A comparative study of farm management systems and influence on milk quality in Ngaka Modiri Molema District, North West Province, South Africa

M.L.G. Mutle

orcid.org 0000-0002-1104-7635

Dissertation accepted in fulfilment of the requirements for the degree *Masters of Science in Agriculture in Animal Health* at the North West University

Supervisor: Professor M Mwanza

Co-supervisor: Doctor L Ngoma

Graduation ceremony October 2018

Student number: 22383034

DECLARATION

I, Motlapele Lynette Gomolemo Mutle, declare that the dissertation entitled: "A comparative study of farm management systems and influence on milk quality in Ngaka Modiri Molema District, North West Province, South Africa", hereby submitted for the degree of Master of Science in Agriculture (Animal Health) has not previously been submitted by me for a degree at this or any other university. I further declare that this is my work in design and execution and that all materials contained herein have been duly acknowledged.

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Signature

Name: Motlapele Lynette Gomolemo Mutle

Student number: 22383034

ABSTRACT

Dairy management is an important tool to improve animal health, productivity, quality of milk as well as to reduce the cost of production. The aim of this study was to assess different dairy management systems used by dairy farmers in the North West Province, South Africa and their impact on milk quality and occurrence of mastitis.

To achieve this, seven (7) dairy farms were identified and selected using convenience sampling method in the North West Province, and a structured questionnaire used to assess the knowledge, attitudes, practices and management of the dairies with regard to mastitis in particular. In addition, milk samples were collected from individual animals and from the bulk tanks of each farm for the determination of mastitis. Milk samples (169), quarter (162) and bulk (7) were collected from 7 farms, and from 41 lactating cows and tested for subclinical mastitis (using California mastitis test) and cultured for bacterial isolation and identification. All milk samples were analysed for milk quality and content (fat; solids non-fats; lactose; solids and protein) using a speedy lab test machine, South Africa. Catalase and Indole tests were performed for identification of bacteria and biochemical identification. For confirmation, DNA was extracted, Polymerase chain reaction performed and gel electrophoresis run, followed by sequencing at INQABA. Data analysis was done using the Statistical Package for the Social Sciences (SPSS); Chi-square and Multivariate Analysis of Variance (MANOVA) test to analyse the relationship between results of quarter milk and different aspects of the dairy farm management.

The analysis of data obtained from the questionnaires revealed that all three farming systems (intensive, extensive and semi-extensive) were applied by dairy farmers in the North West Province. There was a significant impact (p-value<0.05) mainly between the quantity of feed given to lactating cows, method used to clean the teats of the cows, and use of antibiotics and occurrence of mastitis, which was tested using the Chi-square test of association. The occurrence of mastitis, which was found using the CMT method, was confirmed through bacterial culture method to overrule false positive results. Biochemical testing separated the culture results between gram positive and gram negative bacteria using the gram staining testing method. However, the indole and catalase testing methods revealed more prevalence of gram positive bacteria.

Results of the molecular test confirmed that *Bacillus* mainly *Bacillus subtilis*; *Bacillus cereus*; *Bacillus spp.* and *Bacillus thuringiensis*, *Serratia* (*Serratia spp.* and *Serratia liquefaciens*), *Enterococcus* (*Enterococcus hirae* and *Enterococcus mundtti*), *Staphylococcus lentus* and *Clostridium spp.* were the agents responsible for mastitis and that they were mostly environmental mastitis causing pathogens.

In conclusion, it was observed that all farming systems had the potential of experiencing environmental mastitis; however, the study revealed that the best advice for management was for farmers to consider a lot of factors in the type of farming system and management they use such as, applying extensive farming system where daily hygiene cannot be prioritized (as cows will move around freely in a large environment instead of being crowded in a small area where they end up getting more contamination from faeces and concrete floors in some farms); routinely testing the herd for mastitis using CMT which is also inexpensive for small scale farmers, and will prevent misdiagnosis and overuse of antibiotics which could create drug resistance when overused; and preventing environmental mastitis by choosing an appropriate type of bedding which should be low in moisture content to prevent the provision of nutrients to bacteria (sawdust, straw and recycled manure are discouraged).

DEDICATION

I dedicate this study to the following:

My grandmother, Mmathapelo Catharine Mutle, for her love, teachings and for investing in the first step of my tertiary education; and

My entire family and friends, I am grateful for your support and prayers.

ACKNOWLEDGEMENTS

First, I wish to thank the Almighty God for answering my prayers. I would not have made it this far without the support of all the members of my family. I am grateful for your prayers and support. I am sincerely grateful to my supervisors for their guidance and for ensuring that I complete my studies.

I am so grateful to my friends who always had their doors open when I needed their assistance (Volition Montshiwa, Raven Motsewabangwe and my fellow Master's students). Last but not least, I thank the North-West University, the National Research Foundation and HWSETA for the financial assistance.

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Abortion: Foetal death.

Anabolism: Chemical reaction that synthesise molecules in metabolism.

Components: A constituent part or ingredient.

Macronutrients: Type of food required in large amounts in a diet.

Osteoporosis: A condition in which bones become brittle and fragile from loss of tissue caused by lack of calcium.

Prophylaxis: Treatment given or action given to prevent a disease.

Safety: The condition of being protected from danger, risk, or injury.

Synthesis: Combination of components or elements to form a connected whole.

Quality: The standard of something as measured against other things of a similar kind; the degree of excellence of something.

LIST OF ABBREVIATIONS AND ACRONYMS

BCS:	Body Condition Score
BLAST:	Basic Local Alignment Search Tool
Ca:	Calcium
Cl:	Chloride
CP:	Crude Protein
CMT:	California Mastitis Test
DNA:	Deoxyribonucleic Acid
E. coli:	*Escherichia coli*
HACCP:	Hazard Analysis and Critical Control Points
ICT:	Information and Communication Technology
Lb:	Libitum
MSA:	Mannitol Salt Agar
NCBI:	National Centre for Biotechnology Information
PCR:	Polymerase Chain Reaction
PDF:	Precision Dairy Farming
SCC:	Somatic Cell Count
Spp.:	Species
SPSS:	Statistical Package for the Social Sciences
UV:	Ultraviolet
VHHM:	Veterinary Herd Health Management
WHO:	World Health Organisation

CHAPTER ONE

Introduction

A good farming management system is what ensures the health of a cow and the quality of milk. It prevents the farmer from losing money due to having a sick cow (infected with mastitis and other diseases). The purpose of dairy farming begins at farm level and expands to national and global levels. Dairy farming provides income to the farmer, provides and improves the nutrition of the farmer and his/her family (Duguma *et al.*, 2011). Moreover, dairy farming creates employment opportunities, alleviates poverty, improves the livelihood of the whole nation, provides food security and is economically viable (Duguma *et al.*, 2011).

The evolution of dairy farming began when humanity searched for a socio-economically feasible and nutritional superior source of food (Park, 2008). Therefore, mammalian species were domesticated and bred to produce large volumes of milk, which became the foundation of the modern dairy industry (Park, 2008). Thus, dairy producers should have the essential capacity to improve management and environmental conditions of a dairy farm to produce greater quality and large volumes of milk (Park, 2008).

Dairy farming is centred on the needs of a dairy cow to be successful. A dairy cow needs to maintain milk production. In order to maintain the production of good quality milk, the heard should be free from diseases (Health, 2011). This can be established by having a vaccination programme (vaccinating cattle), choosing the right breeds that are suitable for the environment or for the farming system in place, demanding a health certificate of animals before purchasing them and quarantining new animals that just arrived in the farm (Health, 2011).

The other need is proper milking of cows. Cows should not be injured when being milked and contamination of milk should be prevented by all means when milking cows (Health, 2011). This can be done by identifying animals that are injured and require special attention and can be milked before or after the rest of the herd is milked, and should be handled with care (Health, 2011). Clean water should be used in the farm and milking machines used properly to prevent injuries, while applying proper udder management (Health, 2011). The

herd should not be starved. Food and water should always be available and animals should be given enough feed according to their needs (Health, 2011). Cows fed with the right quantity of feed will produce the right quantity of milk (Health, 2011).

Proper management of a dairy farm is needed for dairy farmers to be able to produce milk for human consumption, hence it is very important that they must be confident in the safety and quality of the milk they produce (Nada *et al.*, 2012). Most importantly, dairy management will be sustainable if good farming practices are implemented at the level of the farm by using the appropriate farming system (Nada *et al.*, 2012). A good dairy management system maintains animal health, promotes milking hygiene, ensures that dairy animals get nutritional feed and maintains the economy (Health, 2011). Good dairy management sustains work practices, provides good financial return to the farmer and farm workers (Health, 2011).

A good management in the food business is responsible for the safety of products and having to follow the principles of Hazard Analysis and Critical Control Points (HACCP) system (Nada *et al.*, 2012). HACCP is a systematic tool used in the food industry in order to identify, assess and control hazards, focussing on the prevention of identified hazards (Nada *et al.*, 2012). HACCP is needed to promote food safety (Vilar *et al.*, 2012). It also assists in the implementation of biosecurity measures in the farm, ensuring that everyone involved in the production of milk engages in the programme (Vilar *et al.*, 2012).

Below is an example of the hazard and prevention measure to be considered at the farm level.

Table 1.1: Hazard and prevention measure

Stage	Hazard	Preventative measure
Water supply	Introduction of zoonotic pathogens	Water chlorination treatment
Milking equipment	Introduction of zoonotic pathogens	Post-milking management (cleaning walls, floors, drain and disinfecting)
Udder health	Increased bacterial count	Teat dipping and use of clean water

(Vilar *et al.*, 2012)

In addition to dairy management, there are different management systems in place as follows: extensive system/pasture based system (mainly grass-based where cows only feed on grass and spend most of the time outdoors); semi-intensive system/composite system (mixed approach to feeding and housing, cows feed on pasture and also at the farm, do not stay outside all the time like pasture-based cows); and intensive system/high-output system (cows

are usually housed for more months in the year than other systems and usually fed instead of grazing out in the veld) (March *et al.*, 2014).

The importance of milk quality is that milk presents a great medium for growth of many spoilage and pathogenic microorganisms due to its unique composition and properties (Nada *et al.*, 2012). These compositions are high water content, nearly neutral pH and a variety of available essential nutrients (Amentie *et al.*, 2016). The quality of raw milk is the major determinant that influences its quality and safety (Nada *et al.*, 2012). Generally, milk from healthy cows is sterile inside the mammary gland (Nada *et al.*, 2012). The number and type of bacteria that might occur in milk immediately after milking is associated with direct contact with contaminated sources in a farm environment such as air, soil, workers' hygiene, faeces, grass and excretion from the udder of an infected animal (Nada *et al.*, 2012). The other factors that contribute to the contamination of raw milk are equipment used for storage on the farm, during transportation and at a dairy plant level (Nada *et al.*, 2012).

Contamination of milk by bacteria occurs when the cow develops mastitis, which causes physical, chemical and microbiological changes to it and poses a risk for the transmission of major infections such as tuberculosis, brucellosis, leptospirosis and streptococcal sore throat to human beings (Bachaya *et al.*, 2011).

1.1 Problem statement

Small-scale dairy farming is very successful in other countries, however, it is a challenge in places such as the North West Province (Manzana *et al.*, 2014). According to statistics, the North West Province statistics has 257 000 dairy cattle, with the greatest number in the central region (175 235) and smaller numbers in the western (59 852) and east (21 873) regions (Manzana *et al.*, 2014). These cattle produced approximately 230.4 million litres (L) of milk (12.5 percent (%) of nutritional production), with an estimated value of R304.1 million at R1.32/ L, excluding dairy products (Manzana *et al.*, 2014). On average, the Province produced 14 L per cow per day (Manzana *et al.*, 2014).

Manzana *et al.* (2014) observed that emerging black farmers in the North West Province did not have the knowledge or experience needed to run a highly technical farming business (Manzana *et al.*, 2014). These skills are needed in order to redress the inequalities of apartheid, land redistribution and restriction strategies (Manzana *et al.*, 2014). However, it was also noted in the same study that most farmers did not keep records of milk production

(Manzana *et al.*, 2014). Milk production depends on the health of the cow, which relies on the management of the farm. Management of a farm includes feeding, environment and reproduction control. Most emerging farmers, as mentioned above, do not have the skills or do not apply them correctly.

Furthermore, different dairy farming management systems seem to have an influence on the quality of milk and production, thus the purpose of this study is to highlight such relationship. However, a proper dairy farming management system is needed in inland production areas and farmers should not only depend on pasture for good dairy production. There is a need to conduct research on the influence of dairy farming systems on milk quality, compare the different systems and examine the levels of management, biosecurity and occurrence of mastitis in Ngaka Modiri Molema District.

In addition, coastal areas are known to be more suitable areas for milk production because of the temperature. They are also known to produce good quality natural and artificial pastures, while inland production areas such as the North West Province, are generally climatically less favourable for milk production (Department of Agriculture Forestry, 2012). This is largely due to climatic conditions, such as dry land, less rainfall, resulting in lack of good pasture, especially during the winter season (Department of Agriculture Forestry, 2012).

1.2 Hypothesis of the study

The type of dairy management system used by the farmer affects the milk quality.

1.3 Aim and objectives of the study

The aim of the study was to assess different dairy management systems applied by dairy farmers in the North West Province, assess the quality of milk produce and to provide advice on good farming practices.

The objectives of this study were to:

- Assess and compare different farming management systems using a questionnaire;
- Analyse milk samples collected from farms, understand the quality of milk and test for subclinical mastitis; and
- Study the influence of different farming systems on the quality of milk such as content, microbial bacterial contamination and occurrence of mastitis in farms around

Ngaka Modiri Molema District and making a conclusion based on the results found at the laboratory.

CHAPTER TWO

Literature review

Milk is defined as an emulsion of fats in an aqueous solution containing sugar, proteins and crucial electrolytes (Brüssow, 2013). It is known as nature's single most complete food, valuable and regularly consumed, contained with fats, proteins, carbohydrates, minerals, vitamins and other constituents dispersed in water (Duguma & Janssens, 2015). The first food of every mammal is milk, it provides growth and development during the post-natal period (Pereira, 2014). Furthermore, milk consumption is known to end after the weaning period of mammals but in humans, it is ingested even in adulthood (Pereira, 2014).

Cow milk seems to be the most frequently consumed by humans and more needed compared to other mammals since it contains more proteins, calcium and phosphorus (present in organic and inorganic forms) (Brüssow, 2013). It is composed of 87% of water, 4-5% of lactose, 3-4% fat, 0.8% of minerals and 0.1% vitamins (Brüssow, 2013). As a result, dairy foods are included as an important component of a healthy diet due to their balanced and nutritive value (Brüssow, 2013; Pereira, 2014).

Table 2.1: South Africa's dairy breeds

	Holstein-Friesland	Jersey	Guernsey	Ayrshire
Size	Large-framed with a body mass from 550 to 650 kilogram (kg) (bulls often exceed 1000 kg)	It is the smallest, weighing 380 to 450 kg	It is larger than the Jersey but has some similarities of a Jersey. It has an average weight of 450 kg	Medium sized dairy breed weighing 450 to 500 kg.
Special quality	It has a high milk yield of a relatively low butterfat content and easy to milk, which is influenced by the low temperament	It is characterised by extreme leanness with a very good udder, producing yellow milk which has a very high butterfat content. It has the highest protein content of all the breeds. It is also heat-resistant than some breeds and better forager (adapts well into the extensive farming system)	Ease of calving and milk with a high butterfat content	The milk is white with high butterfat content

(Gertenbach, 2017)

Dairy cows have been the predominant domesticated milk producers, however, other animal species such as goats and sheep have been kept for the production of milk, especially in regions of the world where it is difficult for bovines to adapt to the environments (Park, 2008). Milk of other species has also been used as a substitute for bovine milk where health restrictions are concerned, an example is of lactose intolerant individuals (Silanikove *et al.*, 2010). Goat milk contains less lactose than cow milk (Silanikove *et al.*, 2010).

Below is a table of the different contents of milk of different animal species.

Table 2.2: Comparison of milk of different species

Component	Cow	Goat	Sheep	Buffalo	Camel
Fat %	3.67	3.80	7.62	5.25	2.63
Solid-non-fat (SNF) %	9.02	8.68	10.33	8.79	7.12
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Protein %	3.23	2.90	6.21	3.87	2.54
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Casein %	2.63	2.47	5.16	1.1	2.21
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Phosphorus (P) %	0.235	0.270	0.145	1.4	2.4
Chloride (Cl) %	0.105	0.154	0.270	0.8	0.26

(Jandal, 1996); (Kanwal *et al.*, 2004); (Khaskheli *et al.*, 2005); (Han *et al.*, 2007); (Mehaia *et al.*, 1995); (Ahmad *et al.*, 2008); (Kanwal *et al.*, 2004); (www.fao.org, 2017)

2.1 Importance of milk

Milk has so many benefits that have been discovered over the decades, which also include the provision of macronutrients and minerals to pregnant women (Melnik *et al.*, 2015). It represents an endocrine signalling system of mammals activating the key regulator of cell growth and anabolism (Melnik *et al.*, 2015).

The most known function of milk is the provision of calcium, which is the macro element present in higher numbers in milk (Pereira, 2014). Ca's average concentration is 1200 milligram (mg) in a L of milk (Pereira, 2014). It is crucial for the prevention of fractures and bone mass formation, assisting in the prevention of osteoporosis (Pereira, 2014). Osteoporosis is caused by low bone mass, which is dependable from the peak bone mass achieved during growth, hence milk consumption is encouraged for its value in higher bone density, which is protective (Pereira, 2014). The other minerals provided by milk are magnesium (120 mg available in a L of milk), zinc (3-4mg found in a L), selenium and vitamin A (Pereira, 2014).

2.2 Importance of milk quality

Raw quality milk is in high demand for the production of quality dairy products and direct consumption without bringing any health hazard (O'Connell *et al.*, 2016). Good quality milk is compositionally complete (meaning all the fats, proteins and mineral levels are within the norm), free from odours and not flavoured, free from added water and other adulterants (O'Connell *et al.*, 2016). The right milk temperature of milk produced and stored on the farm must be 8 degree Celsius (°C) and cooled below 2 to 4 °C within 2 to 3 hours (as demanded

by processors) (O'Connell *et al.*, 2016). Milk that is of poor quality, can affect the production of dairy products (O'Connell *et al.*, 2016). Moreover, bulk tank somatic cell count (SCC) is usually used as an indicator of milk quality, herd health and management practice in a farm (Murphy *et al.*, 2016).

2.3 Factors affecting milk quality

Quigley *et al.* (2013) state that factors that contribute to milk quality include microorganisms associated with bedding material, which can contaminate the surface of the teat and thus, potentially enter milk. Milking machines can also contain a reservoir of microorganisms, thus the different types of milking influence the microbial population of the milk collected (Quigley *et al.*, 2013). It has been observed that microorganisms present in the milk of a cow also depends on the type of feeding, whether animals are fed indoors or outdoors, with an increase of organisms during outdoor feeding (Quigley *et al.*, 2013).

Raw milk is more likely to have the following pathogens: *Camphylobacter jejuni, Salmonella* species (*spp.*); *staphylococcus aureus; Listeria monocytogenes;* pathogenic *Escheria coli* (*E. coli);* and *Yersinia enterocolitica* (Claeys *et al.*, 2013). Moreover, the common bacteria present in milk colostrum due to contamination of the mammary gland and post-milking contamination are *Mycoplasma spp., Mycobacterium spp., Campylobacter spp., Salmonella spp., Listeria monocytogenes* and *E. coli* (Saalfeld *et al.*, 2016).

2.4 Occurrence of mastitis and zoonotic diseases

The term 'mastitis' which is derived from the Greek word 'mastos', means breast (mammary gland) and 'itis' means inflammation (Haque *et al.*, 2014). Bovine mastitis is characterised by inflammation of the mammary gland (Miranda-Morales, 2012). Mastitis affects milk secretory cells in the mammary gland causing injury (dos Reis *et al.*, 2013) and interferes with synthesis of lactose, fat and protein (dos Reis *et al.*, 2013). Epithelial cells become damaged as a result and are responsible for the synthesis of milk components as well as changes in the enzymatic actin of somatic cells or microorganisms in the infected mammary gland (dos Reis *et al.*, 2013).

Mastitis is multi-factorial due to various factors such as the environment, management of the farm and genetic make-up of the animal (Kaur *et al.*, 2015). The major causes are usually bacteria (Kaur *et al.*, 2015). In farm management, the type of characteristics considered are

feed, type of floor, education of the owner, type of labour and feeding after milking (Kaur *et al.*, 2015).

Mastitis affects the reproduction of lactating badly, as lactating cows that may experience clinical mastitis may not conceive (Santos *et al.*, 2004). It was also discovered that intra-mammary infections arising from subclinical mastitis also cause reproductive problems (Santos *et al.*, 2004). Mastitis increases culling rates, resulting in cows being culled earlier than their expected date (Santos *et al.*, 2004).

Extensive economic loss due to decreased milk yield, lower milk quality, higher treatment costs and premature culling of cows, highlight the importance of the disease (de Pinho Manzi *et al.*, 2012). The pathogens and toxins have a high potential for spread through the sale of unpasteurized milk and dairy products and failure during the pasteurizing process, causing a public health risk (Barrero *et al.*, 2014). The disease is very contagious, one cow can infect up to seven cows that are milked with the same unit (De Bruin, 2015).

2.5.1 Types and causes of mastitis

There are two forms of the disease (clinical and subclinical mastitis) (Schroeder, 2012).

- Clinical mastitis shows visible signs of mastitis (Schroeder, 2012). The signs include flakes or clots in the milk and slight swelling of the infected quarter as mild signs (Schroeder, 2012). Severe signs include abnormal secretion, hot and swollen quarter or udder, fever, rapid pulse, loss of appetite, dehydration and depression, death may also occur (Schroeder, 2012).
- Subclinical mastitis has no visible signs but the SCC of the milk will be increased (an increased SCC levels in raw milk are associated with mastitis) (Schroeder, 2012; Murphy *et al.*, 2016). It is characterised by an increase in milk SCC due to infiltration of neutrophils in the udder in response to inflammatory chemokines (Karthikeyan *et al.*, 2016). Subclinical mastitis is contagious and could spread if not treated and controlled (Haque *et al.*, 2014). If it lasts longer in the udder, it forms a fibrous tissue barrier between organisms and the antibiotic therapy, thus limiting their efficacy (Haque *et al.*, 2014).

  Animal determinants such as parity, stage of lactation, pregnancy status of the animal, milk yield per day and lactation yield are also associated with the occurrence of

subclinical mastitis (Kaur *et al.*, 2015). Coagulase negative *Staphylococci* and *Corynebacterium spp.* are also the most isolated bacteria (Kaur *et al.*, 2015).

The causes of mastitis are either contagious or environmental (de Pinho Manzi *et al.*, 2012). The infected udder serves as a primary reservoir for contagious pathogens, while contamination of animals by environmental pathogens occurs during the milking process (de Pinho Manzi *et al.*, 2012).

Environmental mastitis

The main causes of environmental mastitis are usually Coliform bacteria, which are gram negative bacteria (Hogan & Smith, 2003). Coliform are usually represented by four genres as follows: *Escherichia; Klebsiella; Citrobacter* and *Enterobacter* (Masiello *et al.*, 2016), although more than twenty bacterial genres are classified as coliforms (Masiello *et al.*, 2016). There are a lot of bacteria that can and have been isolated; some of the most frequently isolated include *Serratia*; *Pseudomonas*, and *Proteus* (Hogan & Smith, 2003). *E. coli* are normally found in the gastrointestinal tract of warm-blooded animals, whereas *Enterobacter* and *Klebsiella spp.* are found in water, soil, and grains, and inside the animal (located in the intestinal tract) (Hogan & Smith, 2003).

Environmental mastitis that cause pathogens are everywhere in the cow's environment (Hogan & Smith, 2003). They can be found in dirty beddings, in the soil, in dirty milking machines and in other sources (Hogan & Smith, 2003). They are not only responsible for causing mastitis but can cause other diseases or infections such as respiratory diseases in a cow (Hogan & Smith, 2003). Coliforms are important in the dairy industry because they are often used as hygiene indicators (Masiello *et al.*, 2016). Coliform bacteria that are capable of growing at refrigerated storage temperatures are of great concern for the dairy industry (Masiello *et al.*, 2016).

Contagious mastitis

Contagious mastitis organisms exist in the udder of the cow. These include *Staphylococcus aureus*, *Streptococcus agalactiae* and Mycoplasma *spp.*, (Riekerink *et al.*, 2006). The existence of contagious pathogens is an indication of intra-mammary infections in the herd (Riekerink *et al.*, 2006). Most pathogenic bacteria are gram-positive. Contagious mastitis pathogens usually cause subclinical mastitis (Riekerink *et al.*, 2006).

Staphylococcus aureus is a cause of both clinical and subclinical mastitis in cows, and *Streptococcus agalactiae* causes subclinical mastitis (Riekerink *et al.*, 2006). Both pathogens are gram-positive bacteria that cause an increase in the SCC in milk (Riekerink *et al.*, 2006). Mycoplasma *spp.* cause clinical mastitis and elevated SCC and do not have a cell wall (Riekerink *et al.*, 2006).

2.5.2 Impact of mastitis on productivity

The occurrence of mastitis limits production in the sense that mastitis infected milk is discarded and cannot be used for human consumption since it causes illness in humans (Costa *et al.*, 1998). A farm that has a prevalence of mastitis is more likely to experience other health problems such as intra-mammary infections, which affect production (Gomes & Henriques, 2016).

The use of antibiotics has been increased in farms exposed to mastitis due to the high prevalence of the disease. Unfortunately, some antibiotics lose their importance when overused, creating resistance of the bacteria to them, which also creates an entrance of resistant bacteria into the food chain (Gomes & Henriques, 2016).

2.5.3 Controlling Mastitis

Teat dipping is one of the control programmes against mastitis (Oliver *et al.*, 1991). The advantages of teat dipping are as follows: it is easy to use; affordable; and effective (Oliver *et al.*, 1991). Teat dipping can reduce intra-mammary infections in lactating cows exposed to contagious mastitis (Oliver *et al.*, 1991). However, teat dipping is less effective in environmental pathogens compared to contagious pathogens (Oliver *et al.*, 1991).

Environmental mastitis does not last for a longer period on the skin but reinfection could occur. Proper control is through proper management of stalls and pastures and application of hygiene practices (Hogan & Smith, 2012). The bedding of cattle should be inorganic and not moist, this is to prevent the creation of an environment for bacteria to grow (Hogan & Smith, 2012). The recommended bedding material is washed sand since it is inorganic, and contains fewer mastitis organisms compared to other beddings such as sawdust, straw and manure (Hogan & Smith, 2012).

Vaccinations have also been developed as a solution to overuse of antibiotics, which creates bacterial resistance. However, vaccines are partly effective as they may work towards the prevention of some pathogens and might not be active towards others (Gomes & Henriques,

2016). An example of one of the effective available vaccines is the *E.coli* J5 vaccine, which was derived from J5 mutant strain of *E.coli* (Gomes & Henriques, 2016). The success rate of the vaccine is 70-80% (Gomes & Henriques, 2016).

2.5.4 Mastitis and bacterial detection in milk

- SCC test: Somatic cells are mostly milk-secreting epithelial cells that have been shed from the lining of the gland and white blood cells that have entered the mammary gland in response to injury (Digest, 2009). SCC, which is less than 100 000 cell/millilitre (ml), is normal in a healthy mammary gland whereas the SCC, which is greater than 200 000 cells/ml, is suggestive of bacterial infection (Trajkovska *et al.*, 2015). In South Africa, the bulk milk SCC limit is 500 000 cells/ml and SCC of <200 000 cells/ml is regarded as an indication of an inflammatory response (the quarter is likely to be infected) (Petzer *et al.*, 2017). California mastitis test (CMT) is an indirect method to measure the somatic cells in milk samples (Salvador *et al.*, 2013). The advantage of the test is that it is not expensive, quick and simple to use (Salvador *et al.*, 2013). It determines if milk has subclinical mastitis but does not give preliminary information about the mastitis (Hoque, 2013). CMT was developed to test individual quarter milk samples but it has been used to test bulk milk samples as well (Hoque, 2013). Fresh unrefrigerated milk can be tested within 12 hours post-milking and refrigerated milk samples can be tested within 36 hours (Hoque, 2013). Results should be seen within 15 seconds of mixing with the reagent because weak reactions disappear after that time (Hoque, 2013). However, the results of CMT are not affected by external factors (Tabidi *et al.*, 2013).

- Bacteriological culture test: The standard method used to identify intra-mammary infections is bacteriological culture (Hoque, 2013). However, due to its financial expenses, the technique has not been widely adopted (Hoque, 2013). Most mastitis control programmes include the use of individual cow cultures to determine which mastitis pathogens are present on a farm (Hoque, 2013). Culturing can be used for surveillance to detect the presence of new or emerging pathogens and to evaluate treatment efficacy and to establish susceptibility patterns to aid in the development of rational treatment strategies (Hoque, 2013).

- Catalase test: In the catalase test, hydrogen peroxide is broken down to water and oxygen. The test differentiates gram positive organisms (Taylor & Achanzar, 1972). It is known to be simple, rapid, and surprisingly accurate, and used as an aid in the identification of certain genera of the *Enterobacteriaceae* (Taylor & Achanzar, 1972). It requires applicability and accuracy (Taylor & Achanzar, 1972).

- Polymerase chain reaction (PCR) test: The test is a molecular method implemented to improve the sensitivity of intra-mammary pathogen detection, in the diagnostics of mastitis (Katholm *et al.*, 2012). In the diagnostics of PCR, all target bacterial species are detected (Hiitiö *et al.*, 2016). PCR-based techniques rely on sequence-specific amplification of nucleic acids (Pulido *et al.*, 2013). From its development, it was initially used for rapid identification of causative agents of sequences specific to a particular pathogen (Pulido *et al.*, 2013). PCR detects the presence of genetic determinants (Pulido *et al.*, 2013).

2.5.5 Management of mastitis

Selective breeding for mastitis-resistant animals is one of the mastitis management practices, producing mastitis-resistant herd (Karthikeyan *et al.*, 2016). However, it is hard to improve conventional breeding due to the polygenetic nature of mastitis-resistant traits, which negatively correlates with production traits (Karthikeyan *et al.*, 2016).

It is believed that pasteurizing milk reduces and protects it from contamination and many bacteria (Masiello *et al.*, 2016). However, the reality is that preventing post-pasteurization contamination from microorganisms remains a major challenge (Masiello *et al.*, 2016). *Pseudomonas spp.* and coliform bacteria are frequently isolated from pasteurized fluid milk (Masiello *et al.*, 2016). Coliforms are defined as aerobic or facultatively anaerobic, gram-negative, non-spore rods capable of fermenting lactose, resulting in gas and acid production within forty eight hours at 35 °C (Masiello *et al.*, 2016).

Antimicrobial use for the management of mastitis

Sensitivity test is important in assessing whether resistant bacteria pose a threat to current mastitis treatment procedure (Aquino, 2015). A microbiological diagnosis, consisting of identification of the causative agent of infection and antibiotic resistance profile, can take between 24 and 72 hours (Pulido *et al.*, 2013). The uses of methods that identify antibiotic resistance, reduce the duration of empirical therapy and facilitate the early initiation of

targeted treatment (Pulido *et al.*, 2013). The rapid availability of results of antimicrobial susceptibility test improves patient outcomes, decreases mortalities of bacterial infections and reduces healthcare costs (Pulido *et al.*, 2013).

The most common antibiotics, which mastitis develops resistance to, are Erythromycin; Tetracycline and Methicillin, especially in dairy herds (Cengiz *et al.*, 2015). It has been found that the rates of multiple resistant strains have variability between herds and countries (Cengiz *et al.*, 2015). However, staphylococcal strains have been reported in most studies to be susceptible to Erythromycin; Tetracycline and Methicillin, especially in dairy herds (Cengiz *et al.*, 2015). It has been found that the rates of multiple resistant strains have variability between herds and countries (Cengiz *et al.*, 2015). Staphylococcal strains have been reported almost in every study (Cengiz *et al.*, 2015).

The attention to factors that potentially contribute to antibiotic resistance is increasing (Pruden *et al.*, 2013). The emergence of antimicrobial resistance has been listed as a complex problem by the World Health Organisation (WHO), which is driven by many interconnected factors (Pruden *et al.*, 2013). The common use of broad-spectrum antibiotics seems to be increasing (Saini *et al.*, 2013). In most cases, broad-spectrum antimicrobials are used to treat coliform mastitis (Saini *et al.*, 2013). The most commonly used antibiotics for the treatment of systemic signs due to coliform mastitis in lactating dairy cattle are Oxytetracycline; Ceftiofur; Ampicillin and Amoxicillin (Saini *et al.*, 2013).

2.5.6 Mastitis and zoonosis

Subclinical mastitis might be the major concern for human health due to its hidden signs, however, there are a number of conditions and diseases caused by bovine milk (Garedew *et al.*, 2012). The rich nutritional contents of milk and the production and processing procedures are issues that make humans to be susceptible to contaminations that cause the diseases (Garedew *et al.*, 2012). Bacteria found in milk also cause respiratory illness such as pneumonia in humans and streptococcal sore throat (Tabidi *et al.*, 2013).

Table 2.3: Zoonotic diseases found in milk

Disease	Cause	Clinical signs	Control
Tuberculosis	*Mycobacterium tuberculosis*	Reduced production of livestock through reduction of milk production and carcass condemnation in cattle (Kidane *et al.*, 2015)	Test and slaughter of positive animals (not for consumption) (Musoke *et al.*, 2015)
Contagious abortus	*Brucella abortus*	Abortion, reduced milk yield, and infertility in wild and domestic animals. Undulant fever and headaches in humans (Njeru *et al.*, 2016)	There is no vaccine for humans, only animal vaccine is available. Therefore, milk pasteurization and other food hygiene measures are the only options of reducing its occurrence in humans (Ducrotoy *et al.*, 2017)
Q fever	*Cociella burnetti* heat-resistant milk-borne zoonotic pathogen (Claeys *et al.*, 2013)	Pneumonia and endocarditis in humans and if the disease is severe, it can lead to mortality (Mattar *et al.*, 2014; Gale *et al.*, 2015)	Immunological assays are available for control but only in critical situations (Lucchese *et al.*, 2015)

2.6 Dairy management

The different types of dairy management systems are: the intensive system; semi-intensive system; and extensive system. Intensive farming system does not always guarantee good welfare as it may reduce productivity since farmers feed animals directly and may reduce feed as a way of economising (Polikarpus *et al.*, 2015). However, cows bred in an intensive indoor system, get fodder of good nutritive value and high supplies of concentrates, which enable them to produce milk rich in protein and relatively low in fat (Morand-Fehr *et al.*, 2007).

The semi-intensive dairy system involves keeping cows indoors after milking them in the afternoon and releasing them in the morning after milking (Hernández *et al.*, 2017). During the period when cows are housed, they receive supplemental feeding (Hernández *et al.*, 2017). The advantage of the system is that it prevents overgrazing because of the balance between natural grazing and concentrate feeding and also saves costs compared to the intensive system. However, due to poor climate change and lack of pasture, cattle might suffer due to lack of adequate nutrients, which is a disadvantage.

In the extensive system, it has been discovered that pasture is suitable for grazing animals, which mainly refers to land covered with grass and other low lying plants (herbage) (O'Driscoll *et al.*, 2015). The system is most efficient when there is maximum grass allowing the farmer to spend less on feed costs (O'Driscoll *et al.*, 2015). The farming system also

produces milk that is rich in fats and in micro-components which are beneficial to human health (Morand-Fehr *et al.*, 2007). However, herbage growth can be low or extremely variable as supply is dependent on prevailing climatic conditions (O'Driscoll *et al.*, 2015). Thus, in times of lack or no rainfall, cows will suffer (O'Driscoll *et al.*, 2015).

Figure 2.1: A farm in Ramatlabama, Ngaka Modiri Molema, making use of a semi-intensive farming system

A good dairy management system improves the living standards (considering the inherent value of a cow), improves the nutritional value associated with milk produced and brings diversity in farming activities (Ndungu *et al.*, 2016). It is expected that by 2025, the demand for milk and dairy products in developing countries would have increased by twenty five percent (Ndungu *et al.*, 2016). The cause of this increase is human population growth, urbanisation, increased disposable income, greater diversity of food products to meet nutritional needs, and increased opportunities for domestic and external trade (Ndungu *et al.*, 2016).

With regard to successful dairy farming, sustainability is used as an indicator of quality (de Ondarza & Tricarico, 2017). Sustainability should return a profit, produce quality milk for consumption, and maintain optimal cow well-being (de Ondarza & Tricarico, 2017). The environment where dairy animals are housed also plays a huge part in animal health and milk quality (de Pinho Manzi *et al.*, 2012).

2.6.1 Nutrition

Nutritional deficits in early lactation have a negative impact on production performance and on animal welfare due to increased feelings of hunger, thus causing risk of health disorders (O'Driscoll *et al.*, 2015). Although extensive grazing is often perceived as being beneficial for cows, due to freedom of movement, the system could pose a challenge to cow welfare if demand for herbage exceeds supply (O'Driscoll *et al.*, 2015). Cows are likely to lose condition in early lactation when fed below their nutritional needs (O'Driscoll *et al.*, 2015). Low feed allowance reduces body condition score (BCS). Therefore, the indoor system has been encouraged a lot in dairy farms (O'Driscoll *et al.*, 2015).

The grazing dairy systems has led to a change of dairy management systems by increasing the use of supplements and the use of total mixed rations (Fajardo *et al.*, 2015). This particularly happens when imbalances between grass production and herd requirements occur through the course of the year (Fajardo *et al.*, 2015). Increased mixed rations result in higher levels of milk production where cows fed 100 % mixed ration, could produce 49 % more milk than cows grazed without supplements (Fajardo *et al.*, 2015). However, cows offered mixed ration ad libitum (Lb), increased milk by 30 % compared to grazing cows provided 8.5 kg dry matter of mixed ration per day and ample herbage allowance (Fajardo *et al.*, 2015). The grazing behaviour of lactating cows influences production responses in terms of feeding strategies based on a combination of herbage and mixed ration (Fajardo *et al.*, 2015). It has been proved that lactating cows during the first three weeks of lactation, spend relatively low proportion of time grazing while at pasture, irrespective of the herbage allowance provided hence, it is highlighted that the diet of cows of different ages and production stages cannot be the same (Fajardo *et al.*, 2015).

Nutritional requirements of cattle of different stages of production

Table 2.4: Nutritional requirements of lactating cows

Lactating cows				
Body Weight	Dry-Matter Intake, pound by weight (lb)	Lb Per Animal Per Day		
		CP %	Ca %	P %
1100	26.2	2.75	0.08	0.05
1200	27.6	2.82	0.08	0.06
1400	30.4	3.01	0.09	0.06

(Smith Thomas, 2017)

Table 2.5: Nutritional requirements of pregnant cows

Pregnant cows				
Estimated mature weight	Dry-matter Intake (lb)	Lb Per Animal per day		
		CP %	Ca%	P%
1100	18.5	1.33	0.041	0.032
1200	19.8	1.42	0.046	0.036
1300	21.0	1.52	0.049	0.038
1400	22.3	1.61	0.054	0.040

(Smith Thomas, 2017)

Table 2.6: Nutritional requirements of breeding bulls

Bulls				
Weight	Dry-matter Intake, (lb)	Lb Per Animal Per Day		
		CP %	Ca %	P %
1300	30.7	1.85	0.057	0.039
1400	32.4	1.88	0.057	0.039
1500	34.1	1.92	0.058	0.040
1600	35.8	1.95	0.059	0.041

(Smith Thomas, 2017)

2.6.2 Biosecurity on the farm

Biosecurity is the management system implemented to reduce the risk of introducing infectious diseases to a herd, and is the cornerstone of disease control, suitably designed with demographically relevant education programmes, required to ensure optimal farmer participation in its implementation (Sayers *et al.*, 2013). The production of high quality raw milk on farms is the main requirement for production of quality dairy products (Nada *et al.*, 2012). Sanitation and udder hygiene also play a role in the production of quality milk (Khalil *et al.*, 2013).

Biosecurity dairy-related diseases of humans result from contaminated milk, which amount to ninety percent (Duguma & Janssens, 2015). Unfortunately, most farmers are not well informed about milk-borne zoonotic diseases (Duguma & Janssens, 2015). The other challenge of preventing many diseases arises from the need of education (Van der Leek, 2015). Available information on the reduction and prevention of the disease is not enough and the problem is in the implementation of the management system (Van der Leek, 2015).

In order to translate emerging knowledge into the farm and to prevent problems, knowledge and understanding of the farm as an integrated system is needed (Van der Leek, 2015). Educating and motivating farmers is necessary in order to implement well designed practices, doing so is both an advance and an ongoing challenge (Van der Leek, 2015). Unfortunately,

in a large dairy business, cow care is usually delegated to employees while the owner is busy with employee management, feed purchases, manure management and farming enterprise. However, the very employees have little or no knowledge of diseases and proper management even if they have more contact with the animals and farm at large (Van der Leek, 2015).

2.6.3 Types of milking methods

There are two most common methods of milking as follows: hand milking; and machine milking (Rajkumar, 2009).

- **Hand milking**

  Hand milking is not encouraged among farmers because of its disadvantages as milkers tend to moisten their fingers with milk, water or even saliva while milking, especially in rural areas (which causes contamination) (Rajkumar, 2009). The advantage of the method is that it is less expensive and makes it easy to put a cow in a milking routine as she considers the milker's hands as one of her calves (cowseatgrass.com, 2014).

- **Machine milking**

  The advantages of milking machines are that they are quick and cause less injury to the udder if used properly (Rajkumar, 2009). However, the disadvantage of machine milking is the cost related to buying it, and the requirement for more cleaning during milking (cleaning of the machine and the teats of the cow) (cowseatgrass.com, 2014). Electricity and proper milking infrastructure are needed for this type of milking method (cowseatgrass.com, 2014).

Figure 2.2: A view of a milking parlour of one of the farms in the District

2.6.4 Milk pasteurization

There is a perception that heating milk destroys nutritional and health benefits and induces some detrimental effects, however, the consumption of raw milk has not been well documented (Claeys *et al.*, 2013). Furthermore, milk is a growth medium and if untreated, more microorganisms will continue to grow in it due to its temperature (Claeys *et al.*, 2013). As a result, to guarantee milk quality that is safe for consumption, milk is heat treated (Claeys *et al.*, 2013). It has been documented that milk pasteurization has a positive history on public health. Before 1938, an estimated 25% of all food-borne and water-borne diseases were associated with milk, while nowadays, the percentage of such outbreaks has reduced due to milk pasteurization (Claeys *et al.*, 2013).

Moreover, it is fully demonstrated that the consumption of raw milk poses a threat because of its possible contamination with pathogenic bacteria (Claeys *et al.*, 2013). However, thermal treatment has been the most frequent and effective method used to increase the microbial safety of milk without substantially changing the nutritional value of milk (Claeys *et al.*, 2013).

Table 2.7: Summary of dairy management

Feed/Nutrition	Biosecurity and hygiene	Knowledge	Treatment
Nutritional management is measured using BCS (which is a 5-point scale). 98% of lactating cows must be between 2.0 and 4.5 and 99% of the cows and must have a BCS that exceeds 2.0.	Biosecurity measures should be in place for new animals entering the herd, which should include action plans (Bergman *et al.*, 2014). The level of microbes in milk is an indication of poor handling from the time of milking to time of consumption. The influential factors are health and hygiene of cows, the environment, cleanness of storage and transport equipment, milk holding temperature, cleanness of water used for hygiene practice, and health and personal hygiene of milkers and milk handlers.	The first step for a successful control programme is knowledge about the diseases and understanding prevailing risk factors. It is important for a farm to have training programmes for animal caretakers because they spend most time at the farm and their knowledge will remedy existing management problems.	A herd plan is required which must include vaccinations and treatment protocols, as well as mastitis control and monitoring of herd performance.

(Bergman *et al.*, 2014); (Amentie *et al.*, 2016)

2.7 Previous studies on dairy management and milk testing

Dairy management has been reaching new developments year after year. One of the dairy management developments is Veterinary herd health management (VHHM), which was developed in the 1990s and has been effective in the past, although new developments are influencing the dairy industry at present due to consequences of high susceptibility to diseases (Derks *et al.*, 2012). The changes have been influenced by urbanisation, which provokes the demand for milk and milk products, that are influenced by increases in capital income and population growth (Duguma *et al.*, 2011). This has also influenced other developments such as selective breeding of high producing cows, influenced by industrialisation and increased international competition (Derks *et al.*, 2012). As a result, small-scale urban dairy production is expanding to improve the livelihoods of farmers through family income and creating employment, achieving food security and poverty alleviation, and also improving the nutritional status of milk (Duguma *et al.*, 2011).

In addition to the developments, in Australia, the use of information and communication technology (ICT) and herd and pasture management decision support systems is increasing (Eastwood *et al.*, 2012). The practice of precision dairy farming (PDF) has evolved (Eastwood *et al.*, 2012). This system shows detailed data recordings about sub-units of the

farming system, such as cows and fields and is used to exert more management control over the system (Eastwood *et al.*, 2012).

Islam *et al.* (2012) also conducted a study on the prevalence of subclinical mastitis in dairy cows in selected areas of Bangladesh. The researchers used three tests in the study (the White Side Test, Surf Field Mastitis Test and CMT). Crossbred dairy cows used in the study were managed under the semi-intensive husbandry systems at the Bangladesh Agricultural University Dairy Farm (provided with green grass in addition to natural pasture and concentrate diet) (Islam *et al.*, 2012).

A questionnaire was used to process data and the parameters used for the study were age, breed, number of parity lactation stage and the production of milk per day (Islam *et al.*, 2012). The statistical analysis of data collected was done using the Statistical Package for the Social Sciences (SPSS) version 11.5 (Islam *et al.*, 2012). The study is similar to this one in that the parameters used focused more on measuring the management systems of farms and SPSS to process the data.

CHAPTER THREE

Materials and methods

3.1 Study area

This study was conducted in selected dairy farms around Ngaka Modiri Molema District, North West Province, Republic of South Africa (Figure 3.1).

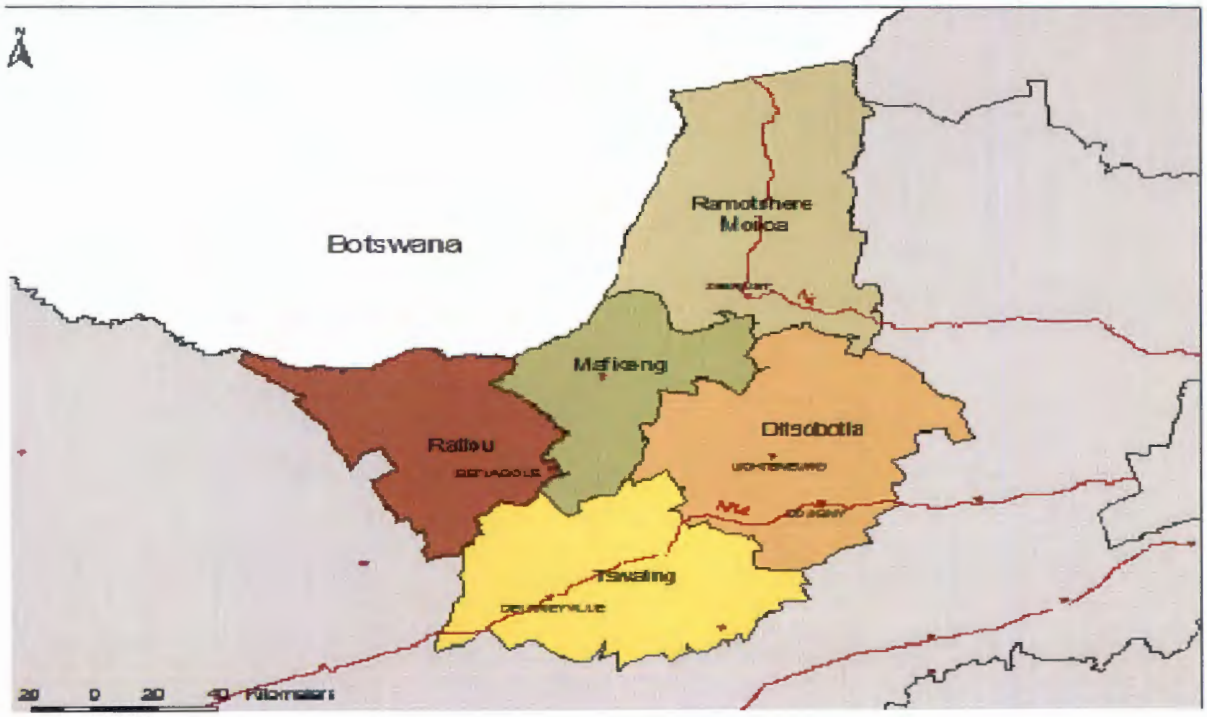

Figure 3.1: Map of Ngaka Modiri Molema District, North-West Province
(Adegboye & Babalola, 2013)

Ngaka Modiri Molema District is one of the four districts of the North West Province, South Africa. The climate is dry with less rainfall, resulting in lack of good pasture, especially during the winter season (https://en.wikipedia.org, 2017).

In order to evaluate farmers' knowledge, attitudes and farming practices, a questionnaire (7) was distributed to each farm (farmers were interviewed) and milk samples collected (see sampling) in different farms identified for the study.

3.2 Sampling

In this study, 169 milk samples were collected from different quarters (162) and bulk tanks in seven dairy farms within Ngaka Modiri Molema District, North West Province, South Africa. The type of sampling used was convenience sampling because dairy farms in the district are not documented, as a results all the farmers that agreed to take part in the study participated. 100 ml milk containers were used to collect the milk and properly labelled and stored in ice immediately after collection. The collection was done from May to June 2015 (samples were only collected once) from all the lactating cows of each farm. Non lactating cows were excluded because mastitis can only be tested from milk as a result, only lactating animals were used. Hand milking and automatic machine were used to collect the milk depending on the farm. There were no special or extra biosecurity measures applied, farmers where allowed to collect milk using their usual method of collection. After collection, samples were transported to the North-West University, Mafikeng Campus (Animal Health Research Laboratory) in a cooler box, where they were stored in a cold room on the point of arrival after performing the CMT and milk quality test, to analyse milk content, fat; SNF; density; lactose; solids and protein as well as to evaluate the quality.

Table 3.1: Summary of total number of samples used

	Number of animals	Total number of samples (quarter and bulk samples)
Farm 1	7	29
Farm 2	5	20
Farm 3	8	32
Farm 4	4	17
Farm 5	5	21
Farm 6	5	21
Farm 7	7	29

(In some cows, only three quarter samples were collected since there was prevalence of clinical mastitis in the fourth quarter. However, there was one clinical mastitis quarter in farms 2 and 3).

3.3 Sampling analysis

In this study, samples were analysed for the following reasons:

- For milk content using a milk quality test; and
- For bacterial contamination and mastitis (using CMT, then further testing CMT positive results using bacteriological culture, biochemical tests to identify bacteria

using catalase, and indole. PCR was used to confirm type of bacteria from Deoxyribonucleic acid (DNA) extracted).

3.3.1 Analysis of quality of milk

The test was used to analyse the quality of milk taking into consideration the content, fat; SNF; lactose; solids and protein using a speedy lab test machine (South Africa) that read the results after consuming milk samples.

3.3.2 California mastitis test

CMT is a screening test that determines the prevalence of subclinical mastitis. It was performed as described by Hogan (1999) and Quinn *et al.*, (2004). A squirt of milk, about 2ml from each milk container, was placed in each of 2 shallow cups in the CMT paddle. An equal amount of the commercial CMT reagent was added to each cup. A gentle circular motion was applied to the mixture in a horizontal plane for 15 seconds. Based on the thickness of the gel formed by CMT reagent milk mixture, test results were scored as 0 (negative/trace), +1 (positive). Milk samples mixed with the reagent, revealed absence of gel formation or clots and were regarded as being negative, while the rest were positive. Positive samples were stored in a cold room for further bacteriological culture test to further identify the causative agent after testing the quality of milk (Hogan, 1999); (Quinn, 2004) .

3.3.3 Bacteriological culture and identification

a) Bacterial culture

Media preparation

In this study, Mannitol Salt Agar (MSA) and MacConkey agar were used and prepared according to the manufacturer's instructions.

Bacteriological culture test was used for positive milk samples post-CMT. MacConkey agar was used to grow gram negative bacteria while MSA was used for the growth of gram positive bacteria. 50 grams (g) of MacConkey agar were suspended in 1 L of distilled water and 111 g of MSA suspended in the same amount of distilled water. Both media were then placed in an autoclave for an hour at a temperature of 121 °C for sterilisation. The media were left to cool down after sterilisation, before being poured into petri dishes.

The media in the petri dishes had to solidify before culturing milk samples. During culturing, 70% alcohol was used to disinfect the working area. The Bunsen burner was switched on in

order to prevent the contamination of samples by microorganisms from the environment. A wire loop was used and disinfected with the flame from the Bunsen burner to transfer the sample into petri dishes. Each sample was placed in both media and later placed in an incubator for 24 hours. They were then observed under a microscope to observe the morphologies.

b) Bacteriological identification

b.1) Biochemical identification

b.1.1) Gram staining

Gram staining was done by taking single colonies of each and every sample using a wire loop and placing them on a microscope slide in a disinfected area. The wire loop was sterilised by flame after taking colonies from each and every sample. The bacteria were fixed on the slide by moving the slide on the flame (produced by the Bunsen burner). The slides were then dipped into solutions. The first solution was crystal violet, which makes gram positive bacteria visible. After dipping the slide into the crystal violet solution for 30 seconds, it was then rinsed with water. The slide was later dipped into lungol iodine for 10 seconds and rinsed with water and alcohol. The slide was also dipped into Safranin fuchsin for 30 seconds, since the solution makes gram negative bacteria to be visible. All the bacteria became visible under a microscope. A microscope was used at ×100 with immersion oil (Haque *et al.*, 2014).

b.1.2) Catalase test

A catalase test was performed according to Haque *et al.* (2014). This was done by preparing hydrogen peroxide, which produces bubbles to show if the test is positive or negative. The bubbling was due to the evolution of oxygen gas. Any cell that uses oxygen or can live in the presence of oxygen, must have a way to get rid of the peroxide by making a catalase hence, positive results (through the presence of bubbles) show that there is a living organism in a colony.

A colony from an MSA culture (since Catalase test identifies Gram positive bacteria) was placed onto a clean microscope slide using a wire loop. Few drops (2 drops) of hydrogen peroxide were added onto the smear with a pipette and mixed with cotton less side of a swab to avoid false positive results. The rapid evolution of oxygen was evident through bubbling for positive results while negative results had no bubbles (Haque *et al.*, 2014).

b.1.3) Indole test

This method was also performed according to Haque *et al.* (2014). The test required the preparation of Nutrient broth first. 25 g of Nutrient broth was mixed in 1L of distilled water and autoclaved for an hour. After autoclaving, colonies from samples were inoculated in the broth and incubated for 24 hours. After 24 hours, 1 ml of Kovac's reagent was added into every sample. Positive samples showed a pink colour ring after addition of the reagent and negative samples did not have any change of colour after addition of the reagent. Kovac's reagent determines the ability of an organism to split amino acid tryptophan to form the compound indole. Indole test helps to differentiate *Enterobacteriaceae* and other organisms (Haque *et al.*, 2014).

b.2) Molecular identification

b.2.1) DNA extraction

In this study, DNA extraction was done using the Zymo kit (INQABA, South Africa) according to the manufacturer's instructions.

Extraction was done as follow: colonies from bacteriological cultures were inoculated in a Nutrient broth and placed in an incubator for 24 hours. They were later placed in centrifuge tubes and centrifuged at 10 000 for a minute. After centrifuging, cells in the centrifuging tubes were transferred into Eppendorf tubes where they were centrifuged again. The liquid was then removed and cells mixed with lysis solution (750 microliter (µl)) in a beat beater tube. They were later mixed with a spinner for 14 minutes before being centrifuged.

Then 400 µl of the supernatant was transferred into a Zymo-Spin™ IV Spin Filter (orange top) tube and centrifuged at 10 000 speed per minute. The filter was then removed and 1 200 µl of Fungal/Bacterial DNA Binding Buffer added to the filtrate in a collection tube. 800 µl of the mixture was transferred into a Zymo-Spin™ IIC Column tube from a collection tube and centrifuged at 10 000 speed for a minute. The liquid was then discarded and the step repeated using the remaining supernatant.

200 µl of DNA Pre-Wash Buffer was added to the Zymo-Spin™ IIC Column tube after discarding the liquid. After, 500µl of Fungal/Bacterial DNA Wash Buffer was added to the Zymo-Spin™ IIC Column tube and centrifuged at 10 000 for 1 minute. Finally, a different clean Eppendorf tube was used, and 100 µl of DNA Elution Buffer added before centrifuging at 10 000 for a minute.

Due to the high costs and limited financial resources, not all the samples were extracted. Samples were selected according to biochemical and morphological similarities.

b.2.2) Polymerase Chain Reaction

Polymerase Chain Reaction (PCR) was performed on pure DNA extract using the ZIMO kit in the Animal Health Molecular Laboratory of the North-West University. The Engine DYAD Peltier thermal cycler (South Africa) was used. The following materials were used:

- 12 µL PCR Master Mix;
- 1 µL template DNA;
- 1µL of each oligonucleotide primer; and
- 10 µL nuclease free water.

They were mixed in PCR tubes to amplify 16S rDNA genes. Universal primers, forward 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and the Reverse 1492R (5'-TGA CTG ACT GAG ACG TTG CGA-3') were used. The denaturation step was set at 95°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 61°C for 30 seconds, then extension at 72°C for 5 minutes, followed by a single extension step at 72°C for 7 minutes in a Peltier thermal cycler (Ngoma *et al.*, 2014).

b.2.3) Gel electrophoresis on extracted DNA

In order to confirm the presence of extracted DNA, a Gel Electrophoresis was run using the method described by Ngoma *et al.* (2014) as follows:

The presence of DNA was analysed by using 1% of agarose gel (South Africa). The agarose gel was prepared as follows: 100 g of agarose gel was weighed and mixed in 100 ml of sterile water plus 10 ml of TE buffer. The gel was then dissolved by using a microwave for 5 minutes, and left to cool down until the bottle was not too hot to be touched. Ethidium bromide (0,5ml) was added for staining and the gel cast and allowed to set. After the gel had set inside the electrophoresis chamber, 5 µL DNA and 5 µL of loading buffer were mixed and transferred to one of the walls in the gel electrophoresis tank. Electrophoresis Bio rad (South Africa) was set for 90 minutes at 80 voltages and 250 Meg amperes (MA). The gel was then visualised under ultraviolet (UV) light at 420 millimetre (mm) wavelength using a BIO-RAD (South Africa), Molecular Imager, Gel Doc™ XRt imaging system. Images were then captured to view the presence of DNA bands using version 6.00.22 software. The presence of a single bright band (DNA bands) for each sample indicated a successful amplification.

Finally, PCR products were sent to Inqaba Biotechnology in Pretoria South Africa, for sequencing (Ngoma *et al.*, 2014).

b.2.4) DNA Sequencing

DNA sequencing was done at Inqaba Biotech, Pretoria, South Africa. Purified fragments of 16S rDNA of the strains were analysed for nucleotide sequence and ABI PRISM® 3500XL DNA Sequencer was used. The sequences were aligned using the Basic Local Alignment Search Tool (BLAST) from the National Centre for Biotechnology Information (NCBI) (Ngoma *et al.*, 2014). Sequences with high similarity were identified before developing a phylogenetical tree.

b.2.5) Phylogenetical tree

Sequences were analysed and edited with Bio Edit Sequence Alignment Editor (Ngoma *et al.*, 2014). Mafft programme 7 was used to align multiple sequences received from BLAST. Aligned 16S rDNA gene sequences were used to construct a phylogenetic tree (Ngoma *et al.*, 2014). However, there was no genetic relationship between all the sequences hence, out groups of sequences were developed instead of developing a single tree. There is a need to further research in order to identify why there was no relationship of the sequences. The Gen-Bank database for accession was used.

3.3.4 Statistical analysis

Descriptive statistics were used, and they provided a clear summary of the samples. SPSS version 21 was used to analyse samples, and to construct the chi-square test for association and the Multivariate analysis of variance test (MANOVA) which identified relationship between the milk quality test results and dairy management. Furthermore, results of CMT were summarised using biographical data for each farmer (obtained from the questionnaire). Due to many dependent variables, there were so many vectors involved that influenced milk quality from the farm management such as provision of veterinary services, use of antiseptics in the farm, knowledge of mastitis, quantity of milk produced, use of antibiotics, etc. The multiple vectors influenced the use of MANOVA test.

CHAPTER FOUR

RESULTS

A: Demographic Variables

All farmers (100%) in the study area were males (Figure 4.1), 43% were aged between 31 and 41 years (Figure 4.2), while 14% of farmers were aged between 21 and 30 years (Figure 4.2).

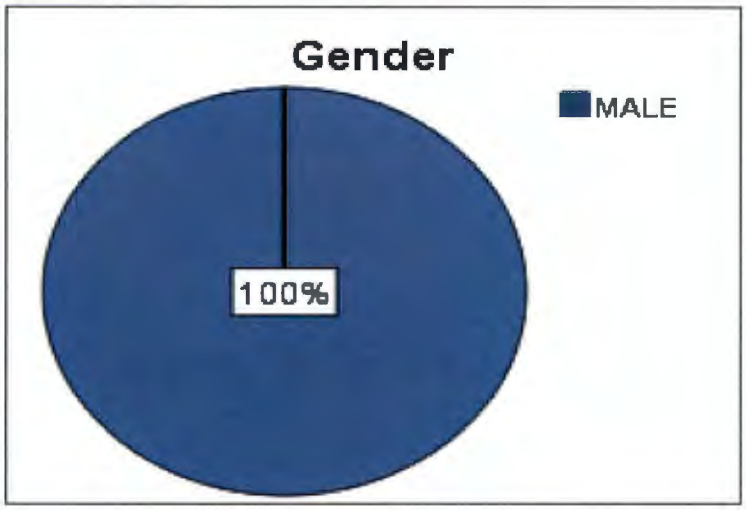

Figure 4.1: Categorisation of farmers based on gender

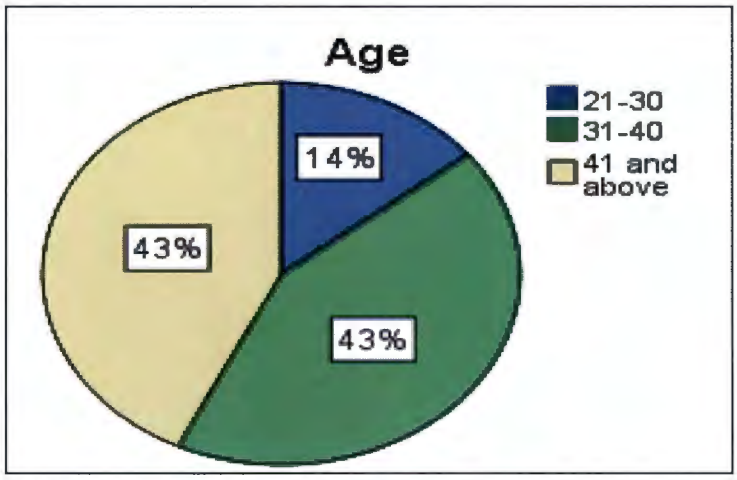

Figure 4.2: Categorisation of farmers based on their age

In this study, 57% of farmers were White (Figure 4.3), 29% were Black (Figure 4.3), while 14% were Coloureds (Figure 4.3). The marital status of farmers was as follows, married (86%) and divorced (14%) farmers (Figure 4.4).

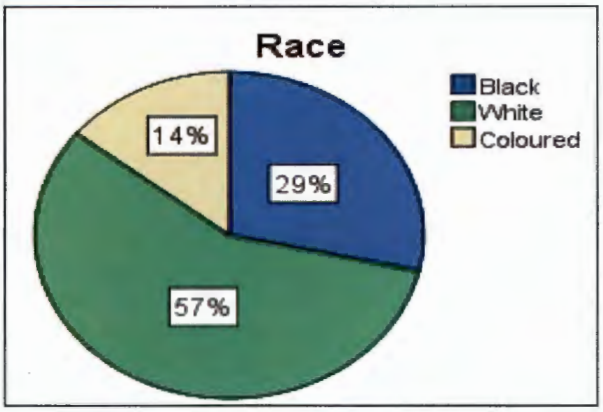

Figure 4.3: Distribution of dairy farmers according to their races

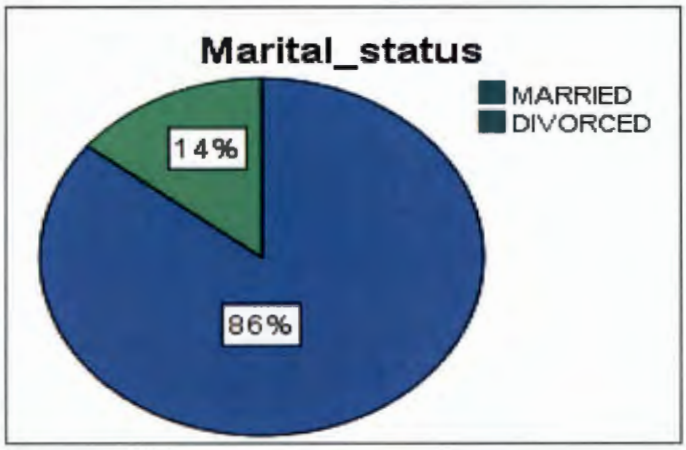

Figure 4.4: Categorisation of dairy farmers according to their marital status

Majority of farmers (71%) attained secondary school education while 14% attained tertiary and postgraduate level (Figure 4.5). In addition, 57% of farmers were self-employed while 43% were employed (Figure 4.6).

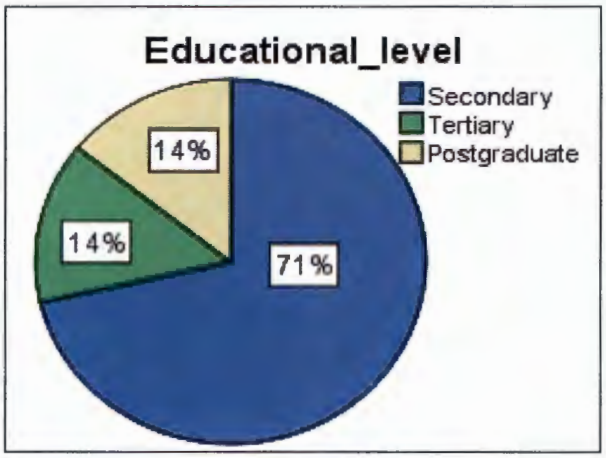

Figure 4.5: Categorisation of farmers according to their level of education

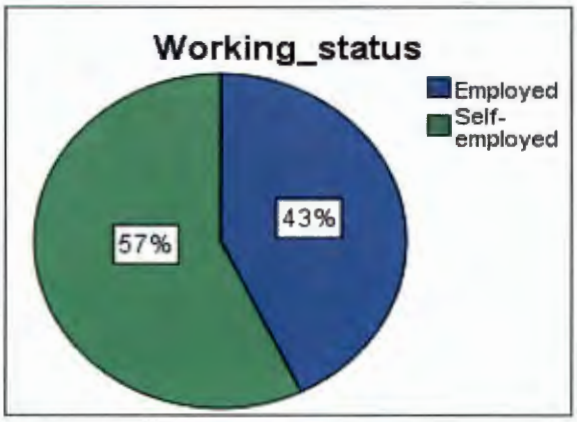

Figure 4.6: Categorisation of farmers according to their employment status

B: Farmers and employees: knowledge of biosecurity

In this study, data obtained revealed that 57%-100% of farmers confirmed that their employees were trained on biosecurity, while 71% revealed their employees had no knowledge on the provision of extension services. However, 14% of farmers confirmed they were not receiving veterinary services while 100% of farmers knew about mastitis. In addition, 14% of farmers confirmed that they did not use antiseptics during milking, 14% did not cull non-productive cattle while 14% maintained there was no good level of hygiene in the farms. 29% of farmers indicated they did not use antibiotics on their animals and 29% of farmers confirmed that the quantity of milk produced by cows was not enough (Figure 4.7).

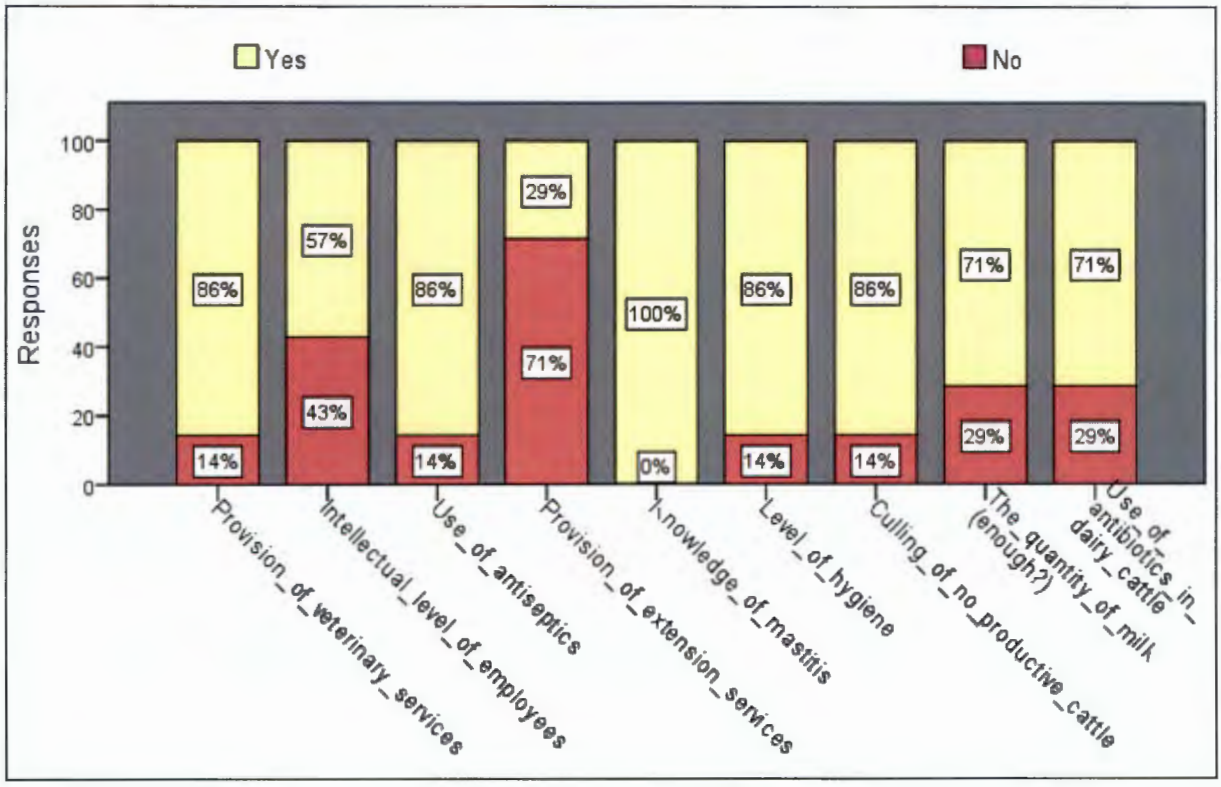

Figure 4.7: Dairy farmers and employees' knowledge about dairy farming

C: Relationship between results of occurrence of mastitis and management factors

The likelihood ratio Chi-square test which is recommended for testing the association between two nominal variables when more than 20% of the expected cell frequencies are less than 5 is used in the table above. The relative risk is not computed for the variables in the table above since they do not produce 2*2 contingency tables.

There is a significant association between quantity of feed given to lactating cows and the results of the CMT test. Cows given at least 11 kg of feed during the lactation period stand a 100% chance of testing negative for CMT, whereas cows given 5-10 kg of feed during the same period stand a 1005 chance of testing positive for CMT.

Table 4.1: Results of occurrence of mastitis caused by management factors

Relationship between the CMT results and the following	CATEGORY	NEGATIVE	POSITIVE	Likelihood Ratio Chi-Square	df	P-value
Type of feed	Pasture	100.00%	0.00%	3.877	2	0.144
	Mixed ration	75.00%	25.00%			
	Full ration	0.00%	100.00%			
Quantity of feed (calves)	5-10 kg	66.70%	33.30%	0.738	1	0.390
	11-20 kg	100.00%	0.00%			
Quantity of feed (lactating cows)	5-10 kg	0.00%	100.00%	8.376*i	2	0.015
	11-20 kg	100.00%	0.00%			
	More than 20 kg	100.00%	0.00%			
Quantity of feed (cows on dry period)	5-10 kg	75.00%	25.00%	1.105	2	0.576
	11-20 kg	50.00%	50.00%			
	More than 20 kg	100.00%	0.00%			
Quantity of feed (weaning and pregnant cows)	5-10 kg	50.00%	50.00%	1.784	2	0.41
	11-20 kg	66.70%	33.30%			
	More than 20 kg	100.00%	0.00%			
Level of disinfection	Daily	100.00%	0.00%	5.603	2	0.061
	Weekly	50.00%	50.00%			
	Monthly	0.00%	100.00%			
Quantity of milk	1-20L/day	66.70%	33.30%	4.557	3	0.207
	21-40L/day	0.00%	100.00%			
	41-50L/day	100.00%	0.00%			
	More than 50L/day	100.00%	0.00%			
Duration of cattle kept	1-<5 years	100.00%	0.00%	3.877	2	0.144
	5-<10 years	75.00%	25.00%			
	10-<15 years	0.00%	100.00%			
Method of storing feed	Indoors	80.00%	20.00%	3.372	2	0.185
	Bags	0.00%	100.00%			
	Outside	100.00%	0.00%			

The likelihood ratio Chi-square test which is recommended for testing the association between two nominal variables when more than 20% of the expected cell frequencies are less than 5 is used in the table above. In addition, the relative risk is computed for the variables in the table above since they have 2*2 contingency tables.

The results show that there is a significant (p-value <0.05) association between the CMT results and the following variables: provision of antibiotics and the method used to clean the teats. Cows provided with antibiotics stand a higher chance (66.7%) of testing positive for CMT than those that are not provided with antibiotics (0%). All the cows which are not provided with antibiotics stand a 100% chance of testing negative to CMT. In addition, cows whose teats are cleaned using water stand a 66.7% chance of testing positive to CMT whereas for those whose teats are cleaned with a disinfectant stand a 100% chance of testing negative to CMT.

Table 4.2: Significant association between the CMT results and dairy management of all the farms

		NEGATIVE	POSITIVE	Likelihood ratio Chi-Square	df	Asymptotic Significance (2-sided)	Relative Risk[ii]	Value	95% Confidence Interval	
Provision of antibiotics	Yes	33.30%	66.70%	4.557*	1	0.033	For cohort RESULTS = NEGATIVE	0.333	0.067	1.652
	No	100.00%								
Milking per day	Once a day	75.00%	25.00%	0.058	1	0.810	For cohort RESULTS = POSITIVE	0.75	0.073	7.73
	Twice a day	66.70%	33.30%							
Type of cleaning teats	Water	33.30%	66.70%	4.557*	1	0.033	For cohort RESULTS = NEGATIVE	0.333	0.067	1.652
	Disinfectant	100.00%								
Type of milking method	Manual	66.70%	33.30%	0.058	1	0.81	For cohort RESULTS = POSITIVE	1.333	0.129	13.743
	Automatic	75.00%	25.00%							
Workers knowledge of biosecurity	Yes	80.00%	20.00%	0.599	1	0.439	For cohort RESULTS = POSITIVE	0.4	0.043	3.737
	No	50.00%	50.00%							
Provision of veterinary services	Yes	66.70%	33.30%	0.738	1	0.390	For cohort RESULTS = NEGATIVE	0.667	0.379	1.174
	No	100.00%								
Intellectual level of employees	Yes	50.00%	50.00%	2.831	1	0.092	For cohort RESULTS = NEGATIVE	0.5	0.188	1.332
	No	100.00%								
Use of antiseptics	Yes	83.30%	16.70%	2.969	1	0.085	For cohort RESULTS = POSITIVE	0.167	0.028	0.997
	No		100.00%							
Provision of extension services	Yes	50.00%	50.00%	0.599	1	0.439	For cohort RESULTS = POSITIVE	2.5	0.268	23.359
	No	80.00%	20.00%							
Level of hygiene	Yes	83.30%	16.70%	2.969	1	0.085	For cohort RESULTS = POSITIVE	0.167	0.028	0.997
	No		100.00%							
Culling of no productive cattle	Yes	83.30%	16.70%	2.969	1	0.085	For cohort RESULTS = POSITIVE	0.167	0.028	0.997
	No		100.00%							
	No	100.00%								

1

[1] [1] * significant at 5%

[1] Only performed for 2*2 tables (i.e variables with dichotomous categories)

D. Occurrence of subclinical mastitis based on knowledge of biosecurity by farmers and employees

In this study, the relationship between the knowledge of biosecurity by farmers and employees and the occurrence of subclinical mastitis in the farms was observed. Farms that received veterinary services had 33.30% prevalence of mastitis, while the level of education of employees impacted with 50% prevalence of mastitis. The use of antiseptic had 16.70% impact on the prevalence of subclinical mastitis, while the following percentages were recorded for the following criteria: impacted with the respective percentages, provision of extension services (50%); knowledge of mastitis by farmers and employees (28.60%); level of hygiene (16.70%); culling of non-productive cattle (16.70%); and use of antibiotics (40.00%) (Table 4.3).

Table 4.3: Occurrence of subclinical mastitis based on knowledge of biosecurity by farmers and employees

	POSITIVE
Provision of veterinary services to farmers by the government	33.30%
Level of education of employees	50.00%
Use of antiseptics	16.70%
Provision of extension services	50.00%
Knowledge of mastitis	28.60%
Level of hygiene	16.70%
Culling of non-productive cattle	16.70%
Use of antibiotics in dairy cattle	40.00%

All farmers used antibiotics that are in the Tetracycline group

E: Chi-square test for association and results of MANOVA test for significance

The results show the significant association between quarterly results and farms (P<0.05), but not between quarterly results and quarter (Table 4.4).

Table 4.4: Results of Chi-square of quarter samples of farms

	Chi-square tests		
	Value	Df	Asymp. Sig. (2-sided)
Results of quarter and farm management	59.492	6	0.000
Results of quarter and farm management	2.709a	3	0.439

In this study, MANOVA was used for data analysis and the results obtained showed a significant difference (P<0.05) among elements associated to milk (fat; SNF; lactose; protein and solids) (Table 4.5).

Table 4. 5: The Effects of farm management on the quality of milk

	Effect	Value	F	Hypothesis df	Error df	Sig.
FARM	Pillai's Trace	0.393	2.178**[2]	30.000	765.000	0.000
	Wilks' Lambda	0.655	2.220**	30.000	598.000	0.000
	Hotelling's Trace	0.455	2.234**	30.000	737.000	0.000
	Roy's Largest Root	0.201	5.121**	6.000	153.000	0.000

(Pillai's Trace, Wilks' Lambda, and Hotelling's Trace are all test statistics in MANOVA, they test whether there are differences in means of identified groups of subjects on a combination of dependent variables. They pool the variance from all dimensions to create the test statistic while Roy's largest root only uses the variance from the dimension that separates the differences of the groups).

Results obtained and analysed showed that all components measuring the quality of milk differ significantly (P< 0.05) across farms, except for fat content, that is the fat content was almost the same regardless of where the milk sample was collected (Table 4.6).

[2] ** significant at 1%

Table 4. 6: Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	Df	Mean Square	F	Sig.
FARM	Fat	9.500	6	1.583	1.620	0.145
	SNF	25.893	6	4.315	4.960**	0.000
	Lactose	20.446	6	3.408	3.763**	0.002
	Solids	14.353	6	2.392	2.530**	0.023
	Protein	19.607	6	3.268	3.587**	0.002

The data obtained in this study also showed a significant association (P< 0.05) between the Quantity of feed given to lactating cows and the occurrence of mastitis (Table 4.7).

Table 4. 7: Association (relationship) between feeding practices and the quality of milk

	Value	Df	Asymp. Sig. (2-sided)
Type of feed *	3.877	2	0.144
Quantity of feed calve *	0.738	1	0.39
Quantity of feed lactating *	8.376	2	0.015**1
Quantity of feed dry period *	1.105	2	0.576
Quantity of feed weaning and pregnant *	1.784	2	0.41

Results obtained also revealed a significant association (P< 0.05) between the methods used to clean the teats of cows by farmers and the occurrence of mastitis (Table 4.8).

Table 4. 8: Significance of biosecurity practices on the occurrence of mastitis

	Value	Df	Asymp. Sig. (2-sided)
Type of milking method *	0.058	1	0.81
Use of antiseptics *	2.969	1	0.085
Level of hygiene *	2.969	1	0.085
Duration of cattle kept *	3.877	2	0.144
[illegible]	[illegible]	[illegible]	[illegible]
Type of method used to clean teats *	4.557	1	0.033*
Workers knowledge of biosecurity *	0.599	1	0.439
Method of storing feed *	3.372	2	0.185
Provision of veterinary services *	0.738	1	0.39
Intellectual level of employees *	2.831	1	0.092
Provision of extension services *	0.599	1	0.439
Culling of non-productive cattle *	2.969	1	0.085
[illegible]	[illegible]	[illegible]	[illegible]
Use of antibiotics in dairy cattle *	1.646	1	0.2

1* significant at 1%
** significant at 1%

F: Screening and biochemical results of other lab tests

The results of mastitis positive quarterly samples using CMT were observed. The results showed that 62.5; 60; 57; 25; 20 and 43 % of animals (for which samples were analysed respectively) from farms 3; 2; 7; 4; 5 and 1, had 4 quarters positive for mastitis. Farm 3 had 25 % of animals with 3 quarters positive for mastitis. Farms 2; 1; 5 and 7 had 40; 28.5; 20 and 29% of animals with 2 quarters positive for mastitis, and farms 3; 4; 5 and 7 had 12.5; 25; 20 and 14 % of animals with 1 quarter positive for mastitis. Farms 4; 5 and 1 had 50; 40 and 28.5 % of animals not showing any signs of mastitis (Table 4.9).

Table 4. 9: Summary of animals that showed positive results towards mastitis using the CMT method presented per quarter infected

Farm	n	4 quarters positive	3 quarters positive	2 quarters positive	1 quarter positive	0 quarters positive
Farm 1	7	3 (43%)	0 (0%)	2 (28.5%)	0 (0%)	2 (28.5%)
Farm 2	5	3 (60%)	0 (0%)	2 (40%)	0 (0%)	0 (0%)
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Farm 4	4	1 (25%)	0 (0%)	0 (0%)	1 (25%)	2 (50%)
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Farm 6	5	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Farm 7	7	4 (57%)	0 (0%)	2 (29%)	1 (14%)	0 (0%)

n (number of cows)

The milk quality of bulk samples of all farms represented in Figure 4.8 shows that the fat content of farm 1 was 4.82%, farm 2 was 5.74% and farm 3 was 4.21%, all above the normal content of 3.67% (see Table 2.2 for normal percentages of milk content). Farms 4; 5; 6 and 7 had a reduced milk fat content. The normal SNF is 9.02% (Table 2.2). Farm 1 had a normal SNF, and all the other farms had a reduced SNF below the normal percentage.

The normal content of lactose is 4.78% (Table 2.2). All the farms had a normal or close to the normal content of lactose. The normal percentage of solids ranged between 13.43 and 14.34% (Table 2.2). The percentage of solids for farm 3 was normal, while for the rest of the farms, it was below normal. The normal percentage of protein is 3.23% (Table 2.2). The protein content was normal or close to normal in all the farms except for farms 2 and 3 where it was slightly below normal.

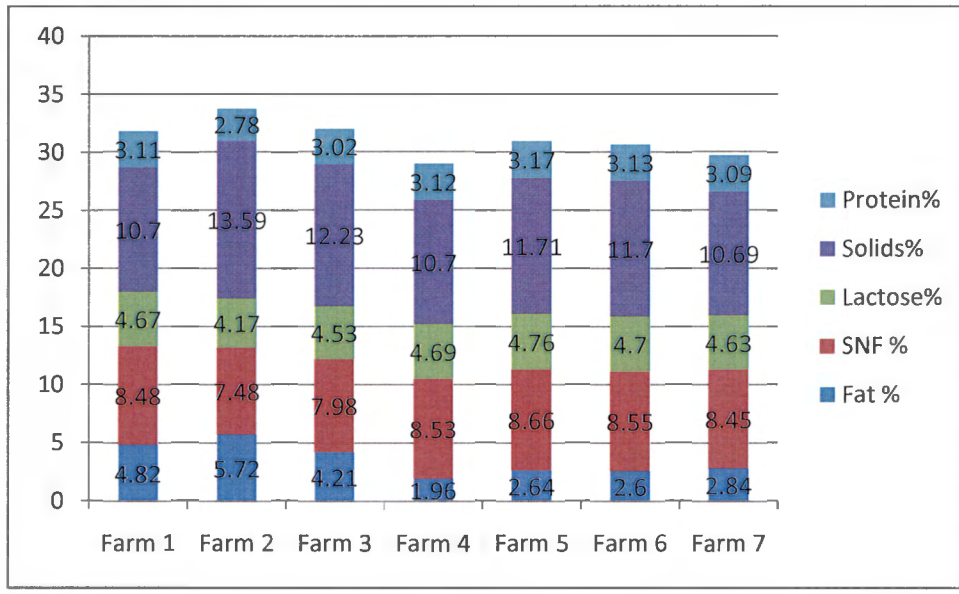

Figure 4.8: Summary of milk content obtained from bulk samples in different farms

Microbial identification

Gram staining

Gram straining is the first biochemical test that was used in this study. The test separates bacteria into gram positive and gram negative bacteria, making it easy to further test or treat microorganisms in milk. Gram positive (21) and gram negative (45) bacteria were divided into cocci and bacilli organisms. Samples that tested positive showed a confirmation of existing contamination in milk samples that were tested using CMT method.

Table 4. 10: Summary of results of gram staining for samples that tested positive for CMT method in all the farms

	n	Cocci gram negative bacteria	Cocci gram positive bacteria	Bacilli gram negative bacteria	Bacilli gram positive bacteria	Total number of results of gram staining accumulated
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Farm 2	17	6 (35%)	1 (6%)	6 (35%)	1 (6%)	14 (82%)
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Farm 4	5	2 (40%)	1 (20%)	0 (0%)	0 (0%)	3 (60%)
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Farm 7	21	5 (24%)	5 (24%)	2 (10%)	3 (14%)	15 (71%)

There were no bacterial growths in some of the bacterial cultures (could represent false positive results from CMT method). Table 4.8 presents only samples that showed some results. The n represents the total number of samples that tested positive for mastitis (quarterly and bulk samples).

Results of Catalase and Indole tests

The Indole test is effective in testing anaerobic bacteria and is reliable in the testing of aerobic and facultative bacteria too (Sutter & Carter, 1972). The catalase test also proved to be useful, as it reacts by breaking hydrogen peroxide into water and oxygen (Taylor & Achanzar, 1972). Gram positive results of gram staining bacteria discovered in the gram staining test were further tested using the catalase test and gram negative bacteria were tested using the Indole test. The numbers below the tests represent the number of samples that tested positive (Table 4.11).

Table 4. 11: Results of Catalase and Indole tests of all farms from where samples were tested using gram staining method

	n (gram positive)	Catalase (gram positive microorganisms)	n (gram negative)	Indole (gram negative microorganisms)
Farm 1	4	2 (50%)	8	2 (25%)
Farm 2	2	1 (50%)	12	5 (42%)
Farm 3	4	3 (75%)	13	5 (39%)
Farm 4	1	1 (100%)	2	2 (100%)
Farm 5	2	0 (0%)	3	2 (67%)
Farm 7	8	5 (63%)	7	4 (57%)

An image showing the presence of DNA bands, which appeared in the gel electrophoresis tank.

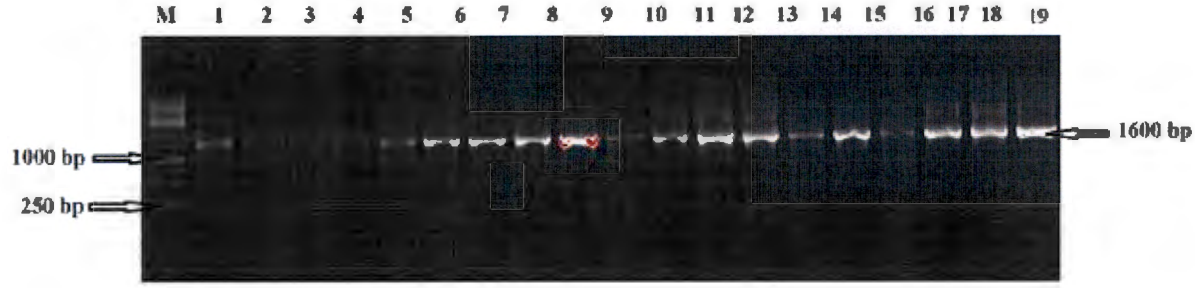

Figure 4.9: Electrophoresis in a 2 % agarose gel of PCR amplified 16S rDNA; Lane 1-19 (16S rRNA gene fragment from DNA extracted; Lane M (1kb DNA ladder)

Results of PCR test after blasting

In this study, bacteria were isolated and identified from different farms using the PCR test (see appendix for their reference numbers). Farm 1 had a prevalence of *Bacillus subtilis* and *Serratia spp.*, while Farm 2 had different *Serratia spp.* and a *Staphylococcus lentus*. All bacteria from Farm 3 were *Enterococcus* and for both Farms 4 and 5, there was a prevalence of *Bacillus*. Farm 7 had a prevalence of *Serratia spp.* and *Clostridium spp.* (Table 4.12).

Table 4. 12: Results of PCR tests of all farms after blasting

Farms	Sample number	Isolate code of organisms identified	Reference from NCBI database
Farm 1	1	NWU 1	*Bacillus subtilis*
	2	NWU 2	*Serratia spp.*
	3	NWU 3	*Bacillus subtilis*
Farm 2	4	NWU 4	*Serratia spp.*
	5	NWU 5	*Serratia psp.*
	6	NWU 6	*Serratia liquefaciens*
	7	NWU 7	*Serratia spp.*
	8	NWU 8	*Staphylococcus lentus*
Farm 3	9	NWU 9	*Enterococcus hirae*
	10	NWU 10	*Enterococcus mundtii*
	11	NWU 11	*Enterococcus mundtii*
	12	NWU 12	*Enterococcus mundtii*
	13	NWU 13	*Enterococcus hirae*
Farm 4	14	NWU 14	*Bacillus cereus*
	15	NWU 15	*Bacillus spp.*
	16	NWU 16	*Bacillus cereus*
	17	NWU 17	*Bacillus cereus*
Farm 5	18	NWU 18	*Bacillus spp.*
	19	NWU 19	*Bacillus thuringiensis*
Farm 7	20	NWU 20	*Clostridium spp.*
	21	NWU 21	*Serratia spp.*

Phylogenetical tree

Tree of Bacillus *spp.*

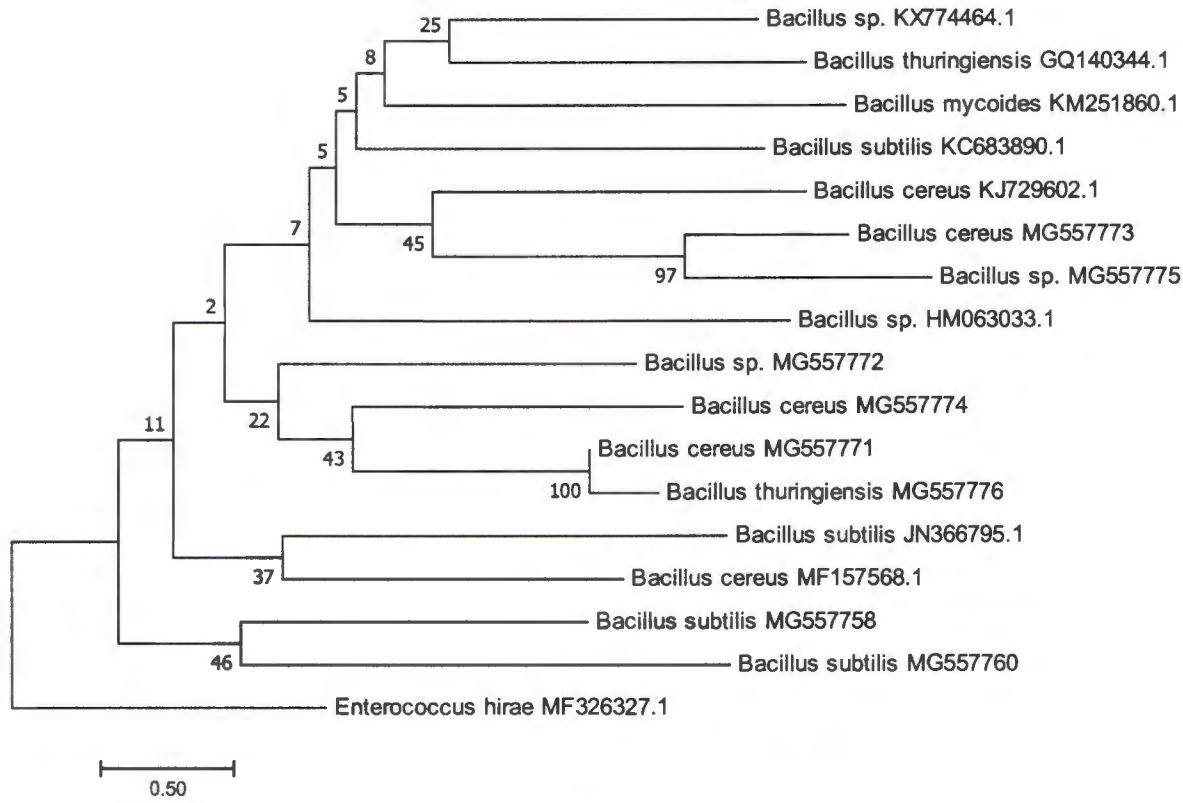

Figure 4.10: Tree of Bacillus *spp.*

Evolutionary relationships of taxa

The evolutionary history was inferred using the Neighbour-Joining method (Nei, 1987). The optimal tree with the sum of branch length = 24.45399791 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates), is shown next to the branches (Felsenstein, 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura, 2004) and are in the units of the number of base substitutions per site. The analysis involved 16 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 553 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar, 2016).

Tree of Enterococcus isolates

Figure 4.11: Tree of *Enterococcus* isolates

Evolutionary relationships of taxa

The evolutionary history was inferred using the Neighbour-Joining method (Nei, 1987). The optimal tree with the sum of branch length = 13.19280476 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura, 2004) and are in the units of the number of base substitutions per site. The analysis involved 10 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 715 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar, 2016).

Tree of Serratia *spp.*

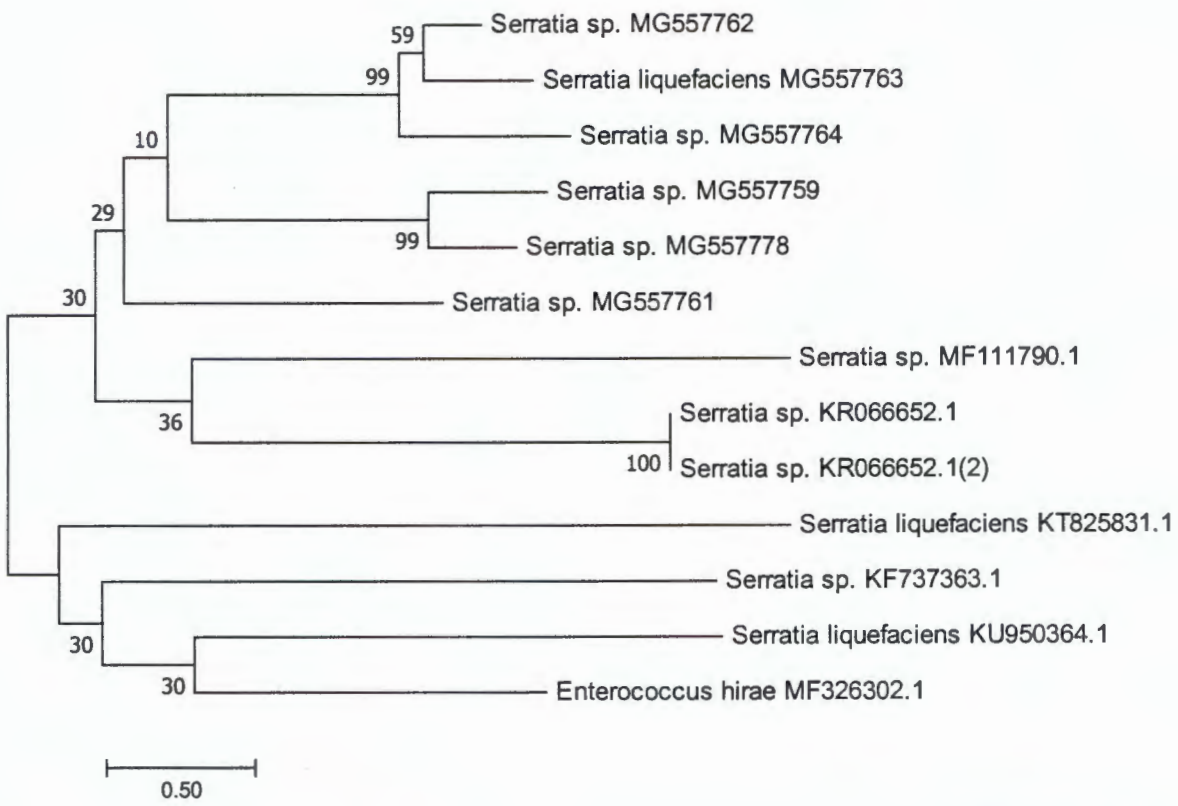

Figure 4.12: Tree of Serratia *spp.*

Evolutionary relationships of taxa

The evolutionary history was inferred using the Neighbour-Joining method (Nei, 1987). The optimal tree with the sum of branch length = 16.07588299 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura, 2004) and are in the units of the number of base substitutions per site. The analysis involved 12 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 940 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar, 2016).

The trees excluded *Staphylococcus lentus* and *Clostridium sp.* because there were no similarities between the bacteria and other bacteria found although they were also identified in the study.

CHAPTER FIVE

Discussion

The aim and objectives of this study were to: assess the quality of milk per farm against the management system put in place; ensure that farmers improve their productivity by assessing and comparing different farming management systems they use by using a questionnaire; analyse milk samples collected from farms in order to examine the quality of milk and test for subclinical mastitis; and study the influence of farming systems on milk quality such as content, microbial bacterial contamination and occurrence of mastitis in farms around Ngaka Modiri Molema District.

The results obtained showed that 100% of dairy farmers who participated in the study were male (Figure 4.1). This could be due to the cultural and social challenges that women faced in the past, thus changes that involve women empowerment take time. Hart (2012) criticises post-apartheid policies of agriculture. The author argues that the policies did not support poor and vulnerable farmers, especially females. This is also a factor that contributes to male dominance in dairy farming. Njuki *et al.* (2006) conducted a study in Kenya and found that when women get more education, they shift their focus from farming activities and focus more on non-farming and income-generating, thus abandoning farming activities in the hands of farm labourers, which could be one of the reasons why women are not fully involved in dairy farming.

86% of farmers were aged 31 years and above (Figure 4.2). This is an indication that there is a lower percentage of young farmers who take up dairy farming. This is in line with the findings of Kunene (2006), who found that a large number of farmers were above the age of 30 (only 4% were less than 30 years old). This is also similar to Rigg's (2006) finding which confirmed that young people are moving away from the farming sector, wishing to invest in non-farming sectors. This is an indication that people who show interest in farming are the older generation. The issue of age of dairy farmers is not only a concern for the African continent, in Canada and the United States of America, older farmers still dominate the sector (Jelinski *et al.*, 2015) (www.agcensus.usda.gov, 2014). In Canada, nearly half (45.8%) of dairy farmers are $\geq$ 50 years of age (Jelinski *et al.*, 2015).

Majority of farmers who participated in this study were white (Figure 4.3). This could be explained by the history of South Africa's blacks who were excluded from the accumulation of capital; education; land and employment, and which did not allow them to be involved in commercial and dairy farming (Francis, 2002). In addition, Lahiff (2005) maintains that agricultural land belongs to about 45 000 corporate and individual white owners. Black people who have land, do not have the capital to maintain or work on it. This statement is supported by Francis (2002). According to Crowley (2017), about three-quarters of South Africa's land is owned by white people, an indication that white people still own most of the land currently in the whole country even after apartheid. In 1994, whites owned 85.1% of the land and currently, the number has dropped to 73.3% (Crowley, 2017). This might explain why there are few black people involved in dairy farming. In addition, most dairy farmers inherited their business from their families.

71% of farmers had secondary school education (Figure 4.5). The study revealed that education does not affect farming as long as farmers are literate; this is with reference to Farm 6 which was owned by a farmer with secondary education (Figure 4.5). This is in line with a study by Lockheed *et al.* (1979), who found that farm productivity increased by 7.4% as a result of a farmer completing 4 years of primary school education rather than none. This shows that the most important factor is for farmers to become literate and not to have a post-secondary educational qualification that does not have a negative impact on farm management.

The results of the occurrence of mastitis using the CMT methods per quarter showed that 100% of farms with the exception of Farm 6 showed positive signs of mastitis (Table 4.9). However, after using the bacteriological culture method on all mastitis positive samples, it was discovered that some samples resulted to false positive results (Table 4.10). This showed that the CMT method for mastitis is not a definitive test but a screening test for mastitis (Rice, 1981). Screening tests are sensitive hence, taking in false positive results as well. Rice (1981) confirmed that false positive results of CMT method might be explained by testing cows that are approaching their dry period, which could be the cause of false positive results obtained in this study.

It is important to indicate that the intensive management system is also more likely to contribute to the prevalence of mastitis (www.ciwf.org, 2017). Cows that are kept indoors (on concrete floors, with inadequate bedding), are more likely to contract mastitis than those kept

on pastures, due to crowded conditions, poor ventilation and high humidity increase (www.ciwf.org, 2017). Farm 6 could be used as a reference to this because it had 0% mastitis prevalence and used the extensive management system, while other farms that used semi-intensive (14% of the farms) and intensive management systems (57% of the farms) had more than 50% of prevalence of mastitis. In contrast, a study by Iraguha *et al.* (2015) revealed that the extensive farming system had a higher prevalence of mastitis due to the teat-end condition. Cows that are grazing in savannah vegetation (which is extensive) may get damaged at the teats, causing mastitis infection (Iraguha *et al.*, 2015). However, Romain *et al.* (2000) confirmed that there was no difference in the prevalence of subclinical mastitis in cows managed intensively, semi-intensively and extensively. It is reported that intensive and extensive management systems have different exposures to environmental and contagious pathogens of mastitis (Romain *et al.*, 2000). The extensive management system exposes cows to pathogenic exposure as they are left in pastures, even milked there (out-on-field), thus increasing the risk of infection through teat contamination, while the intensive farming system exposes contamination of infection through dirty dairy equipment (Romain *et al.*, 2000).

The standard quality of the contents of milk that are within normal ranges was observed (Table 2.2). However, all the farms in the study area had milk contents that are normal or close to normal (Figure 4.8). Cows from farm 6 showed good health and body condition compared to the other farms in terms of the occurrence of mastitis. Cows from farm 6 were given pasture and more feeding attention was given to lactating, pregnant and weaning cows, which were given more feed. The study also showed a significant association (p-value<0.05) in the quantity of feed that was given to lactating cows and the occurrence of mastitis. However, Morand-Fehr *et al.* (2007) disagree with the findings of this study.

According to Morand-Fehr *et al.* (2007), natural pasture-based farming system produces milk that is rich in fats and the cows tend to have a reduced BCS, producing less quantity of milk while getting exposed to high climatic temperatures. Hill and Wall (2015) emphasise this and maintain there are also changes in the availability of fodder and pasture and pests and parasites increase due to increased temperatures. West (2003) also concurs with the statement that temperatures above critical thresholds are related to a decrease in dry matter intake and milk yields. Apparently, intensive farming produces milk that is rich in protein and low in fat (Morand-Fehr *et al.*, 2007). This study revealed that there is no correlation between the farming system and protein and fat contents. With regard to farms 1; 2 and 3, different

farming systems were used but the fat content was above the normal range. The same applies to farms 4; 5; 6 and 7 which had a reduced fat content (Figure 4.8). Heinrichs (2017) maintains that the age of cows may cause milk fat to decline since each year, lactating cows use about 0.2% of fat drops. This might also explain the slight differences between farms since the age of animals in different farms was not taken into account in this study.

From the findings of this study, the different types of farming systems did not affect the lactose content of milk, which was normal or slightly normal. The SNF of most of the farms (6 out of 7 farms) was below the normal range (Figure 4.8). The low SNF percentage could be due to subclinical mastitis, thus causing a decrease in the synthetic activity of mammary glands due to pathogens that invade the mammary tissue (Hassan, 2013). In cases like the one in farm 6, where there was a 0% prevalence of mastitis, the reduced SNF could be due to feeding. Cows given 130% of high energy recommended feed, are more likely to have a reduced SNF (Harris, 1988).

The percentage recorded for solids in farm 3 was normal while for the rest of the farms, it was below normal (Figure 4.8). This could be due to less provision of feed (cows should eat 3.5-4.0% of their body weight daily) (Looper, 2012). Cows in farm 3 were given pasture, which was more likely to be ad lib, meaning feed was not limited. The protein content was normal or close to normal in all the farms, except for farms 2 and 3 where it was slightly below normal, which could have been influenced by the prevalence of mastitis. Mastitis does affect milk protein, which loosens the connection between cells thus, decreasing proteins that are synthesised in the mammary gland (caseins, beta-lactoglobulin and alpha-lactalbumin) (Linn, 1988).

Linn (1988) also argues that administering concentrates to animals increase the average milk yield by 11%. In this study, 5 farms used concentrates and out of the 5, 2 (40%) produced 1-20L milk per day, which was the lowest production. The other farms could be doing well because their cows were also given pasture. Farm 7 produced far better compared to all the 5 farms that used concentrates. Clerk *et al.* (2007) state that milking a cow once a day decreases the milk yield of a cow by 20-30% compared to milking a cow twice a day. This is in line with this study as in the study, all animals that were milked twice a day, produced at least 21-40L of milk or more (farm 3; 6 and 7), while some of the animals that were milked once a day, were able to produce 1-20L per day (farm 1; 2 and 5).

De Peters *et al.* (1985) maintain that feeding cows with a mixed ration diet ad libitum increases the percentages of milk fats and solids but decreases the percentage of protein. However, older lactating cows produced milk that was lower in fat, solids and protein content (DePeters *et al.*, 1985). It is important to mention that farms 1; 2; 4 and 7, which showed high percentage of fat in their milk, used the mixed ration farming system. However, the solid contents of the farms were lower than the normal content, except for farm 2; this could be due to the fact that the animals were not given the right quantity of feed (Figure 4.8).

With regard to milking hygiene, results of this study showed that farms that used machine or hand milking, had 66.70% for hand milking and 75% for automatic milking chances of contracting mastitis (Table 4.7). However, it is confirmed by cowseatgrass.com (2014) that any type of milking method could have negative and positive effects on the health of a cow. Based on the advantages and disadvantages of both methods, any of them could be used (though properly without putting the cow at risk) (cowseatgrass.com, 2014).

Farmers that disinfected daily reduced the risk of mastitis prevalence compared to farmers that disinfected weekly or monthly (Table 4.1). According to Health (2011), the milking area should be clean at all times meaning that milking equipment must be cleaned and disinfected after each and every milking. However, the type of method used to clean the teats of a cow did have a significant association to the occurrence of mastitis (p-value<0.05) (Table 4.8). Health (2011) confirmed this by putting an emphasis on teat disinfection of every teat after milking.

Bacillus mainly *Bacillus subtilis; Bacillus cereus; Bacillus spp.* and *Bacillus thuringiensis, Serratia* (*Serratia spp.* and *Serratia liquefaciens) Enterococcus (Enterococcus hirae* and *Enterococcus mundtti*), and *Staphylococcus lentus* and *Clostridium spp.* were identified in milk isolates used in this study (Table 4.12). All bacteria are environmental bacteria; this is in line with the studies of Tuazon (2017); Schwendener (2012); Brook (2017) and www.ubrocare.com (2017). It was confirmed by Petersson-Wolfe *et al.* (2011) and Petersson-Wolfe *et al.* (2012) that bacteria of the *Serratia spp.* and *Enterococcus spp.* are found in feed in a dairy farm, while *Staphylococcus lentus* is isolated from the skin (Foster, 2012), which could be a result of transmission of the bacteria from dirty hands of the milker. However, *Bacillus spp.* are usually found in manure (Banykó & Vyletělová, 2009), which could land from the feed or floor. *Clostridium spp.* are isolated from feed and may be a result of poor management in terms of storing of feed (McGuirk, 2018).

Bacillus spp. causes infections such as bacteraemia and septicaemia (Tuazon, 2017). *Staphylococcus lentus* colonises the skin of the animal and has been found in dairy animals. People working with animals could be carriers, although it has caused infections in humans in rare cases (Schwendener, 2012). Brook (2017) posits that about 30 *Clostridium spp.* are associated with human diseases. *Enterococcus* affects milk quality without affecting the overall health of the animal until it develops into clinical mastitis, and *Serratia spp.* also affects milk quality, forming clots in milk (http://www.ubrocare.com, 2017).

The use of antibiotics for the treatment of cows is more likely to increase the prevalence of mastitis since farmers tend to abuse drugs/overuse drugs, which may make the cows to develop antibiotic resistance and contract mastitis anyway despite being treated (Table 4.2). The study showed the significant association (p-value<0.05). This is supported by a study by Gomes & Henriques (2016), who found that the use of antibiotics has increased in farms exposed to mastitis, but end up losing their importance when overused, thus creating some drug resistance.

CHAPTER SIX

Conclusion and recommendations

6.1 Conclusion

The aim of this study was to assess different farming management systems and their correlation with milk quality and the occurrence of mastitis. To achieve this, 7 farms (using different management systems) were chosen in the Ngaka Modiri District of the North West Province. A questionnaire was issued to farmers, and milk samples collected and analysed using the CMT method and bacterial culture to confirm the presence of mastitis. The results of the study indicate that although most of the farmers were aware of mastitis and adhered to some management practices such as teat cleaning, disinfection and feeding practices, there was a significant impact on the occurrence of mastitis on dairy farms. There was a significant impact (p-value<0.05) mainly between the quantity of feed given to lactating cows (Table 4.7), method used to clean the teats of the cows (Table 4.8), and use of antibiotics (Table 4.2) and occurrence of mastitis. The occurrence of mastitis, which was found using the CMT method, was confirmed through bacterial culture method to overrule false positive results (Table 10). Biochemical testing separated the culture results between gram positive and gram negative bacteria using the gram staining testing method. However, the indole and catalase testing methods revealed more prevalence of gram positive bacteria (Table 4.11).

Data obtained also revealed there is still a need to improve feeding and hygiene management practices in most of the farms used in the study. There might have been a lot of bias due to lack of record-keeping, especially in terms of feeding records of the quantity of feed given to different cows at different stages of production. Most of the management standards and hygiene practices were relatively poor in most of the farms studied.

It is, therefore, concluded that the extensive farming system is the most suitable farming system as cattle are fed ad libitum, which not limit feed consumption. Based on the level of mastitis that was prevalent in the farms, it was discovered that all bacteria resulted from environmental mastitis. Hence, there is a need for more training on environmental management of dairy farms for both farmers and farm workers. This will help reduce the level of mastitis on farms. Most cattle in semi intensive farms and intensive farms (especially intensive farms) are prone to getting infected with environmental mastitis which result to or

caused by the farmers or farm workers themselves. The level of hygiene in the farms seems to play a huge role in the transmission of mastitis more than the type of feeding given to the animals. It is concluded that there are similarities in the farm management of the farms in the district and since the farms are all in the same area, there is also a similarity in the bacteria that were isolated from the farms. The mastitis causing bacteria reduce the milk quality hence it is important to apply the recommended measures. The type of farming system used in a farm does play a huge role in milk quality.

6.2 Recommendations

The following recommendations were suggested:

- Farmers should consider a lot of factors in the type of farming system and management they use. Extensive farming system can be applied where daily hygiene cannot be prioritized (as cows will move around freely in a large environment instead of being crowded in a small area where they end up getting more contamination from faeces and concrete floors in some farms);
- In a herd that has environmental mastitis, it is advisable to routinely test the herd for mastitis using CMT which is also inexpensive for small scale farmers, this will prevent misdiagnosis and overuse of antibiotics which could create drug resistance when overused;
- For prevention of environmental mastitis, the type of bedding should be considered, which should be low in moisture content to prevent the provision of nutrients to bacteria (sawdust, straw and recycled manure are discouraged);
- The use of teat dips is encouraged pre-milking, which reduces intra-mammary infection caused by environmental mastitis;
- Provision of clean water to cows, removing manure frequently and not overstocking cattle is the best way of ensuring the control of environmental hygiene;
- Animal health workers (veterinarians or animal health technicians) should be consulted as well in terms of the use of the relevant antibiotics to use and when to use them, to avoid drug resistance; and
- It is advisable not to treat cows that have had three unsuccessful treatments; this could be followed by culling or allowing the affected quarter to dry permanently.

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Appendix

Questionnaire

Comparative study of farm management systems and influence on milk quality in Ngaka Modiri Molema District, North West Province, South Africa

SECTION A: DEMOGRAPHIC CHARACTERISTICS

1. Gender a) Male b) Female	4. Marital status a) Married b) Single c) Divorced d) Widowed
2. Age a) Below 20 b) 21-30 c) 31-40 d) Above 41	5. Level of education a) Primary level b) Secondary level c) Tertiary d) Post-graduate
3. Race a) Black b) White c) Coloured d) Indian	6. Employment status a) Employed b) Unemployed c) Self-employed d) Pensioner

SECTION B: BIOSECURITY AND FEEDING OF CATTLE

Place a tick next to your answer.

1. What are your cows raised on?

Pasture	
Mixed ration	
Full ration	

2. How much feed is given to calves (1-5kg) daily?

5-10kg	
11-20kg	
More than 20kg	

3. How much feed is given to lactating cattle?

5-10kg	
11-20kg	
More than 20kg	

4. How much feed is given to cattle during the dry period?

5-10kg	
11-20kg	
More than 20kg	

5. How much feed is given to weaning and pregnant cattle?

5-10kg	
11-20kg	
More than 20kg	

6. Are your cows given any antibiotics on a continual basis?

Yes	
No	

If yes, which antibiotic?

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7. How many times do you milk your cows?

Once a day	
Twice a day	
Thrice a day	

8. How much milk do the cows produce?

1-20L/day	
21-40L/day	
41-50L/day	
More than 50L	

9. How long do dairy cows stay in your herd?

1-5years	
5-10years	
10-15years	

10. How often do you disinfect your animals?

Daily	
Weekly	
Monthly	

11. What type of milking method do you use?

Manual	
Automatic	

12. What is used to clean the teats of the cow pre- and post-milking?

Water	
Disinfectant	

13. Are your workers trained on biosecurity and mastitis?

Yes	
No	

14. How do you store your food?

Indoors	
Bags	
Outside	