic digest of casein, agar, sodium chloride, sodium sulphate and phenol red. The sugars used were trehalose, arabinose, ribose, sorbitol, mannitol, inulin, raffinose and lactose. To prepare the individual sugars, CTA base medium was used. Using an electronic balance (RB 153, Stanton, UK), 7.2g was weighed and dissolved in 230ml distilled water. The mixture was made to dissolve completely by placing it in a water bath at $100^0$C. After dissolving, the media was autoclaved at $118^0$C for 15 minutes at 1 bar. The sugars, which were made to a final concentration of 0.5% of the total preparation, were weighed using the same balance into appropriate containers. Carbohydrate sugar weighing 1.5g was dissolved in 20ml distilled water, sterilized using a Prox-X filter unit 0.22µm hydrophilic cellulose acetate membrane (Lida Co). Each sugar was treated separately and was filtered into the base media after these had been cooled down to $50^0$C in a water bath. The mixture was shaken gently and then dispended into sterile test tubes at the rate of 5 to 6 ml per tube using a sterile 50ml pipette. The tubes were left at room temperature for the media to solidify ready for inoculation. The inoculation was made by making a suspension of overnight culture in 3ml of sterile water in a test tube. Using a 1ml pipette, 0.1 ml of the culture suspension was dispensed into each reagent tube of the sugar medium. Since the medium was solid, a straight sterile wire was used to stab the medium for the culture to percolate. These were incubated at 370C for 48 hours. The reading was taken after 24 hours, and the culture re-incubated for a further 12 hours to 24 hours for the second reading.

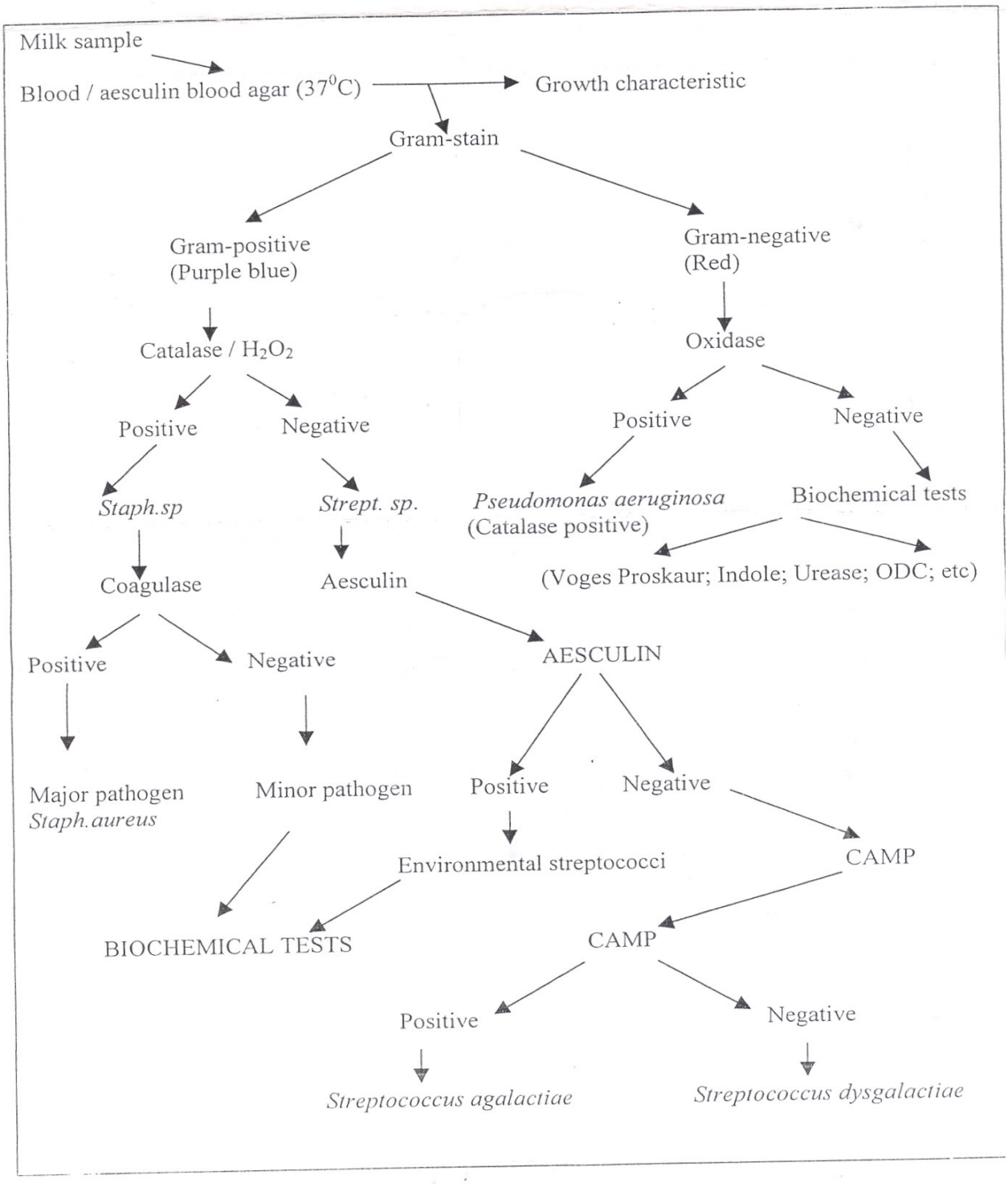


**Figure 12: Laboratory Identification of Bacteria in Milk (Carter, 1990)**

### iii. Statistical Analysis

One way analysis of variance, using ANOVA was used to determine CMT, SCC, bacterial isolation against breed, location, parity and stage of lactation. CMT, SCC and bacterial isolation against breed and location was analysed using chi-square test (x2), test for association.

## 4.3 Results

### 4.3.1 California Mastitis Test (CMT)

Table 11 shows that CMT was conducted on 138 milk samples (17) 12.3% of the samples graded as negative, (42) 30.4% were graded as 1, (32) 23.2% were 2, while (47) 34.1% were graded as 3.

**Table 11: California Mastitis Test**

| CMT Level | Frequency (N) | Percentage (%) |
|---|---|---|
| Negative (0) | 17 | 12.3 |
| Mild (1) | 42 | 30.4 |
| Moderate (2) | 32 | 23.2 |
| Heavy almost solidifies (3) | 47 | 34.1 |
| **Total** | **138** | **100** |

### 4.3.2 Somatic Cell Counts (SCC)

A total of 239 milk samples were analyzed for SCC. Table 12 summarizes the SCC in actual counts, and the corresponding $\log^6$. The lowest SCC was 248,371 (248 x $10^6$) the highest was 1,693,440 (1693 x $10^6$), with a mean count of 869,522.87(86592 x $10^6$).

**Table 12: Somatic Cell Counts (SCC)**

| | SCC | SCC $\log^6$ |
|---|---|---|
| N | 239 | 239 |
| Mean | 869,522.87 | .86,952 x $10^6$ |
| Standard deviation | 206,609.32 | .206,609 x $10^6$ |
| Range | 1,445,069 | 1.455 x $10^6$ |
| Minimum | 248,371 | .248 x $10^6$ |
| Maximum | 1,693,440 | 1.693 x $10^6$ |

Table 13 shows the distribution of SCC based on classes of 500,000 cells /ml.

**Table 13: Distribution of SCC**

| Levels of SCC | Frequency (n) | Percentage (%) |
|---|---|---|
| <500,000 | 4 | 1.7 |
| 500,000 - 1,000,000 | 172 | 72 |
| 1,000,000 - 1,500,000 | 62 | 25.9 |
| 1,500,000 - 2,000,000 | 1 | 0.4 |
| **Total** | **239** | **100** |

### 4.3.3 CMT and SCC

Pearson's correlation co-efficient was applied to determine the strength and direction of the association between CMT and SCC as seen in Table 14.

**Table 14: Correlation of CMT and SCC**

| | | Somatic Cell Count (SCC) | California Mastitis Test |
|---|---|---|---|
| Somatic Cell Count (SCC) log $^{6}$ | Pearson Correlation | 1 | 0.08 |
| | Sig. (2 - tailed) | | 0.417 |
| | n | 239 | 104 |
| California Mastitis Test | Pearson Correlation | 0.08 | 1 |
| | Sig. (2 - tailed) | 0.417 | |
| | n | 104 | 138 |

This analysis showed the CMT and SCC, r = 0.080, $p(0.417) > 0.05$. There was a positive geometrical correlation, even though not statistically significant. Further analysis using independent samples t-test with CMT scores 1, 2, 3 collapsed into one category as +ve CMT, and 0 as another category was tested against SCC mean scores.

Table 15 summarizes the t-test results. The t-test results showed that combined CMT (1, 2, 3) had a higher SCC, mean score of 0.895 x $10^6$, compared to CMT categorized as 0, which had SCC mean score of 0.870 x $10^6$, however, a P(0.667) >0.05 was not statistically significant.

**Table 15: T-test Results**

| CMT Levels | n | Somatic Cell Count ($log^6$) mean score | Std. Dev. | t-value | df | Sig.(2 tailed) |
|---|---|---|---|---|---|---|
| Negative / None (-) | 13 | 0.87017 | 0.255266 | -0.431 | 102 | 0.667 |
| Positive (+) | 91 | 0.89546 | 0.188979 | | | |

### 4.3.4 Bacterial Analysis

In Table 16 the occurrence of various groups of microorganisms based on morphology and physiology were isolated from each half-udder. The gram positive *cocci* group constituted 42% while gram negative were about 27%.

**Table 16: General Bacterial Isolates**

| | Frequency (n) | Percentage (%) |
|---|---|---|
| *Escherichia coli* | 53 | 21 |
| *Staphylococcus aureus* | 76 | 30 |
| *Bacilli* | 48 | 19 |
| *Streptococcus* | 2 | 1 |
| No growth | 71 | 28 |
| **Total** | **250** | **100** |

### 4.3.5 Bacterial Isolates

The key bacteria isolated from 131 milk samples are indicated in Table 17, with *Staphylococcus aureus* as the most dominant at (76) 58%, *Escherichia coli* (53) 40.5%, *Streptococcus* (2) 1.5%.

**Table 17: Key Bacterial Isolates**

| | Frequency (n) | Percentage (%) |
|---|---|---|
| *Escherichia coli* | 53 | 40.5 |
| *Staphylococcus aureus* | 76 | 58 |
| *Streptococcus* | 2 | 1.5 |
| **Total** | **131** | **100** |

### 4.3.6 CMT and Correlation with Key Bacteria / Isolates

Table 18 summarizes descriptive statistics of CMT scores across the key bacteria isolates based on one way analysis of variances (ANOVA). All bacteria isolated recorded CMT scores of between 2 and 3.

**Table 18: Relationship between CMT Scores and Key Bacteria / Isolates**

| | n | CMT Mean | St. Dev. | St. Error | Minimum | Maximum |
|---|---|---|---|---|---|---|
| *Escherichia coli* | 24 | 2.17 | 0.963 | 0.197 | 0 | 3 |
| *Staphylococcus aureus* | 37 | 2 | 0.972 | 0.16 | 0 | 3 |
| *Streptococcus* | 2 | 3 | 0 | 0 | 3 | 3 |
| **Total** | **63** | **2.1** | **0.962** | **0.121** | **0** | **3** |

### 4.3.7 Relationship between SCC Scores and Key Bacteria / Isolates

Table 19 is a cross-tabulation of the SCC against the two key bacterial isolates, *Escherichia coli* and *Staphylococcus aureus*. An independent sample t-test was used to determine if the SCC mean scores between the two unrelated bacteria differed significantly.

**Table 19: Cross-tabulation of SCC summary against the two key bacterial isolates**

| Statistics | *Escherichia coli* | | *Staphylococcus aureus* | |
|---|---|---|---|---|
| | Somatic Cell Count (SCC) | Somatic Cell Count (SCC) $\log^6$ | Somatic Cell Count (SCC) | Somatic Cell Count (SCC) $\log^6$ |
| N | 46 | 46 | 54 | 54 |
| Mean | 861690.99 | 0.86169 | 881008.8 | 0.88101 |
| St. Deviation | 193298.911 | 0.193299 | 206127.4 | 0.206127 |
| Range | 824141 | 0.824 | 835430 | 0.835 |
| Minimum | 485453 | 0.485 | 462874 | 0.463 |
| Maximum | 1309594 | 1.31 | 1298304 | 1.298 |

Table 20 summarizes the t-test result which indicates $p(0.632)$ at >0.05 significance level. The SCC showed no significant difference due to the type of bacteria.

**Table 20: Mean of SCC across the Types of Bacterial Isolates**

| | | Somatic Cell Count | | | | *Sig. (2* |
|---|---|---|---|---|---|---|
| **Bacteria** | **N** | **$\log^6$ mean score** | **St. Dev.** | ***t-value*** | ***df*** | ***tailed)*** |
| *Escherichia coli* | 46 | .86169 x $10^6$ | 0.193299 | -0.481 | 98 | 0.632 |
| *Staphylococcus aureus* | 54 | .88101 x $10^6$ | 0.206127 | | | |

### 4.3.8 Variation of CMT across Locations

Table 21 shows the CMT scores across locations, in order to find out if the mean scores for locations were significantly different.

**Table 21: Descriptive Statistics of CMT Scores across Locations**

| **Location** | **n** | **CMT Mean** | **St. Deviation** | **Std. Error** | **Minimum** | **Maximum** |
|---|---|---|---|---|---|---|
| Njoro | 30 | 1.43 | 0.971 | 0.177 | 0 | 3 |
| Kasambara | 24 | 1.79 | 1.141 | 0.233 | 0 | 3 |
| Elburgon | 31 | 2.16 | 0.898 | 0.161 | 1 | 3 |
| Kwale | 25 | 1.52 | 1.194 | 0.239 | 0 | 3 |
| Bomet | 28 | 2 | 0.943 | 0.178 | 0 | 3 |
| **Total** | **138** | **1.79** | **1.05** | **0.089** | **0** | **3** |

ANOVA was used, as shown in Table 22, which indicates significant differences in CMT mean scores across the locations, $p(0.036)$ at $\alpha$ 0.05 significant level. Table 23 indicates significant differences in CMT mean scores across type of breeds.

**Table 22: ANOVA Comparing CMT Mean Scores across Location**

| | **Sum of Squares** | **df** | **Mean Square** | **f** | **Sig.** |
|---|---|---|---|---|---|
| Between groups | 11.147 | 4 | 2.787 | 2.652 | 0.036 |
| Within groups | 139.759 | 133 | 1.051 | | |
| **Total** | **150.906** | **137** | | | |

Means significant at $\alpha = 0.05$ significant level ($p < 0.05$)

**Table 23: ANOVA Comparing CMT Mean Scores across Type of Breed**

| | Sum of squares | df | Mean square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 2.512 | 3 | 0.837 | 0.756 | 0.521 |
| Within Groups | 148.394 | 134 | 1.107 | | |
| Total | 150.906 | 137 | | | |

### 4.3.9 Variation of SCC across Locations

Table 24 shows the SCC across locations, in order to find out if these mean SCC were significantly different across locations.

**Table 24: Descriptive Statistics of SCC Scores across Location**

| Location | N | Mean | St. Deviation | St. Error | Minimum | Maximum |
|---|---|---|---|---|---|---|
| Njoro | 51 | 0.91645 | 0.20675 | 0.028951 | 0.531 | 1.693 |
| Kasambara | 31 | 0.90161 | 0.135596 | 0.024354 | 0.621 | 1.106 |
| Elburgon | 38 | 0.99259 | 0.169991 | 0.027576 | 0.576 | 1.332 |
| Kwale | 33 | 0.76222 | 0.162853 | 0.028335 | 0.463 | 1.185 |
| Homabay | 16 | 0.69572 | 0.172986 | 0.043247 | 0.248 | 1.039 |
| Siaya | 29 | 0.75485 | 0.205346 | 0.038132 | 0.485 | 1.287 |
| Nyando | 30 | 0.90016 | 0.221507 | 0.040441 | 0.598 | 1.343 |
| Bomet | 11 | 0.92985 | 0.216294 | 0.065215 | 0.644 | 1.242 |
| **Total** | **239** | **0.86952** | **0.206609** | **0.013364** | **0.248** | **1.693** |

Table 25 shows ANOVA comparisons of SCC across locations, $p$ value $0.000 < 0.05$. Post HOC multiple comparisons using Least Squares Difference (LSD) was used to establish differences between specific locations. Table 26 shows statistically significant differences.

**Table 25: ANOVA Comparing SCC Mean Scores across Location**

| | Sum of squares | df | Mean square | F | Sig. |
|---|---|---|---|---|---|
| Between groups | 2.033 | 7 | 0.29 | 8.254 | 0 |
| Within groups | 8.127 | 231 | 0.035 | | |
| **Total** | **10.16** | **238** | | | |

Means significant at α = 0.05 significant level ($p < 0.05$)

**Table 26: LSD Test Comparison SCC Mean Scores across Location**

| (I) Location | (J) Location | Mean Difference (I-J) | Std. Error | Sig. |
|---|---|---|---|---|
| Njoro | Kwale | .154230 | 0.041904 | 0 |
| | Homabay | .220731 | 0.053747 | 0 |
| | Siaya | .161607 | 0.043623 | 0 |
| Kasambara | Elburgon | -.090986 | 0.045395 | 0.046 |
| | Kwale | .139385 | 0.046915 | 0.003 |
| | Homabay | .205886 | 0.057739 | 0 |
| | Siaya | .146761 | 0.048457 | 0.003 |
| Elburgon | Kwale | .230371 | 0.044631 | 0 |
| | Homabay | .296872 | 0.055899 | 0 |
| | Siaya | .237748 | 0.046249 | 0 |
| | Nyando | .092436 | 0.04581 | 0.045 |
| Kwale | Nyando | -.137935 | 0.047316 | 0.004 |
| | Bomet | -.167630 | 0.065303 | 0.011 |
| Homabay | Nyando | -.204436 | 0.058065 | 0.001 |
| | Bomet | -.234131 | 0.073466 | 0.002 |
| Siaya | Nyando | -.145311 | 0.048846 | 0.003 |
| | Bomet | -.175006 | 0.066419 | 0.009 |

### 4.3.10 Bacteria and Location

Table 27 shows the two key bacterial isolates across the locations in a chi-square statistical test used to compare the frequency of bacterial isolates in two or more unrelated locations. This analysis shows there was a significant association between the type of bacteria and location *Escherichia coli* was common in Njoro, Elburgon, Homa Bay and Siaya, while *Staphylococcus aureus* was more common in Kasambara, Kwale, Nyando and Bomet. In this case contingency coefficient value of 0.490 indicates a moderate and significant association between type of bacteria and location.

**Table 27: Type of Bacteria across Location**

| Bacteria isolates | Location | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Njoro** | **Kasambara** | **Elburgon** | **Kwale** | **Homabay** | **Siaya** | **Nyando** | **Bomet** | **Total** |
| *Escherichia coli* | 8 | 2 | 17 | 8 | 5 | 8 | 3 | 2 | **53** |
| | 53.3 | 33.3 | 70.8 | 32 | 83.3 | 66.7 | 15.8 | 8.3 | **40.5** |
| *Staphylococcus aureus* | 7 | 4 | 7 | 17 | 1 | 4 | 16 | 20 | **76** |
| | 46.7 | 66.7 | 29.2 | 68 | 16.7 | 33.3 | 84.2 | 83.3 | **58** |
| *Streptococcus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | **2** |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 8.3 | **1.5** |
| **Total** | **15** | **6** | **24** | **25** | **6** | **12** | **19** | **24** | **131** |

$x^2 = 41.475$ $df = 14$ $p = 0.000$ Contingency coefficient = 0.490

### 4.3.11 SCC and Breed

Table 28 shows descriptive statistics of SCC across breeds.

**Table 28: Descriptive Statistics of SCC Scores across Types of Breeds**

| Types | N | Mean | Std. Dev. | Std. Error | Minimum | Maximum |
|---|---|---|---|---|---|---|
| Toggenburg | 29 | 0.97474 | 0.162282 | 0.14379 | 0.621 | 1.253 |
| Toggenburg Alpine Cross | 77 | 0.91972 | 0.1911 | 0.10368 | 0.531 | 1.693 |
| Saanen | 123 | 0.80266 | 0.205719 | 0.08830 | 0.248 | 1.343 |
| Alpine | 10 | 1.00026 | 0.166876 | 0.25150 | 0.768 | 1.31 |
| **Total** | **239** | **0.86952** | **0.206609** | **0.06367** | **0.248** | **1.693** |

ANOVA was used to establish if the above differences are significant or not, as shown in Table 29. SCC across breed types was significant (*P* value, 0.000 < 0.050).

**Table 29: ANOVA Comparing SCC Mean Scores across Type of Breed**

| | Sum of Squares | df | Mean square | F | Sig. |
|---|---|---|---|---|---|
| Between groups | 1.236 | 3 | 0.412 | 10.848 | 0 |
| Within groups | 8.924 | 235 | 0.038 | | |
| **Total** | **10.16** | **238** | | | |

Means significant at α = 0.05 significant level ($p < 0.05$)

Post HOC Multiple Comparison using Least Squares Differences (LSD) test was used to establish the SCC against specific types of breeds, as shown in Table 30. The LSD tests shows significant differences in SCC between some breed type, while others are not significantly different.

**Table 30: LSD Test Comparing SCC Mean Scores across Types of Breeds**

| (I) Breeds | (J) Breeds | Mean Difference (I-J) | Std. Error | Sig. |
|---|---|---|---|---|
| Toggenburg | ToggenburgXAlpine Cross | 0.055026 | 0.042457 | 0.196 |
| | Saanen | .172079* | 0.040226 | 0 |
| | Alpine | -.025516 | 0.071462 | 0.721 |
| ToggenburgX Alpine Cross | Toggenburg | -.055026 | 0.042457 | 0.196 |
| | Saanen | .117053* | 0.028318 | 0 |
| | Alpine | -.080542 | 0.065502 | 0.22 |
| Saanen | Toggenburg | -.172079* | 0.040226 | 0 |
| | ToggenburgXAlpine Cross | -.117053* | 0.028318 | 0 |
| | Alpine | -.197595* | 0.064079 | 0.002 |
| Alpine | Toggenburg | .025516 | 0.071462 | 0.721 |
| | ToggenburgXAlpine Cross | .080542 | 0.065502 | 0.22 |
| | Saanen | .197595 | 0.064079 | 0.002 |

*The mean difference is significant at the 0.05 level.

The Toggenburg was significantly different from Saanen. Toggenburg X Alpine cross were significantly different from Saanen, while Alpine and Saanen showed significant difference in their SCC.

### 4.3.12 Bacteria and Breed

To establish if there was statistically significant association in the type of bacteria and breed types, Chi-square statistical test was used as shown in Table 31. It was observed that Toggenburg, Toggenburg X Alpine Cross and Alpine had a higher *Escherichia coli* infection of 56.3%, 60.0% and 75% respectively compared to the Saanen which had a higher *Staphylococcus aureus* of 67.4%. There was however no significant statistical association.

**Table 31: Type of Bacteria Isolate across Types of Breeds**

| | Breeds | | | | |
|---|---|---|---|---|---|
| **Bacteria Isolate** | **Toggenburg** | **Toggenburg X Alpine Cross** | **Saanen** | **Alpine** | **Total** |
| *Escherichia coli* | 9 | 15 | 26 | 3 | 53 |
| | 56.3 | 60 | 30.2 | 75 | 40.5 |
| *Staph. aureus* | 7 | 10 | 58 | 1 | 76 |
| | 43.8 | 40 | 67.4 | 25 | 58 |
| *Streptococcus* | 0 | 0 | 2 | 0 | 2 |
| | 0 | 0 | 2.3 | 0 | 1.5 |
| **Total** | **16** | **25** | **86** | **4** | **131** |

### 4.3.13 SCC and parity

The study sought to establish a relationship between parity and SCC. Table 32 shows the Correlation Coefficient between the two, which shows r = 0.145, $p(0.380) > 0.05$, a positive but not statistically significant.

**Table 32: Correlation of Parity with SCC**

| | | Parity | Somatic Cell Count ($log^6$) |
|---|---|---|---|
| Parity | Pearson Correlation | 1 | -.145 |
| | Sig. (2 - tailed) | | .380 |
| | N | 45 | 39 |
| Somatic Cell Count ($log^6$) | Pearson Correlation | -.145 | 1 |
| | Sig. (2 - tailed) | .380 | |
| | N | 39 | 239 |

### 4.3.14 SCC and Lactation Length

The study sought to establish the relationship between SCC and Lactation length using Pearson's Correlation Coefficient. Table 33 shows the relationship, r – 0.0880 $p(0.617) > 0.05$, a positive but not statistically significant.

**Table 33: Correlation of Lactation with SCC**

| | | Somatic Cell Count (SCC) | Lactation |
|---|---|---|---|
| Somatic Cell Count (SCC) | Pearson Correlation | 1 | .088 |
| | Sig. (2 - tailed) | | .617 |
| | N | 239 | 35 |
| Lactation | Pearson Correlation | .088 | 1 |
| | Sig. (2 - tailed) | .617 | |
| | N | 35 | 40 |

### 4.3.15 Bacteria and Parity

T-test was used to determine if parity mean scores between the two species of bacteria differed significantly. Table 34 indicates that udder halves infected with *Escherichia coli* had a higher mean parity score of 2.67 in comparison with those infected with *Staphylococcus aureus* with a parity mean score of 1.60.

**Table 34: Parity across the Type of Bacteria**

| Bacteria | N | Parity mean score | Std. Dev. | t-value | df | Sig. (2 tailed) |
|---|---|---|---|---|---|---|
| *Escherichia coli* | 9 | 2.67 | 1.871 | 1.186 | 12 | 0.259 |
| *Staphylococcus aureus* | 5 | 1.6 | 0.894 | | | |

### 4.3.16 Bacteria and Lactation Length

The study sought to establish any statistical differences in lactation length and the type of bacteria isolated, using the independent t-test. Table 35 shows lactation mean scores against bacteria isolates (*Escherichia coli* and *Staphylococcus aureus*).

*Staphylococcus aureus* isolates had generally a high lactation mean of 4.75, compared to *Escherichia coli* with a mean lactation mean score of 3.25, even though there was no significant statistical difference.

**Table 35: Lactation across the Type of Bacteria**

| Bacteria | N | Lactation mean score | Std. Dev. | *t-value* | *df* | *Sig. (2 tailed)* |
|---|---|---|---|---|---|---|
| *Escherichia coli* | 4 | 3.2500 | 2.06155 | - 1.414 | 6 | 0.207 |
| *Staphylococcus aureus* | 4 | 4.7500 | .50000 | | | |

**Table 36: Regression Analysis of SCC against Location and Breed**

| Independent variable | Standardized regression coefficient (Beta) | Significance F. Change |
|---|---|---|
| Constant | 1.025 | 0.000 |
| Breed | -0.232* | 0.000 |
| Location | -.016* | 0.000 |

*Beta is significant at $p < 0.01$ significance level (1-tailed)

N=239

The derived model is:

$$y = 1.025 - 0.232x_1 - 0.016x_2 + e$$

**$R^2 = 0.354$**

Table 36 indicates that when SCC was regressed on the combined predicted independent variables (location and breed), they all showed significant relationships. The observed relationships suggest that both breed and location significantly influenced SCC. These variables (location and breed) combined explain or contribute to 35.4 percent of the variations in the levels of SCC. However, through stepwise procedure, breed contributed 29.1 percent of the change in SCC and thus the most important of the two variables.

**Table 37: Regression Analysis of bacteria against Location, Breed and parity**

| Independent variable | Standardized regression coefficient (Beta) | Significance F. Change |
|---|---|---|
| Constant | 1.244 | 0.000 |
| Location | .077* | 0.000 |
| Breed | -0.093 | 0.533 |
| Parity | 0.049 | 0.236 |

*Beta is significant at $p < 0.01$ significance level (1-tailed)

N=131

The derived model is:

$$y = 1.244 - 0.093x_1 + 0.077x_2 + 0.049x_3 + e$$

$$\mathbf{R^2 = 0.120}$$

Table 37 indicates that when bacteria was regressed on the combined predicted independent variables (location, breed and parity), only location showed significant relationships. The others (breed and parity) showed no significant relationship. The observed significant relationship suggest that only location influenced the presence of bacteria. The three variables combined explain or contribute to 12.0 percent of the variations in the bacteria. However, through stepwise procedure, location alone contributed 12.0 percent of the change in bacteria and thus the most important of the three variables. The others have no significant relationship.

### 4.4 Discussion

Leukocytes (Somatic Cells) migrate into the mammary tissue to provide the first immunological line of defense against bacteria that penetrate the physical barrier of the teat canal. It is therefore generally accepted that concentration of somatic cells in the milk is directly to the infection

status of the udder, and no other factor(s) influences milk somatic cell count to the degree bacterial infections do. Therefore in the day to day management of the dairy, infection status of the herd can be monitored effectively by monitoring the SCC in bulk or individual animals (Escobar, 2007). Normal dairy goat milk has a higher SCC than cow milk due to cyplasmic particulate debris and epithelial cells shed along with milk (Haskell, 2005). Other factors known to increase SCC in goats include duration of lactation stage of lactation and parity (Moroni *et al.,* 2007).

### 4.4.1 California Mastitis Test (CMT)

California mastitis test scores conducted on 138 milk samples ranged from 0 (12.3%), 1(30.4%), 2 (23.2%) and 3 (34.1%). According to Haskell (2005) a CMT score of trace or 1 (one) indicates a healthy udder half, but at 2(two) and 3 (three) one must consider it infected (Shearer and Harris, 2003) it was also stated that scores of $2 \geq$ or $\leq 3$ are indicative of mastitis. Pearson and Olofsson (2011) and McDougall & Prosser (2010), in their evaluation of direct and indirect measurement of somatic cell count as indicator of intramammary infection (IMI) in dairy goats concurred with the view that CMT score of 1 was associated with freedom from intramammary infection (IMI) while CMT score of 2 was indicative of IMI.

In this study, considering the CMT scores in relation to the key bacterial isolates, i.e. *Staphylococcus aureus and Escherichia coli* had a CMT score of between 2 and 3 (Table 18). CMT was a reliable measure of intramammary infection in relation to the key bacterial isolates. ANOVA comparing CMT mean across the breed types showed significant differences amongst Toggenburg, Saanen, Alpine X Toggenburg and Alpine (Table 23). There have been mentions of breed as a factor causing different somatic cell counts in other studies (Mannasmith, 1981; Haskell, 2005), in which the Nubian scored higher than other breeds. In this study the Toggenburg scored highest mean CMT of 2.20, while Alpine scored 1.83 and Saanen 1.82, reaffirming significant breed variations in SCC.

### 4.4.2 Somatic Cell Count

The SCC conducted on 239 udder halves ranged between 248,371 cells/ml and 1,693,440, with a mean of 869,522. The use of SCC is one of the most established methods for diagnosis of udder health in cows (Paape *et al.,* 2007; Stuhr & Aulrich, 2010). Unfortunately SCC has not yet been

established as a proven marker for Sub Clinical Mastitis (SCM) in dairy goats due to factors like parity, stage of lactation, estrus and breed which contribute significantly to levels of SCC in milk. Furthermore, Mycoplasma infections can lead to higher SCC in goat milk (Corrales *et al.,* 2004). It has also been documented that Caprine Arthritis Encephalitis virus (CAE) may lead to higher SCC, though regarded as a minor contributor (Bergonier *et al.,* 2003).

Souza *et al.,* (2009) examined bulk milk samples of 1,400 dairy goats resulting in a mean score of 779,000 cells/ml, while in a different study by Jendretzke (Stuhr & Aulrich, 2010) a mean score of 990 $10^3$ was established.

In the European Union (EU) the SCC threshold for raw cow milk was set at 400 x $10^3$ (EC, 2004), but so far no limit values in EU exist for goat milk (Paape *et al.,* 2007). Nevertheless, some national thresholds exist for bulk milk ranging between 750 $10^3$ to 1 million cells/ml (Pirisi *et al.,* 2007).

A universal definition of a cell number threshold in goat milk to distinguish between healthy and infected udder does not exist. Only in the United States the SCC in bulk goat milk is not allowed to exceed 1 million cells / ml (US/Public Health Service, 2003). The findings in this various study and elsewhere on SCC studies and the factors mentioned above that might influence SCC in goat milk must be considered when setting SCC criteria for assessing the quality of goat milk Leitner *et al.,* (2008) proposed that differentiation between high, medium and low quality of bulk goat milk needs to be established as follows: high quality milk should have a SCC of < 800 $10^3$ cells/ml, associated with infection rate of 25%, medium quality. Milk should have < 1.5 $10^6$ cells/ml, associated with infection rate ranging between 25% and 50% while low quality milk should have a SCC of > 1.5 $10^6$ cells/ml. Goat milk of > 3.5 $10^6$ should be regarded as unsafe for human consumption. Each one of the proposed categories should be verified under different production systems / conditions, in various countries.

In this study 73.9% of *Escherichia coli* and 68.5% of *Staphylococcus aureus* infection fell within the SCC range of 500,000 and 1 million cells/ml, with mean SCC for each of these key organisms 861,690 cells/ml and 881,008 cells/ml respectively. These figures are in agreement with findings elsewhere, especially the proposed quality grading by Leitner *et al.,* (2008). The

range of SCC in this study in general, and the mean values for determining the key bacterial isolates therefore concur with results from studies elsewhere.

Among the factors reported elsewhere to influence SCC is breed. In this study ANOVA comparing SCC between the various breeds i.e. Toggenburg/Saanen, Toggenburg X Alpine / Saanen, Saanen / Alpine were significantly different. Studies in USA and European countries for a long time now have documented significant breed differences especially the Nubian, in SCC (Mannasmith, 1981; Haskell, 2005; Stuhr & Aulrich, 2010). Rupp *et al.,* (2012) in a study estimated the SCC heritability in French Alpine and Saanen dairy breeds to be 0.20, and it may be possible in future to improve mastitis resistance by selection based on SCC.

This is the first study to document this breed differences amongst the smallholder farmers in Kenya with the above combinations of breeds. It is noteworthy that in this study SCC varied amongst study sites significantly. It is not possible in this study to establish the factors in the various study agro-ecological zones which could have influenced the SCC. It is, however, probable that the clustering of breeds in the various sites of study, e.g. Njoro, Kasambara, Elburgon had a concentration of the Toggenburg breed supplied by the Farming Systems Kenya (a Catholic Organization), while the sites in Nyanza and Coast regions had a predominance of the Saanen breed could have influence on SCC. Other factors climate and diet could also have influence on SCC in different study locations. Parity and lactation length have been documented to affect SCC in goat milk by many researchers (Mannasmith, 1981; Haskell, 2005; Stuhr & Aulrich, 2010). Both parity and lactation length in this study impacted on SCC. Even though not statistically significant, the trends are noteworthy. These results concur with studies done elsewhere confirming the effect of parity and lactation length on SCC. In a study in Spain (Sanchez – Rodriguez *et al.,* 2012) it was concluded that as lactation days ran the SCC increased, more so in multiparous than primiparous goats.

### 4.4.3 Bacterial Isolates

The preliminary bacterial culture, showed predominance of gram positive (+ve) colli, 42%, and gram negative (–ve) rods, 27%. The objective of this study was to focus on key mastitis causing organisms. Therefore, as shown in Table 15 *Staphylococcus aureus*, 30% and *E. coli,* 21% (Total

51%) became the focus. The contagious *Staphylococcus aureus* has been documented as one of the most dominant and serious cause of goat mastitis.

In Kenya, a study was carried out in goats in Nyeri established *Staphylococcus* as the dominant isolates (63.6%), with *Staphylococcus aureus* constituting 22.7% of all bacterial isolates (Ndegwa *et al.,*2000). Studies elsewhere reaffirm the dominance of *Staphylococcus aureus* (Moroni *et al.,* 2007; Pearson & Olefsson, 2011, Stuhr & Aulrich, 2010). In Europe and USA the contagious IMI tends to be controlled by high level of standards of milking hygiene. However, amongst smallholder goat farmers in Kenya standards of hygiene are low and therefore the high incidence of *Staphylococcus aureus*. *Escherichia coli* a coliform, is documented as an environmental bacteria present at all times on all dairy farms, as faecal bacteria and capable of living in bedding, saw dust, shavings especially in hygienic environments (Ingalls, 2003). The significance of the two key bacterial organisms, *Staphylococcus aureus* (30%) and *E. coli* (21%) in this study lies in the fact that the Kenyan smallholder dairy farmer has little or no experience in managing a dairy animal especially milking hygiene, which leads to the high prevalence of the contagious organisms passed from one animal to another by milkers hands and the faecal bacteria.

# CHAPTER FIVE

# MOLECULAR IDENTIFICATION OF BACTERIA

## 5.1 Introduction

Mastitis remains one of the most common diseases of the dairy goats, causing the biggest economic loss to the industry (Halasa *et al.,* 2007). This chapter describes DNA extraction, PCR amplification utilizing gene specific primers and subsequent identification of the PCR products by gel electrophoresis. The study unveils the potential for rapidly identifying vaccine antigens IMI causing pathogens in goats, a tool that has been developed for IMI in cows.

## 5.2 Materials and Methods

Among the bacterial isolates, *Escherichia coli* and *Staphylococcus aureus* were identified the key mastitis causing organisms. These were subjected to PCR

### Chemicals and Enzymes

Diethyl pyrocarbonate (DEPC) (Amresco), r-Taq DNA polymerase, Dntp mixture, and DL 2000 molecular DNA marker

### Special Equipment

Centrifuge machine (12000 rpm, Eppendorf, 5417R), Thermocycler PCR machine (Biorad DNA Engine®), Mortar and pestle, Rotating shaker, Biorad mini protean system for gel electrophoresis, U-2800 spectrophotometer, Boiling water bath, shaking incubator, Blood agar plates, MacConkey agar plates, and Polaroid 667 – film.

### Primers

Two gene specific primers for amplification of the *Staphylococcus aureus* sequence targeting the *nuc* gene were designed with PRIMER PREMIER computer program. The forward primer was designated F 5'GCGATTGATGGTGATACGGTT-3' while the reverse primer was designated R 5'AGCCAAGCCTTGACGAACTAAAGC-3' respectively. The primer concentrations of 10 pMol were used in the amplification reaction of the partial sequence of the *nuc* gene.

**Culture Bacterial Cells**

Pure bacterial colonies were obtained for bacteriological analysis as described (Carter, 1990; Hogan *et al.,* 1990). The plates were incubated aerobically at $37^0$C and examined after 24 hours. The colonies were provisionally identified on the basis of Gram Stain, morphology and haemolysis patterns; the number of each colony type was recorded. The representative colonies were then sub-cultured on blood agar plates and incubated aerobically at $37^0$C for 24 hours to obtain pure cultures. Catalase and coagulase production was tested for gram positive *cocci.* Gram negative isolates were identified by use of colony, morphology, gram-staining characteristics, oxidase and biochemical reactions on MacConkey's agar and API 20E (BioMèrieux). Identification of *Escherichia coli* and *Staphylococcus aureus* colonies were based on morphological characteristics.

**DNA Extraction from *Staphylococcus aureus* and *Escherichia coli***

A few colonies of the positively identified *Escherichia coli* and *Staphylococcus aureus* were transferred into Eppendorf tubes containing 500µl of sterile distilled water and vortexed thoroughly. A total of 16 samples were processed. The test samples were then transferred to pre-heated water bath at $100^0$C, allowed to heat for 10 minutes, then cooled to room temperature. The samples were then spun for 10 minutes at 5000rpm.

*Staphylococcus aureus* and *Escherichia coli* DNA was extracted from the supernatants by alkaline lysis plasmid SDS procedure as previously described (Sambrook *et al.,* 1995). The DNA pellet was dissolved in 30µl of TE (10mMol/L Tris-Cl, pH 8.0; 1mMol/L EDTA Ph8.0) containing DNase-free RNase A (pancreatic RNase) and used as template DNAs for amplification reaction.

**PCR Amplification of *Staphylococcus aureus***

The forward primer F 5'-GCGATTGATGGTGATACGGTT-3'and reverse primer R 5'-AGCCAAGCCTTGACGAACTAAAGC-3' were used to amplify the partial sequence of the *nuc* gene. The PCR mixture was assembled in thin walled 0.5ml tubes to a final 50µl volume as follows;

| | |
|---|---|
| Template (*Staphylococcus aureus)* | 2.0µl |
| 10x LA PCR buffer µ($Mg^{2+}$free) | 5.0µl |
| $MgCl_2$ (25mM) | 5.0µl |
| dNTP mixture (2.5mM) | 8.0µl |
| 1st PCR primer F(10pMol/µl) | 0.5µl |
| 1st PCR primer R (10pMol/µl) | 0.5µl |
| Taq Polymerase (5 U/µl) | 0.5µl |
| $dH_2O$ | 28.5µl |
| | 50.0µl |

Template used was the DNA product diluted 10 times in TE pH 8.0. The thermocycler amplification conditions were initial denaturation at $94^0$C for 5 min followed by 25 cycles at $94^0$C, 30s for denaturation, $55^0$C 30s for annealing and $72^0$C, 5 min for polymerization.

**PCR Amplification for *Escherichia coli***

The forward primer F 5' ATTCTTCTGGCTGGCATTCC 3' and reverse primer R 5' CGGGATTAGAGACTATTGTTGC 3' were used to amplify the *pic* marker gene for *Escherichia coli.* The PCR mixture was assembled in thin walled 0.5ml tubes to a final 50µl volume as follows:

| | |
|---|---|
| Template (*Escherichia coli)* | 2.0µl |
| 10 x LA PCR buffer µ($Mg^{2+}$free) | 5.0µl |
| $MgCl_2$ (25mM) | 5.0µl |
| dNTP mixture (2.5mM) | 8.0µl |
| 1st PCR primer F(10pMol/µl) | 0.5µl |
| 1st PCR primer R (10pMol/µl) | 0.5µl |
| Taq Polymerase (5 U/µl) | 0.5µl |
| $dH_2O$ | 28.5µl |
| | 50.0µl |

Template used was the DNA product diluted 10 times in TE Ph 8.0.

The PCR conditions were initial denaturation $94^0$C (5 min) followed by 35 cycles at $94^0$C (30s), $72^0$C (45s), $72^0$C (2 min), and a final extension for 10 min at $72^0$C in the last cycle. Amplification was performed using the automated cycler (Biorad, CA, USA).

**Identification of the *Staphylococcus aureus* and *Escherichia coli* PCR Products**

The *Staphylococcus aureus* and *Escherichia coli* PCR products were separated on 1% (w/v) agarose gels (sigma), stained with 0.5mg/ml of ethidium bromide using the "Biorad Mini Protean System." Gels were visualized under ultraviolet illumination and recorded on a Polaroid 667-film.

### 5.3 Results

The following Table 38 shows the results of molecular detection of *Escherichia coli* and *Staphylococcus aureus* strains in 16 milk samples representing about 10% of the total samples. Six of the samples were positive for the *pic* gene of *Escherichia coli,* while five were positive for *nuc* gene of *Staphylococcus aureus,* with distribution of the two genes in all the three regions of Kenya namely; Coast, Nyanza and Rift Valley.

**Table 38: PCR Results by Regions**

| **Region** | ***Escherichia coli*** | ***Staphylococcus aureus*** |
|---|---|---|
| Coast | 2(1, 14) | 2 (1, 2) |
| Rift Valley | 2 (10, 11) | 1 (11) |
| Nyanza | 2 (6, 16) | 2 (6, 16) |
| **Total** | **6** | **5** |

The gel pictures in Figure 13 and Figure 14 below show the amplification of the results in the 16 samples for *Staphylococcus aureus* and *Escherichia coli.*

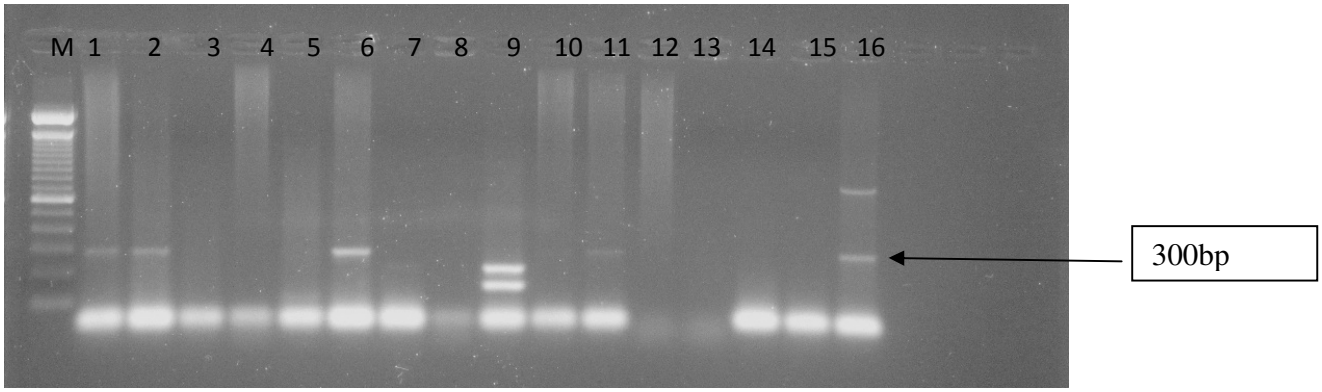


**Fig 13: Gel picture showing amplifications on some test samples for the detection of *Staph. aureus* (55)**

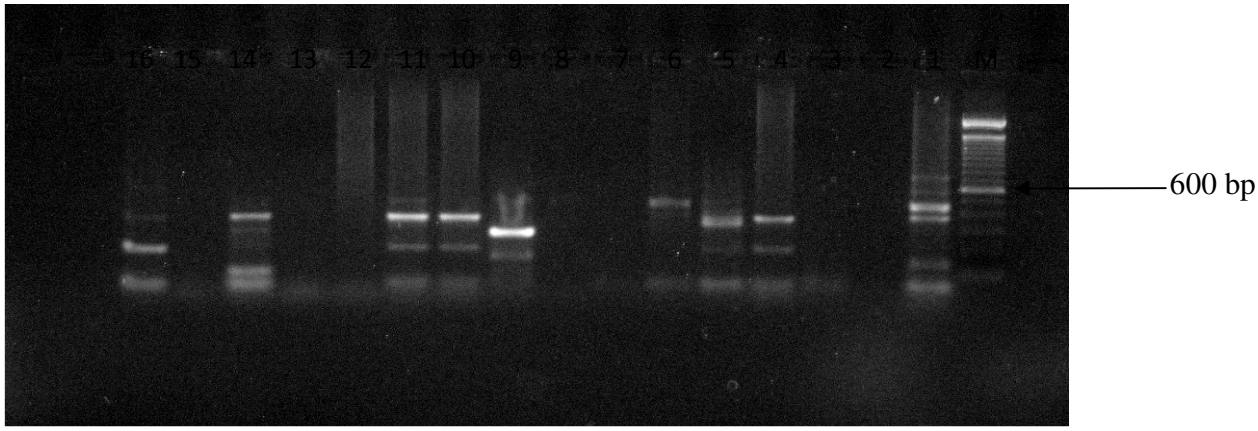


**Fig 14: Detection of *E. coli* in samples 1-16 listed above. Arrow indicating expected fragment**

### 5.4 Discussion

The positive identification of the pathogenic genes of the two key mastitis causing organisms, *Staphylococcus aureus* and *Escherichia coli* in the 11 samples indicates that they are significant causative agents of mastitis in goats in Kenya. These two genes occurred in dairy goats across the three regions of Coast, Nyanza and Rift Valley. This means that the two strains are widespread geographically and control measures for mastitis would not be based on regions.

This is the first attempt to identify mastitis pathogens in goat milk using PCR. In cow mastitis PCR as diagnostic procedure has taken root especially in Europe. Taponen *et al.,* (2009) in a

study named Real-time PCR based identification or bacteria in milk samples which targeted 11 most common bacterial species and groups in mastitis, and the *Staphylococcal* blaZ gene – responsible for penicillin resistance a procedure which could identify and quantify bacterial cells even if dead or growth inhibited.

Balson (2010) reported that the (British) National Milk Record's in February 2010 converted fully to use of PCR analysis to test and determine cause of mastitis infection to boost producer confidence in results compared with traditional bacterial culture analysis, especially in cases of "no growth" which were common and frustrating for farmers.

In Kenya in dairy goat industry is young, rapidly growing, but with no established quality control standards to guide the growth. Considering the progress in the use of PCR as a diagnostic tool in Europe and America (Taponen *et al.,* 2009; Balsom, 2010) more research work needs to be done to develop the use of PCR in characterization of goat mastitis causing pathogens in Kenya and indeed the rest of the world.

# CHAPTER SIX

# GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

The first objective of this study was to demonstrate the impact of management practices on levels of mastitis in goats in the various study areas. The demographic profiling indicated high ratio of women to men of 4:1, out of this 36.8% of the women were widows. The dairy goat sponsors, especially the Heifer Kenya Dairy Goat Project, targeted poor families with the objective to improve nutrition and create a source of income generating activity for women. Due to the lowly economic cadre of these farmers, ability to invest in improved management was therefore limited. All farmers who received goats were expected to construct houses based on a standard plan. It was, however, noted that houses actually constructed varied from fairly good to poor, depending on the farmers ability to afford construction materials. Good structures were designed to facilitate cleaning of droppings and urine, while poor structured represented a health hazard due to accumulation of droppings and urine. Other factors impacting on mastitis were frequency of removal and proximity of storage of manure in relation to the house. Animal contact with faecal / environmental bacteria like *E. coli* depended on the quality of the house structures, cleaning frequency and proximity of storage in relation to the house.

There were four sources of extension education, namely the extension worker (55%) group advice (60%) from some of amongst the farmers who had been selected for specialized training (56%), field days (34%) and occasional field training sessions. It was established that none of these sources of extension relied on a package specific to dairy goats, consequently, the farmers were not aware of hygienic milking procedure like use of clean / warm water, detergent, towel and clean utensils. The poor extension advice clearly impacted on incidence of mastitis at farm level.

About 91% sources of water relied upon by farmers were borehole, well, river, lake, dam, mostly regarded as unsafe. Only 9% of the farmers had capacity to harvest roof catchment, regarded as safest. Considering farmers poor knowledge of hygienic milking procedure, poor quality / contaminated water would increase to chances of mastitis infection.

CMT has been documented as the most widely used cheap and available first line detection of IMI in cattle, sheep, and goats. It is an indirect measure of levels of somatic cells in milk. Even though, it has been documented somatic cells in goats are higher influenced by many factors such as stage of lactation, parity and breed (Haenlein, 2002). The usefulness of CMT is for early detection of inflammation so that; laboratory testing can be done on inflamed halfs; treatment can be administered promptly and effectively; udder damage can be minimized and milk losses can be reduced.

Even as many researchers have documented varying CMT scores in goat milk, according to Shearer and Harris (2003) the test can be performed with reasonable accuracy, and, in general milk from non infected glands will yield negative (0), trace / or 1 ≥. Scores of 2 ≥ or 3 ≥ are indicative of mastitis.

In this study, CMT was used as a preliminary / animal side IMI detection test in goats, prior to further laboratory testing as stated above. The score of 1< was associated with freedom from IMI, while scores of 2 ≥, 3 ≥ were indicative of infection as established by the presence of the two organisms isolated.

The Somatic Cell Counts (SCC) is a direct measure of SCC in the laboratory. Due to demand for high quality milk products in the recent years, regulatory limits on bulk milk SCC have been imposed in various countries (Barkenia, *et al.,* 1998). SCC is also intended to encourage monitoring of udder health (Boor, *et al.,* 2001; Barkena, *et al.,* 1998). United States of America

is one of the very few countries in the world with dairy goat milk quality standards, while bulk tank the SCC for the cow is set at 750,000/ml, the count in goat milk is set at 1,000,000/ml. (Escobar, 2007). According to Shearer and Harris (2003), Somatic Cell Counts in goats of 1,500,000/ml are suggestive of intra-mammary infection. In this study SCC ranged between 248,371/ml. and 1,693,440/ml. with mean of 861,690/ml for *E. coli* infection and 881,008/ml for *Staph. aureus* infection.

Somatic Cell Counts have been adopted as a quality control measure in dairy industry for low milk by developed countries, including European Union, USA, New Zealand, Australia, however, dairy industry in developing countries, including Kenya has not set such standards. The goat milk is lagging behind in the development of quality standards based on SCC, except the USA which has set 1,000,000IMI as the highest in bulk tank.

This study has demonstrated that even though there are significant variations in levels of SCC amongst breeds and locations, SCC in relation to bacterial isolates represents a reliable mastitis marker, which can be developed to monitor bulk tank quality control.

The two key bacteria isolated, *Staph. aureus* and *E. coli* were indicative of the contagious IMI and environmental organism both commonly associated with poor standards of milking hygiene. This fact vindicated the findings in the first objective of this study implicating poor structures, poor quality of water, poor extension education as impacting on incidence of IMI.

DNA extraction of bacteria, PCR, DNA hybridization and colorometric reaction was carried out for the first time to test its potential as a rapid and accurate diagnostic tool in goat IMI. As stated by Kuo-itua Leel *et al.* (2008), DNA-based assay might circumvent some major drawbacks such as time too low numbers in milk for culture and identification of unique genetic information of bacterial genome. In this study pathogenic strains of both *Staph. aureus* and *E. coli* were

demonstrated in samples collected from all the three key goat keeping regions of Coast, Rift Valley and Nyanza. DNA-based assay as a diagnostic tool has great potential in goat IMI.

**Recommendations**

The dairy goat subsector in Kenya is young and rapidly growing. These being dairy animals IMI will continue to be a major production challenge. It would therefore, be necessary for "a dairy goat" production / herd health package, which would include milking hygiene to be developed for extension service providers, university curricula needs to include goat herd health.

Considering that the Kenya Dairy Board (KDB) has not yet developed quality standards for goat milk, further studies to develop SCC-based quality standard would be prudent. The use of DNA-based assay, as demonstrated in this study and it current growing application in dairy cow milk, would be a worthwhile effort by KDB.

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## APPENDICES

**Plate 1: Toggenburg**

The Toggenburg is a breed of goat, named after the region in Switzerland where the breed originated, the Toggenburg valley. Toggenburgs are medium in size, moderate in production, and have relatively low butterfat content (2-3%) in their milk.

The color is solid varying from light fawn to dark chocolate with no preference for any shade. Distinct white markings are as follows: white ears with dark spot in middle; two white stripes down the face from above each eye to the muzzle; hind legs white from hocks to hooves; forelegs white from knees downward with a dark line (band) below knee acceptable; a white triangle on either side of the tail. Wattles, small rudimentary nubs of skin located on each side of the neck, are often present in this breed. The Toggenburg underwent a development program

when introduced to Britain - the resulting British Toggenburg being heavier and having improved milk quality. By the middle of the year 2002, 4146 Toggenburgs had been registered with the New Zealand Dairy Goat Breeders Association, representing 8.10% of registered dairy goats. They perform better in cooler conditions. They are the oldest known dairy breed of goats.

**Plate 2: Alpine**

The Alpine Dairy Goat is also referred to as the French Alpine and registration papers for this dairy goat use both designations and they are synonymous. The Alpine dairy goat is a medium to large size animal, alertly graceful, and the only breed with upright ears that offers all colors and combinations of colors giving them distinction and individuality. They are hardy, adaptable animals that thrive in any climate while maintaining good health and excellent production. The

hair is medium to short. The face is straight. A Roman nose, Toggenburg color and markings, or all-white is discriminated against.

Alpine colors are described by using the following terms:

- **Cou Blanc** (coo blanc) - literally "white neck" white front quarters and black hindquarters with black or gray markings on the head.
- **Cou Clair** (coo clair) - literally "clear neck" front quarters are tan, saffron, off-white, or shading to gray with black hindquarters.
- **Cou Noir** (coo nwah) - literally "black neck" black front quarters and white hindquarters
- **Sundgau** (sundgow) - black with white markings such as underbody, facial stripes, etc.
- **Pied** - spotted or mottled.
- **Chamoisee** (shamwahzay) - brown or bay characteristic markings are black face, dorsal stripe, feet and legs, and sometimes a martingale running over the withers and down to the chest. Spelling for male is chamoise.
- **Two-tone Chamoisee** - light front quarters with brown or gray hindquarters. This is not a cou blanc or cou clair as these terms are reserved for animals with black hindquarters.
- **Broken Chamoisee** - a solid chamoisee broken with another color by being banded or splashed, etc.

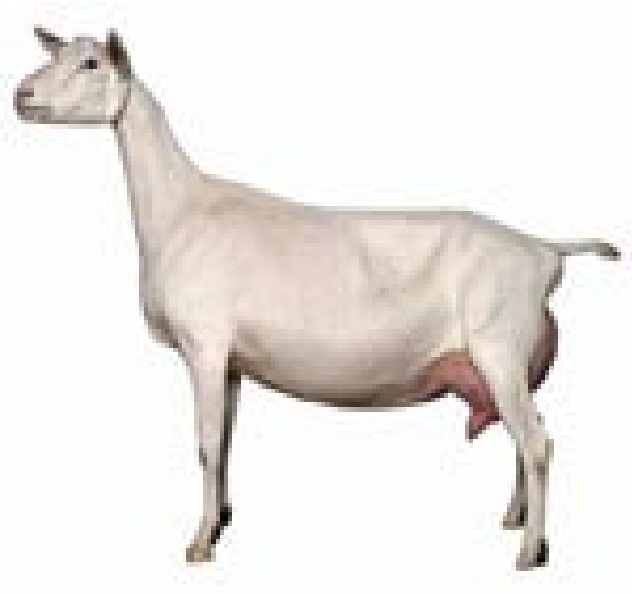


**Plate 3: Saanen**

Saanens are the largest and one of the calmest of the dairy breeds. The Saanen breed also produces the most milk on average and tends to have a lower butterfat content, about 2.5%-3%.

The Saanen temperament is as a rule, calm and mild mannered; breeders have been know to refer to them as living marshmallows. Saanen goats are easier for children to handle and are popular in the showmanship classes due to their calm nature. They originated in the Saanen valley in the south of Canton Berne, Switzerland. In 1893 several thousand head were taken out of the valley and spread throughout Europe. Between 1904 and the 1930's approximately 150 Saanens were imported into the United States from Switzerland.

Saanens are medium to large in size (weighing approximately 145 lbs/65kg) with rugged bone and plenty of vigor.

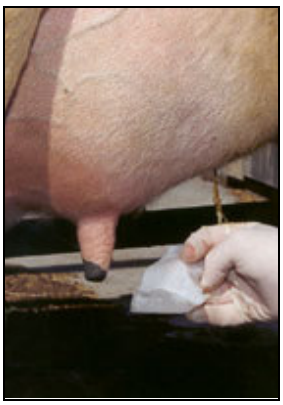
Does should be feminine, and not coarse. Saanens are white or light cream in color, with white preferred. Spots on the skin are not discriminated against. Small spots of color on the hair are

allowable, but not desirable. The hair should be short and fine, although a fringe over the spine and thighs is often present. Ears should be erect and alertly carried, preferably pointing forward. The face should be straight or dished. A tendency toward a roman nose is discriminated against. The breed is sensitive to excessive sunlight and performs best in cooler conditions. The provision of shade is essential and tan skin is preferable.

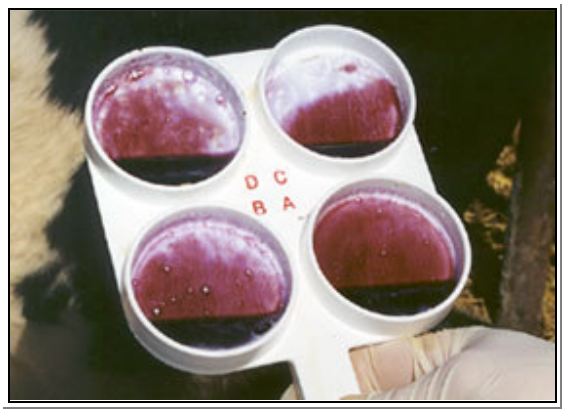

**Plate 4: California Mastitis Test (CMT)**

The California Mastitis Test (CMT) is a rapid, accurate, cow-side test to help determine Somatic Cell Counts (SCC) in a specific cow. The test was developed to sample individual quarters to determine the presence of subclinical mastitis. A cow with subclinical mastitis does not have abnormal looking milk or other clinical signs such as a swollen or painful udder. The test can also be conducted on bucket and bulk tank milk samples to help determine Somatic Cell Counts (SCC) of the entire herd.

**How to Perform the Test:** (See figures 1-5.) A small sample of milk (approximately ½ teaspoon) from each quarter is collected into a plastic paddle that has 4 shallow cups marked A, B, C and D. An equal amount of CMT reagent is added to the milk. The paddle is rotated to mix the contents. In approximately 10 seconds, read the score while continuing to rotate the paddle. Because the reaction disappears within 20 seconds, the test must be read quickly.

| | |
|---|---|
|  |  |
| Clean each teat with alcohol. | Squirt a small amount of milk from each quarter into the appropriate quadrant of the paddle. |

| | |
|---|---|
|  |  |
| 1/2 teaspoon of milk is sufficient. | Mix an equal ratio of reagent to milk. |

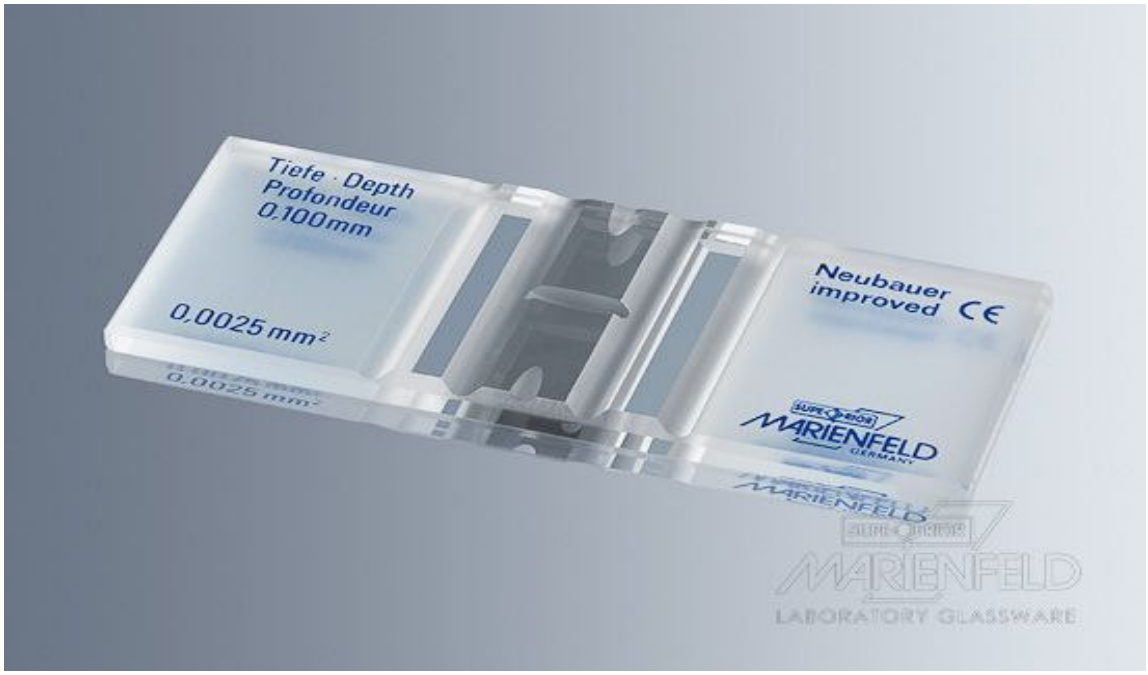



Read and record the results.

**How to Read the Results:** The CMT reagent reacts with the white blood cells and the mixture thickens or gels in proportion to the amount of infection present. To become accurate and consistent, practice this test on cows with a known SCC.

| **CMT Score** | **Average Somatic Count (Cells per milliliter)** | **Description of reaction** |
|---|---|---|
| N (negative) | 100,000 | No thickening, homogeneous. |
| T (trace) | 300,000 | Slight thickening. Reaction disappears in 10 seconds. |
| 1 | 900,000 | Distinct thickening, no gel formation. |
| 2 | 2,700,000 | Thickens immediately, begins to gel, levels in the bottom of cup. |
| 3 | 8,100,000 | Gel is formed, surface elevates, with a central peak above the mass. |

**Treatment:** Refer to the mastitis information found on page F500.

- 

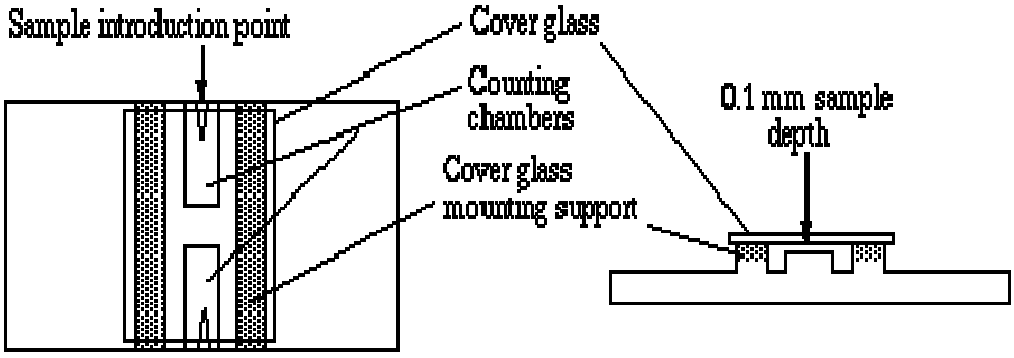
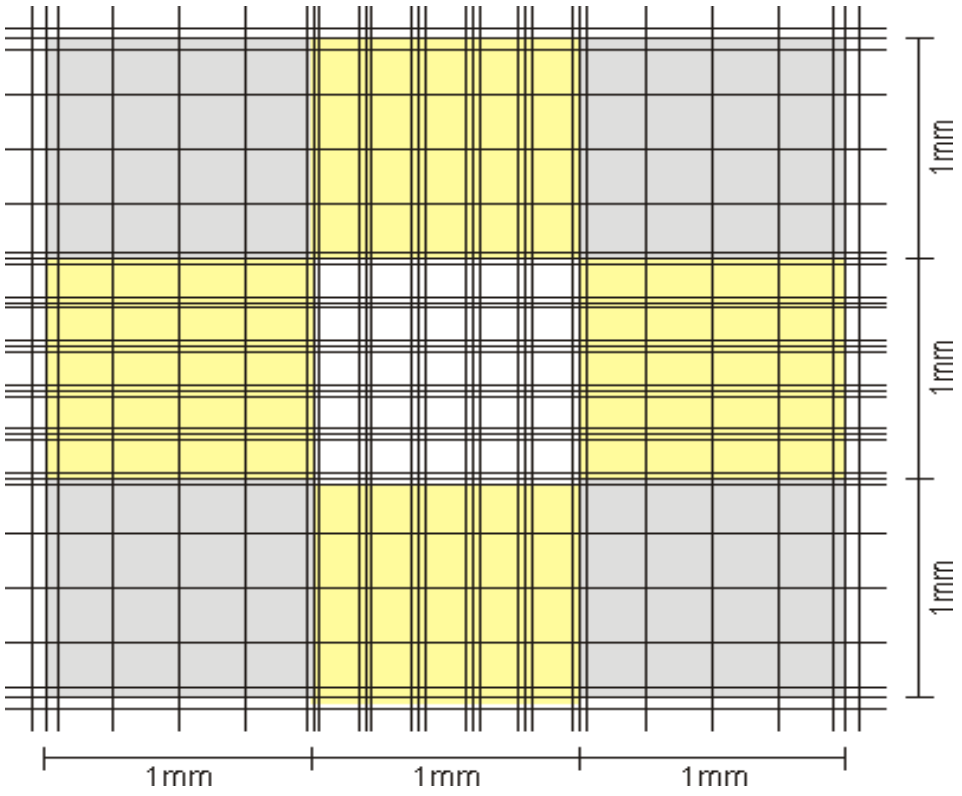
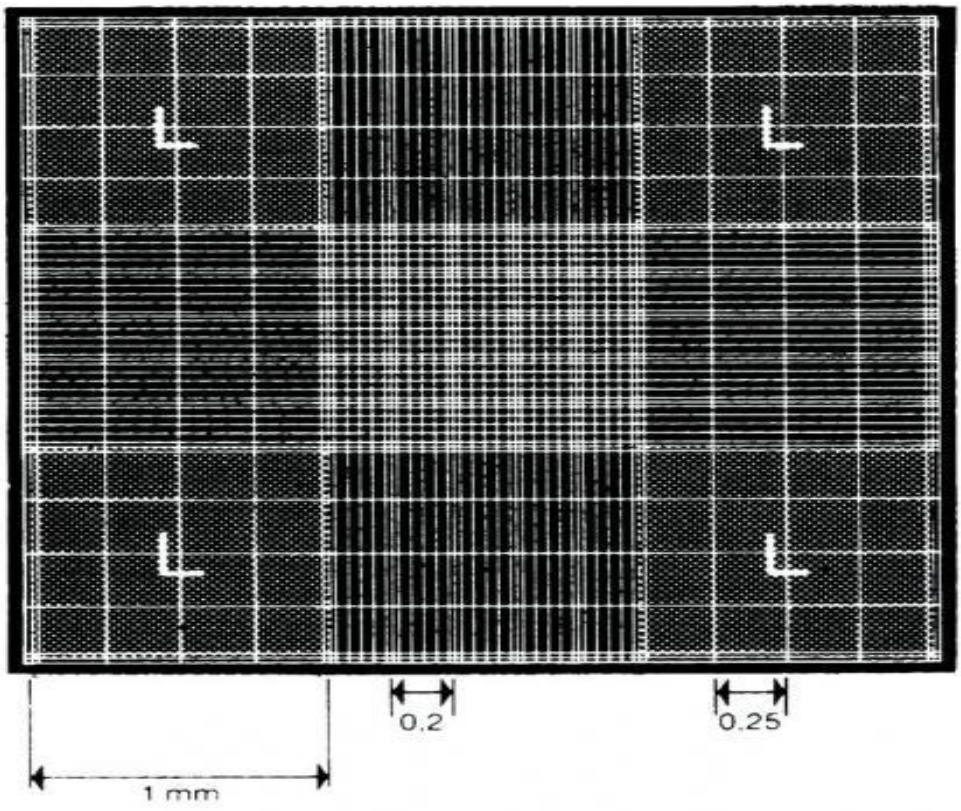
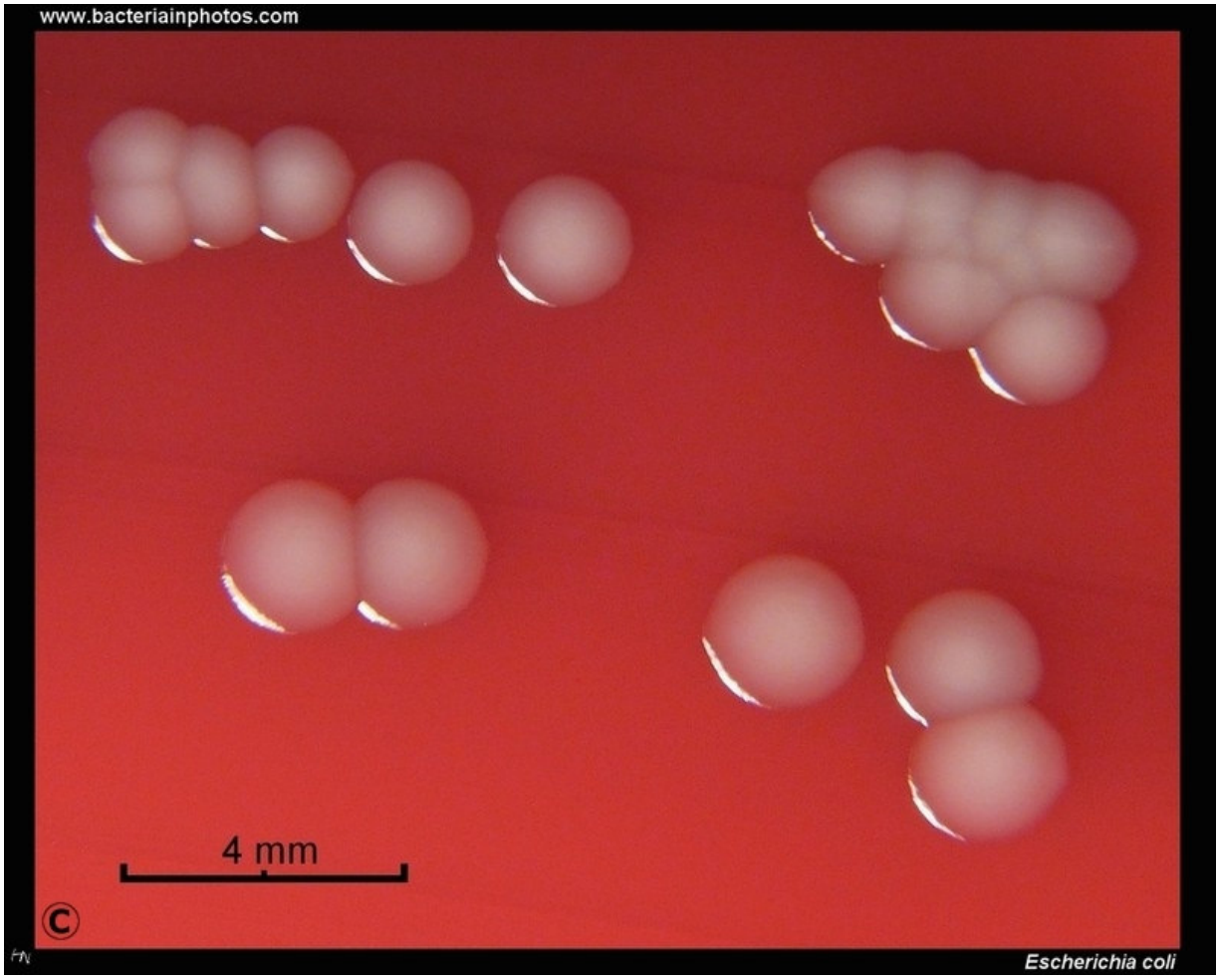




**Plate 5: Improved Neubauer Chamber**


1mm
1mm
1mm
1mm
1mm
1mm


0.2
0.25
1 mm



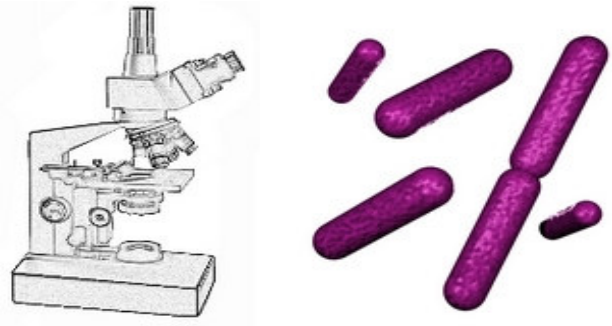



**Plate 6:** ***Escherichia coli***

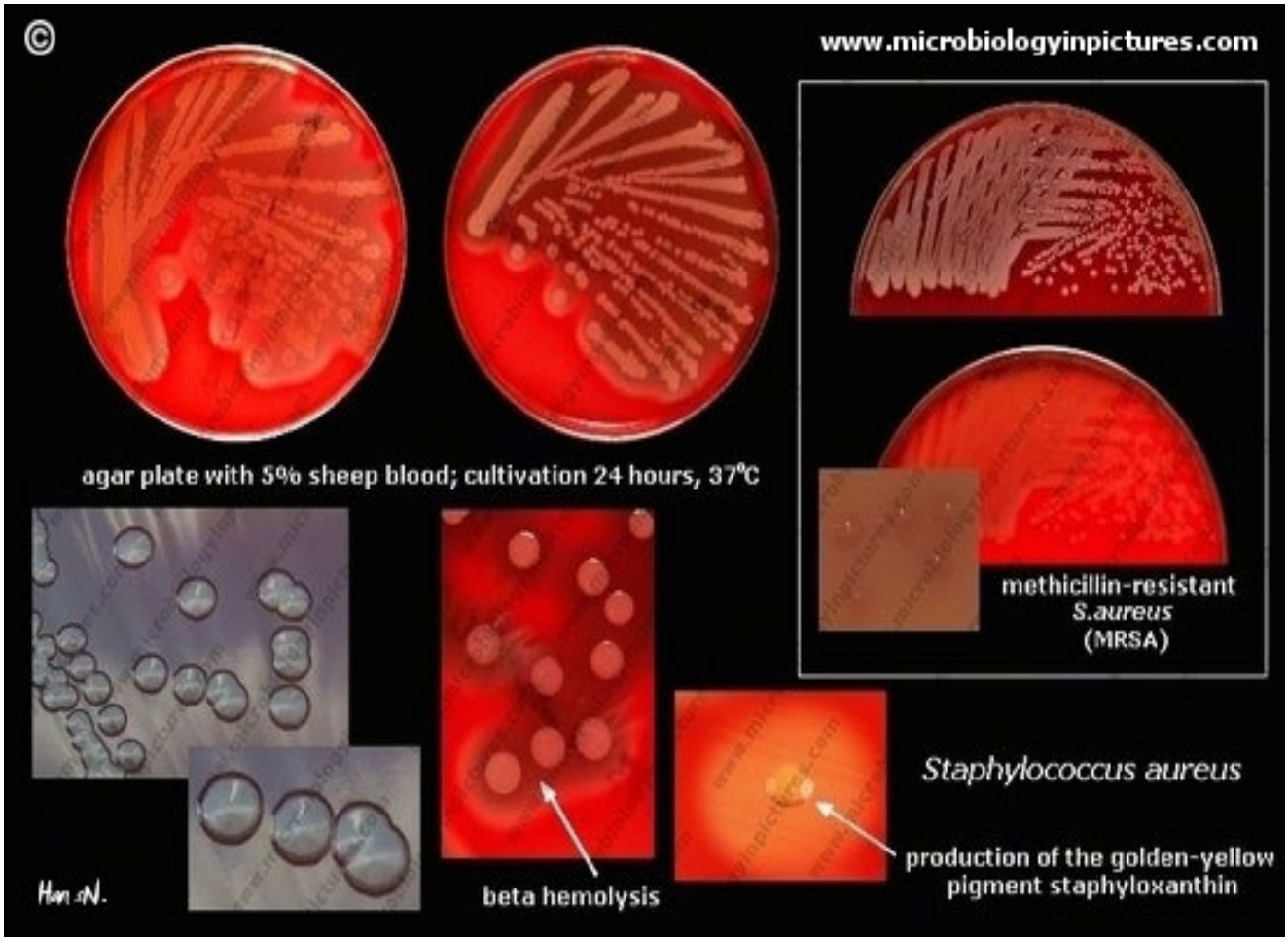



**Plate 7:** ***Staphylococcus aureus***

**Plate 8: Housing Structures**

**Plate 9: Water Sources**

## Appendix 1: Clinical Mastitis Evaluation Form

**Location.....** **Date.....**

**Name.....**

| **Doe ID** | **Kidding Date** | **Parity** | **Date of Clinical Mastitis** | **CMT Result Halves** | | **Treatment Drugs used** | **Dose & Route** | **Days Treated** | **Date milk OK to sell** | **Days milk withheld** |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | LH | RH | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |

| **Clinical Mastitis Cost Estimates per Month** | |
|---|---|
| Treatment Costs | |
| Milk Loss | |
| Cull Loss | |
| Death Loss | |
| Total | |

Total days Treatment.....

Average Treatment Cost.....

Total days Withheld.....

Average Milk Loss..... Milk Price.....

Milk Loss.....

## Appendix 2: Structured Questionnaire

Questionnaire No. .....Date of interview / /

Enumerator Name ..... Code No.....

1. Province Name ..... Code No.....

2. District Name ..... Code No.....

3. Division Name ..... Code No.....

4. Location Name ..... Code No.....

5. Sub-location Name ..... Code No.....

6. Farm type: .....

Small-scale Commercial ..... Large scale commercial .....

Small Scale subsistence .....

7. GPS .....(to be filled in later)

8. Household No. .....

Wealth category | Rich/Medium/ Poor/ Not Classified | (tick one)

**PRODUCTION / MANAGEMENT SYSTEM**

1. **Grazing / Feeding (tick most common)** **Form of housing (tick of present)**

| | Dry | Wet |
|---|---|---|
| 1. Herded ..... | ☐ | ☐ |
| 2. Paddock ..... | ☐ | ☐ |
| 3. Tethered..... | ☐ | ☐ |
| 4. Stall Fed..... | ☐ | ☐ |
| 5. Yarded..... | ☐ | ☐ |
| 6. Free grazing..... | ☐ | ☐ |

7. Local feeds available.....

| | |
|---|---|
| 1. Roof..... | ☐ |
| 2. Solid wall..... | ☐ |
| 3. Floor a) concrete..... | ☐ |
| b) Earth..... | ☐ |
| 8. Open Wall (rails/wire).. | ☐ |

**Housing type full day or overnight (tick most common)**

| | | |
|---|---|---|
| 1. Kraal/Boma | ☐ | ☐ |
| 2.Stall/shed | ☐ | ☐ |
| 3. Yard | ☐ | ☐ |
| 4. None | ☐ | ☐ |
| Other specify | | |
| 5. ..... | ☐ | ☐ |

**cleanliness of housing structure (tick as observed by numerator)**

| | |
|---|---|
| 1. Clean | ☐ |
| 2. Satisfactory | ☐ |
| 3. Unclean | ☐ |

**How they are watered**

| | Dry | Wet |
|---|---|---|
| 1. Animal go to water | ☐ | ☐ |
| 2. Water is provided | ☐ | ☐ |
| 3. Both | ☐ | ☐ |

2. **Materials used for housing (tick one or more)**

1. Untreated wood/bush ☐
2. Treated wood ☐
3. Iron sheets ☐
4. Bricks ☐
5. Mud ☐
6. Wire ☐
7. Thatch (grass, makuti) ☐
8. Other (specify) ☐
9. ..... ☐

**3. Distance to farthest watering point**

| | dry | wet season |
|---|---|---|
| 1. At household | ☐ | ☐ |
| 2. < 1 km | ☐ | ☐ |
| 3. 1 – 5 km | ☐ | ☐ |
| 4. 6 – 10 km | ☐ | ☐ |
| 5. > 10 km | ☐ | ☐ |

**Sources of water**

| **Tick one or more** | **dry season** | **wet season** |
|---|---|---|
| 1. Borehole | ☐ | ☐ |
| 2. Dam /pond | ☐ | ☐ |
| 3. River | ☐ | ☐ |
| 4. Water well | ☐ | ☐ |
| 5. Spring | ☐ | ☐ |
| 6. Municipal /piped | ☐ | ☐ |
| 7. Rain /Roof catchment | ☐ | ☐ |
| 8. Other (specify) | ☐ | ☐ |

**Frequency of watering** (tick one or more)

| | **dry** | **wet** |
|---|---|---|
| 1. Freely available | ☐ | ☐ |
| 2. Once a day | ☐ | ☐ |
| 3. Twice a day | ☐ | ☐ |
| 4. Every other day | ☐ | ☐ |
| 5. Once in 3 days | ☐ | ☐ |
| 6. Other (specify) | | |
| ..... | ☐ | ☐ |

**Water quality (observation by enumerator)**

| | Dry | Wet |
|---|---|---|
| 1. Good /clear | ☐ | ☐ |
| 2. Muddy / not clear | ☐ | ☐ |
| 3. Salty | ☐ | ☐ |
| 4. Smelly | ☐ | ☐ |

**Young Stock Management Household** **Herd Health**

**General Information**

**1. Respondent name..... 2. Household head/chief decision maker's name**

Position in household

1. Household head ☐
2. Spouse of head ☐
3. Brother ☐
4. Sister ☐
5. Son ☐
6. Daughter ☐
7. Manager ☐

Other (specify)

8. ..... ☐

Sex of head Male

Female

Age (yrs) < 30 ☐

31 -40 ☐

41 - 50 ☐

51 - 60 ☐

61 - 70 ☐

> 70 ☐

Not known ☐

**3. Ethnic group of farm owner**

Name .....

Code ☐

**4. Number of people residing in household**

Adult males ☐

Adult females ☐

Children < 15 years ☐

Total ☐

**5. Land holding / farm size**

(Enter X in box first column if not known)

Total size (area) ☐ Acres ☐

Crop ☐ Hectares ☐

Grazing ☐

Forest ☐

Homestead ☐

Other than communal ☐

**6. Land Ownership**

(tick one or more)

Own..... ☐

Lease..... ☐

Other..... ☐

**7. Livestock activity**

Is livestock the major activity on your farm

Yes ☐ No ☐

**8. Livestock kept**

Most important

(enter numbers in first column

species (Rank -3)

| | Numbers | (1, 2, 3) |
|---|---|---|
| 1. Cattle | ☐ | ☐ |
| 2. Sheep | ☐ | ☐ |
| 3. Goats | ☐ | ☐ |
| 4. Chicken | ☐ | ☐ |
| 5. Pigs | ☐ | ☐ |
| 6. Donkeys | ☐ | ☐ |
| 7. Camel | ☐ | ☐ |
| 8. Other specify | | |
| ..... | ☐ | ☐ |

**Sources of income** (tick first column as appropriate, rank level of sources of income in second column – highest)

1. Crop ☐ ☐
2. Livestock and products ☐ ☐
3. Dairy goats ☐ ☐
4. Home industries ☐ ☐
5. Salary / wages ☐ ☐
6. Relative's remittances ☐ ☐
7. Other specify ☐ ☐

**9.Livestock Production Strategy**

(divide numbers given in question 8. into the;)

| | Dairy | Meat | Wool | dual purpose |
|---|---|---|---|---|
| 1. Cattle | ☐ | ☐ | | ☐ |
| 2. Sheep | ☐ | ☐ | ☐ | ☐ |
| 3. Goat | ☐ | ☐ | | ☐ |

**HEALTH**

**1. Access to veterinary / extension services**

(Tick as appropriate)

1. Government veterinarian…… ☐
2. Private Veterinarian …. ☐
3. Veterinary Drug supplier…… ☐
4. Governemnt extension service ☐
5. NGO …… ☐
6. Community Assistant…… ☐
7. None…… ☐
8. Other Specify…… ☐

NAME

**2. Key areas of advice to farmers**

1. Vaccination…… ☐
2. Deworming…… ☐
3. General…… ☐

**3. Number if extension visits initiated by extension agent or arranged by the farmer / course on dairy goats attended in the last 12 months**

| | | | |
|---|---|---|---|
| | 1 month ☐ | | 1 month ☐ |
| visits | 3months ☐ | course | 3 months ☐ |
| | 6 months ☐ | | 6 months ☐ |
| | 1 year ☐ | | 1year ☐ |

**4. Prevalent dairy goat disease that occur on the farm**

(i.e . disease that are seen by the farmer on his animals)

If none tick this box ☐ **Are animals treated when sick?**

**Local name or symptom of disease**

| (rank, most common first) | Code | Yes | No | Treatment given (if known) | code |
|---|---|---|---|---|---|
| 1…… | ☐ | ☐ | ☐ | …….. | ☐ |
| 2…… | ☐ | ☐ | ☐ | …….. | ☐ |
| 3…… | ☐ | ☐ | ☐ | …….. | ☐ |
| 4……..... | ☐ | ☐ | ☐ | …….. | ☐ |
| 5……..…….. | ☐ | ☐ | ☐ | …….. | ☐ |
| 6……..……. | ☐ | ☐ | ☐ | …….. | ☐ |

*(codes to be entered later from list x of diseases and treatments)*

**5. Vaccination / preventive treatments given to the dairy goats**

If none, tick this box ☐

| Local name or symptoms of disease | Code | Done routinely | Done when need arises (*Tick as appropriate)* |
|---|---|---|---|
| …….. | ☐ | ☐ | ☐ |
| …… | ☐ | ☐ | ☐ |
| …… | ☐ | ☐ | ☐ |
| …… | ☐ | ☐ | ☐ |

**6. Ectoparasite control for dairy goats**

| Method | done when need arises | | done routinely | | if done routinely | | specify how often | |
|---|---|---|---|---|---|---|---|---|
| | Dry | Wet (season) | Dry | wet | | | Dry | Wet season |
| 1. None | ☐ | ☐ | ☐ | ☐ | every ☐ | weeks | every ☐ | weeks |
| 2. Dip | ☐ | ☐ | ☐ | ☐ | every ☐ | weeks | every ☐ | weeks |
| 3. Spray | ☐ | ☐ | ☐ | ☐ | every ☐ | weeks | every ☐ | weeks |
| 4. Pour-on | ☐ | ☐ | ☐ | ☐ | every ☐ | weeks | every ☐ | weeks |
| 5.Hand dressing | ☐ | ☐ | ☐ | ☐ | every ☐ | weeks | every ☐ | weeks |
| 6. Injectable | ☐ | ☐ | ☐ | ☐ | every ☐ | weeks | every ☐ | weeks |
| 7.Hand-Picking | ☐ | ☐ | ☐ | ☐ | every ☐ | weeks | every ☐ | weeks |
| 8. Traditional | ☐ | ☐ | ☐ | ☐ | every ☐ | weeks | every ☐ | weeks |

If traditional method specify…… Code ☐

(to be entered from a lost of traditional methods)

9…… ☐ ☐ ☐ ☐ every ☐ weeks every ☐ weeks

**7. Trypanosomias control in dairy goats**

| Method | Done when need arises (Tick) | | Done routinely | | if done routinely specify how often | | | |
|---|---|---|---|---|---|---|---|---|
| | dry | wet season | Dry | wet season | | | Dry | wet season |
| 1.None | ☐ | ☐ | ☐ | ☐ | ☐ | every ☐ weeks | every ☐ | weeks |
| 2.Chemotherapy | ☐ | ☐ | ☐ | ☐ | ☐ | every ☐ weeks | every ☐ | weeks |
| 3.Pour-on | ☐ | ☐ | ☐ | ☐ | ☐ | every ☐ weeks | every ☐ | weeks |
| 4.Traditonal | ☐ | ☐ | ☐ | ☐ | ☐ | every ☐ weeks | every ☐ | weeks |

If traditional method specify……...…… Code ☐ (to be entered from list of traditional methods)

Other (Specify)

5. ……. ☐ ☐ ☐ ☐ ☐ every ☐ weeks every ☐ weeks

**8. Endoparasites control for dairy goats**

| Method | Done when need arises (Tick) | | | Done routinely | | if done routinely specify how often | |
|---|---|---|---|---|---|---|---|
| | dry | wet | | Dry | wet season | Dry | wet season |
| 1.None (n/a) | ☐ | ☐ | ☐ | ☐ | ☐ | every ☐ weeks | every ☐ weeks |
| 2.Drench | ☐ | ☐ | ☐ | ☐ | ☐ | every ☐ weeks | every ☐ weeks |
| 3.Injections | ☐ | ☐ | ☐ | ☐ | ☐ | every ☐ weeks | every ☐ weeks |
| 4.Traditonal | ☐ | ☐ | ☐ | ☐ | ☐ | every ☐ weeks | every ☐ weeks |

If traditional method specify…… Code ☐ ( to be entered from list of traditional methods)

5. ……. ☐ ☐ ☐ ☐ ☐ every ☐ weeks every ☐ weeks

**D. ENTRY / EXIT**

**1. Number of entries within the last 12 months**

Question 4, first ask for information on kids and others ( i.e. weaners and adult total). Then complete individual columns for weaners and adults if known . Enter X in a box if not known , 0 if answer is none.

| | Kids | Weaners | | Adults | | | Total |
|---|---|---|---|---|---|---|---|
| | Males | Females | Males | females | Males | females | W+A |
| 1. Born | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| 2. Bought | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| 3. Donated / gift | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| 4. Exchanged/ Lent | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| Total | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |

Weaners and adults.

** To include bride price and dowry*

If bought, at what age? ☐ ☐ ☐ ☐ ☐ ☐

(in months)

**2. Numbers if exits within last 12 months**

Weaners and adults.

| | Kids | | weaners | | Adults | | Total |
|---|---|---|---|---|---|---|---|
| | Males | Females | Males | females | males | females | W + A |
| 1.Died | | | | | | | |
| 2.Sold | | | | | | | |
| 3.Slaughtered | | | | | | | |
| 4. Donated/ gift | | | | | | | |
| 5.Exchanged / Lent | | | | | | | |
| 6.Stolen | | | | | | | |

**To include bride price and dowry*

**3. Sales outlet (if sold in last 12 months)**

Were animals sold? Yes ☐ No ☐

If yes tick one or more boxes

1. Sold at auction……. ☐
2. Sold to butcher……. ☐
3. Sold privately to other farmer ☐
4. Sold to abattoir……. ☐
5. Other (Specify)……

7. Seasonality in sales
   1. More in wet seasons… ☐
   2. More in dry seasons…. ☐
   3. Always…… ☐

Would goats ever be exchanged for cattle

Yes ☐ No ☐

**4. Use of income from dairy goats (indicate fraction i.e. % within last 12 months**

1. Food purchase for home…….. ☐
2. Farm expenditure…… ☐
3. Hospital medical expenses…….. ☐
4. Social miscellaneous ( e.g donations / gifts) ☐
5. Buying more animals…… ☐

6. Investing (in farm or out)…….. ☐

7. School fees/ education…….. ☐

8. Other (specify…… ☐

Total amount ( Kshs.) ☐

**5. Reasons for culling / disposal**

Ask an open question and tick any answer given in first half of box, one or more boxes to be ticked. Then rank top three by writing in second half of box 1 for primary reason for culling, 2 for second and 3 for third.

| | Males | | Females | |
|---|---|---|---|---|
| | Tick | Rank | Tick | Rank |
| 1. Size……. | ☐ | ☐ | ☐ | ☐ |
| 2. Conformation shape…… | ☐ | ☐ | ☐ | ☐ |
| 3. Colour……. | ☐ | ☐ | ☐ | ☐ |
| 4. Temperament…….. | ☐ | ☐ | ☐ | ☐ |
| 5. Health……. | ☐ | ☐ | ☐ | ☐ |
| 6. Body condition…….. | ☐ | ☐ | ☐ | ☐ |
| 7. Performance…… | ☐ | ☐ | ☐ | ☐ |
| 8. Old age……. | ☐ | ☐ | ☐ | ☐ |
| 9. Poor fertility…….. | ☐ | ☐ | ☐ | ☐ |
| 10. True to breed type……. | ☐ | ☐ | ☐ | ☐ |
| 11. Other specify…… | | | | |
| …… | ☐ | ☐ | ☐ | ☐ |
| …….. | ☐ | ☐ | ☐ | ☐ |

**E. BREEDING**

**BUCKS**

**1. Primary reasons for keeping Bucks**

(tick one)

Breeding…….. ☐

Social-cultural…….. ☐

Capital revenue (hire)…….. ☐

Other (Specify)

…… ☐

**2. Mating**

(Tick one) or more boxes)

1. Uncontrolled…… ☐
2. Hand mating…… ☐
3. Group mating…….. ☐
4. A.I…….. ☐
5. Other specify…….. ☐

Age at first mating (in months)male….

If breeding not done proceed to next page

Ask an open question and tick any reason for choice considered in first half of box, one or more boxes to be ticked. Then rank top three any writing in second half of box 1 for primary reason for choice, 2 for second and 3 for third.

Size…… ☐

Conformation/ shape……. ☐

Color……. ☐

Horns……. ☐

Temperament…… ☐

Performance…….. ☐

Availability……... ☐

True to breed type……. ☐

Other specify

……. ☐

**3. Prolificacy Breeding**

Consider the total number if kids in your flock

Kids…….. ☐

From how many does were they born? ☐

How many of these does had

Singletons……. ☐

Twins…….. ☐

Triplets…… ☐

**4. Source of breed(s) of buck (s) used in the flock**

Breed name(s) ( Specify if known crosses can be included.)

Tick one or more boxes

| | Breed 1 **common name** | | Breed 2 **common name** | |
|---|---|---|---|---|
| | | **Code*** | | **Code*** |
| 1. Own Buck (bred)……. | ☐ …….. | ☐☐ | …… | ☐☐ |
| 2. Own Buck ( bought)…… | ☐ …….. | ☐☐ | …… | ☐☐ |
| 3. Buck donated…….. | ☐ …….. | ☐☐ | …… | ☐☐ |
| 4. Buck borrowed……... | ☐ …….. | ☐☐ | …… | ☐☐ |
| 5. Communal area buck…… | ☐ …….. | ☐☐ | …… | ☐☐ |
| 6. A.I……..… | ☐ …….. | ☐☐ | …… | ☐☐ |
| 7. Project buck……..… | ☐ …….. | ☐☐ | …… | ☐☐ |

Code to be entered from list of breeds-use first box if pure breed, two boxes if buck is a crossed breed)

**5. Number of daily goat breeds ( pure)**

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|

(Tick)

If crossing of two goat breeds has resulted in a genotype that is recognized and maintained as a breed. Then count this as a separate breed and include it on this form. If no pure breeds tick 0 in a box and complete section on mixed crosses form. If more than two pure breeds. Third breed can be entered on mixed crosses form.

**BREED 1 Code** **Origin/ source of dairy goat breed**

(from list of breeds) Inherited…… ☐

Common breed name…….. Communal area farm ☐

Local breed name…… Commercial farm ☐

specify location if known

**Market……** ☐

**6. Trend of dairy goat breed within flock ( tick one)**

Increasing ☐ Decreasing ☐

Stable ☐ Unknown ☐

Number by age and sex of dairy goat breed

(Enter X in box if not known)

quality traits perceived by owner for goat breed 1

(Ask each question and for each traits one box poor, average, good, no opinion/ not important).

| | Kids | Weaners | Adult |
|---|---|---|---|
| Total | ☐ | ☐ | ☐ |
| Intact male | ☐ | ☐ | ☐ |
| Castrate | ☐ | ☐ | ☐ |

**BREED 2** **Code** ☐

(From list of breeds)

**Common breed name…….. 3.Numbers by age and sex of dairy goat breed**

(enter X in box if not known)

**Local breed name……**

| | Kids | Weaners | Adults |
|---|---|---|---|
| Total……. | ☐ | ☐ | ☐ |
| Intact male… | ☐ | ☐ | ☐ |
| Castrate……. | ☐ | ☐ | ☐ |
| Female……. | ☐ | ☐ | ☐ |

Trend of dairy goat breed within flock (tick one)

Increasing ☐ Decreasing ☐

Stable ☐ Unknown ☐

How old is the oldest goat?

Buck ☐ Doe ☐ Year