MILK QUALITY AND PREVALENCE OF MASTITIS PATHOGENS IN INFECTED BOVINE MAMMARY GLANDS AND THEIR SENSITIVITY TO LOCAL PLANT EXTRACTS

by

Jean Longo Banza

Submitted in accordance with the requirements
for the degree of

MASTER OF SCIENCE

in the subject

Agriculture

at the

UNIVERSITY OF SOUTH AFRICA

Supervisor: Prof. KR Mbatha

Co-supervisor: Dr MC Muya

October 2020

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

DECLARATION

Name: Banza Longo Jean

Student number: 57651043

Degree: Master of Science in Agriculture at the University of South Africa

Exact wordings of the title of the thesis as appearing on the copies submitted for examination:

PREVALENCE OF MASTITIS PATHOGENS IN INFECTED BOVINE MAMMARY GLANDS OF THE ARC DAIRY HERD AND THEIR SENSITIVITY TO LOCAL PLANT EXTRACTS.

I declare that the thesis is my own work and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references

I further declare that I submitted to originality software checking and that it falls within the accepted requirements of the originality

I further declare that I have not previously submitted this work, or part of it, for examination at Unisa for another qualification or at any other higher education institution.

SIGNATURE

03 – 11 – 2020.

DATE

DEDICATION

To Banza Kiseba Anastase my father and Banza Monga Charlotte my mother for directing me in the right path, today I will not depart from it.

To my wife Banza Mimi Boteya, for your unselfish love, for keeping both spiritual and love flame in our lives, you are a gift from God.

To Jehnnifer Banza, Gracia Banza, Elwin Banza, Aeonël Banza and Godwin Banza, my children for decorating our lives with love, smile, understanding and sacrifice. Let this work serves as a standard in your life. The blessing of the Lord, it makes rich, and he adds no sorrow. You are the essence of my living.

I want to thank and praise to Almighty God for this wonderful opportunity He gave me and for His strength and guidance all through my research period.

ACKNOWLEDGEMENTS

My sincere thanks to my supervisors; Prof. Khanyisile R. Mbatha and Dr. Mukengela Claude Muya for their sound advice, encouragement, dedications and for leading me through the intricacies of a master dissertation.

I would like to thank Portia Moshidi, at Agricultural Research Council (ARC-API), Irene. I appreciate the support and prayers received from the families of Pastor Guy Banza, Aaron Banza, Apostle Leon Mwamba, Alain Nkole, Oliver Kahanzi, Tabitha Longo and Rachel Longo. To Congovets community, my family and friends, I really appreciate your support and prayers.

To Emmanuel Mbuyi, for all the moment passed together and encouraging each other may God bless you.

My thanks to Ulrich Mwimbi for his assistance and encouragement throughout this period.

ABBREVIATIONS

ARC-API:	Agriculture Research Council-Animal Production Institute
CMT:	California mastitis test
CNS:	Coagulase negative staphylococcus
CP:	Crude protein
Lact:	Lactose
LSM:	Least-square means
M:	Mild sensitive,
MBC:	Minimum bactericidal concentration
MIC:	Minimum inhibitory concentration
ml:	millilitre
mm:	millimetre
NS:	Not significant
R:	Resistant
S:	Sensitive
SCC:	Somatic cell count
SCS:	Somatic cell score
SEM:	Standard error of means
spp:	Species
TDMY:	Test-day milk yield

ABSTRACT

The presence of pathogenic bacteria and antibiotics residue in milk is of public health concern. Traditional and established tests of mastitis diagnosis including microbial culture-based and somatic cell counts (SCC) microbial methods are standard for good health management in dairy industry. The objectives were: to identify the major etiological agents of bovine mastitis in the dairy herd and evaluate the influence of SCC on cow's udders, to determine the effects of crude extracts of *V. infausta and E. globules* leaves on isolated mastitis causing pathogen 152 samples of milk from 38 milking cows were analysed, two SCC groups (group 1: ≤250 000 and group 2: >250 000 cells/mL) and this categorisation indicated that 76.9 % samples of the milk samples analysed were appurtenant to group 1 and only 24.1 % belonged to group 2. Milk somatic cell score (SCS) ranged from -5.6 to 2.12 (± 2.122) and milk yield ranged from 15.6 to 32.6 kg/d (±4.65). Milk fat, protein and lactose ranged from 2.99 to 4.11%, 2.78 to 3.30 % and 4.17 to 4.95 %, respectively. Mean SCC for each quarter was 2.58, respectively. From all quarter sampled, 62.5% were normal and 9.23 % had mastitis. The majority (62 %) of samples were normal and 17.8 % of teat canals were infected. Prevalence and distribution of isolated pathogens from quarter of the milk samples collected were arranged in 12 different SCC ranges. The results showed that 63.15% of pathogens are found in group 1 where SCC ranged from ≤ 2 ≤ 250^3 and 17.11% of pathogens in group 2 with SCC ranged from > 250 > 2900^3. Among the152 samples, 80.26% were cultured negative and 19.74% cultured positive. The results showed that the common pathogens isolated were coagulase-negative *Staphylococci* (CNS) (17 isolates, 56.66%) followed by *Streptococcus uberis* (5 isolates, 16.68%), *Enterococcus* spp (3 isolates, 9.98%), *Enterococcus faecalis* (3 isolates, 3.35%), *Micrococcus* (3 isolates, 3.35%) and contaminated (3 isolates, 9.98%). Extract of *V. infausta* did not show activity against CNS organisms at concentration lower than 0.020 g/ml and against other organisms at lower concentration than 0.025 g/ml. From 0.025 g/ml and 0.025 g/ml, CNS and other organisms, *Micrococcus*, *E. faecalis* and *S. uberis* were, respectively, inhibited. Extract of *E. globulus* exhibited activity against CNS at a concentration of 0.015 g/ml. *Micrococcus* and *E. faecalis* were inhibited from 0.020 g/ml while *S. uberis* were inhibited at the concentration from 0.025 g/ml.

Key words: *Vangueria infausta, Eucalyptus globules,* milk, teats, *Micrococcus, Streptococcus, Enterococcus*

CHAPTER 1: INTRODUCTION

1.1 General introduction

Mastitis is the most expensive and prevalent disease in dairy cattle worldwide (Castelani *et al*., 2019), showing negative impact on both animal efficiency, welfare and food security (Seegers, 2003; Heikkilä *et al*., 2018). It is multifactorial, being influenced by several factors, including environment, management, udder physiology and cow health (Crowe *et al*., 2018). Sharma *et al.,* (2012) defined bovine mastitis as mammary gland inflammation caused by *Escherichia coli (E. coli), Staphylococcus aureus (S. aureus),* and *Streptococci* and have major financial impacts on the dairy sector the worldwide (Bachaya *et al.,* 2011).

Mastitis is a bacteriological disease divided into clinical mastitis which is the inflammation with visual symptoms in the udder and / or milk and subclinical mastitis involving inflammation with no visual symptoms in both udder and milk (Lundberg, 2015). Bacteria such as *S. aureus*, *Klebsiella* spp., *E. coli*, and *Streptococc*i (*S. uberis, S. dysgalactia*) are common agents of clinical mastitis, while subclinical mastitis are caused by *S. aureus* and *S. agalactiae* (Contreras & Rodríguez, 2011). Subclinical mastitis is often hidden but can be observed through an increase in the milk somatic cell count (SCC), decreasing milk production, and subsequently result in most cases a clinical mastitis and contamination of uninfected mammary glands (Wu *et al.*, 2007).

The early detection of mastitis has been reported as important and can be done using the assessment of the udder inflammation or the organoleptic aspect of the milk (Contreras & Rodríguez, 2011). Bachaya *et al.,* (2005) categorized the udder inflammation as signs for clinical mastitis, into five aspects, which are reddening, hotness, swelling, pain and loss of milk production. The assessment of subclinical mastitis is more important, since it does reduce milk production (Abebe *et al.*, 2016) and poses the risk of transmission of major zoonosis such as brucellosis and leptospirosis (Bachaya *et al.,* 2011). Most mastitis causing pathogens are commensals to humans and animals, while passing undetected (Zadoks *et al.,* 2011). Alternative treatment during lactation period other than the antibiotics are tried in veterinary clinical practice throughout the world (Wu *et al.*, 2007). In South Africa,

contagious mastitis is accountable for approximately 50% of mastitis cases (Blignaut *et al.,* 2018). Therefore, repeated and routine assessment are needed. The bacteria causing clinical and subclinical mastitis are gram-positives and gram-negatives and are commonly treated using antibiotics (Oliver & Murinda, 2012). However, the increased usage of antibiotics to cure bovine mastitis has been a concern due to the residual effects on milk products and perceived undesirable consequences on human health (Sunder *et al.*, 2013). Besides, the use of other chemotherapeutic agents for the treatment of mastitis has not yet yielded desirable results due to several factors such as bacteria, hosts susceptibility, drug, management and other side effects induced by treatments (Du Preez, 2000). However, increased global demand for milk and milk products made elimination of antibiotics use for the treatment of mastitis difficult (Pieterse & Todorov, 2010) hence, the need for alternative treatment measures.

There has been an interest to find antimicrobial alternative products over bacteria without side effects (Chandra *et al.*, 2017). According to World Health Organization (WHO), from 252 antimicrobial drugs, 11% of antimicrobials come from plants (as cited by Rates, 2001). Regardless of a collective belief that extract from plants and their phytoconstituents are harmless, many secondary plant metabolites are toxic (Elisha *et al.*, 2016; Mendonça-Filho, 2006). The screening of *in vitro* toxicity is an obligatory perspective of the pilot safety evaluation of plant extracts and composites preceding any advance in their improvement and marketing (Eloff & McGaw, 2006).

Bacteria have developed complex strategies to evade the antibiotic attack and to survive, in a process likely accelerated by the increased use of antimicrobials (Munita & Arias, 2016). Antibiotic resistance has emerged as one of the most serious public health concerns of the twenty-first century. As a result, efforts to develop antimicrobials and study resistance mechanisms should continue in order to reduce the problem (Lopes *et al*., 2018). Alternative therapies are being researched in order to remedy the issue of bacterial resistance in dairy herds as a result of the constant use of antibiotics. These therapies, which primarily consist of plant extracts and essential oils, have been shown to be effective in controlling mastitis-causing pathogens in vitro (Lopes *et al*., 2018).

According to study run by Moshidi, (2015) a mixture of solvent extracts from leaves of *Vangueria infausta (V. infausta)* and *Eucalyptus globules* (*E. globules)* exhibit antimicrobial properties against range of bacteria including *Staphylococcus epidermis (S. epidermis)* and *Staphylococcus lutentiensis (S. lutentiensis)*. However, the occurrence of mastitis and causative agent identification around South Africa and all over the world are less investigated (Tilahun & Aylate, 2015). Moreover, the major strain of mastitis causal agents might be different from herd to herd. Therefore, the objective is to determine if the plant extracts *(V. infausta* and *E. globules)* can be used against mastitis causal agent at the dairy herd. Furthermore, to know if the extent of differences in nutrient density between mastitis and non-mastitis milk will help the management to implement feeding strategy for dairy calves.

1.2 Aim

To identify mastitis causal agents and determine antimicrobials activity of crude plants extracts from *V. infausta* and *E. globule*s against mastitis of dairy herd.

1.3 Objectives

1) To ascertain the major etiological agents of bovine mastitis in the dairy herd and evaluate the influence of SCC on cow's udders

2) To determine the effects of crude extracts of *V. infausta and E. globules* leaves on isolated mastitis causing pathogen

1.4 Hypothesis

1) Dairy herd does not present bovine mastitis agents

2) *Vangueria infausta, and E. globules* do not have any sensitivity on mastitis pathogens

CHAPTER 2: LITERATURE REVIEW

2.1 Mastitis overview

Bovine mastitis invariably imposes major financial losses on the dairy industry. (Motaung *et al.,* 2017), which is a chief concern for African dairy cattle farmers. The dairy industry in contrast to established countries such as the United States and European Union (EU) countries, the approved herds of dairy production in Africa is less desirable (USAD, 2010; Lacto Data, 2015). Over a decade, the number of milk producers diminished with a rise in average herd size (Karzis *et al.*, 2018). In South Africa milk production 2020 is estimated at 241 million litres, 2.83% less than in 2019. Milk production in 2020 is indicative of the negative farm economics that have been plaguing the sector (MPO, 2020). Milk has emerged as a crucial consumers-goods in African agriculture industries whilst its production provides farmers with income. Dairying can generate income in poor communities through sale of milk and milk products (FAO, 2018). Several factors such as milking practices, distribution, processing and consumption of dairy products might have effects in Africa, on mastitis control procedures and associated policies held in Africa with only about 30% of bovine mastitis cases being reported with a gap of approximately 70 %. (Motaung *et al.,* 2017).

Organisms like bacteria, mycoplasma, viruses, and yeasts may cause mastitis (Osumi *et al.*, 2008); with majority of cases from bacteria such as *E. coli, S. aureus, S. dysgalactiae, S. uberis, S. agalactiae, Klebsiella pneumonia (K. pneumonia)* and *Streptococcus bovis (S. bovis),* (Aarestrup *et al.*, 1995; Kuang *et al.*, 2009). Other less frequent organisms are *Brucella abortus, Pasteurella spp., Klebsiella oxytoca, Proteus spp., Mycoplasma spp., Nocardia, Pseudomonas aeruginosa, Trueperella pyogenes, Prototheca zopfii,* and *Prototheca wickerhamii* (Awale *et al.*, 2012). However mammary gland inflammatory immune system is activated by the presence of the pathogens to eliminate these but not always sufficiently.

2.2. Pathogens

Mastitis causing bacteria has been identified and categorized into distinct classes of pathogens according to the source of the associated pathogens (Pieterse & Todorov, 2010). These include infectious pathogens, opportunistic bacteria from the environment and other organisms. Some organisms are contagious pathogens and other are found in the dairy cow habitat (NMC, 2016). Some studies have shown that certain isolates in the same species may be categorized as extremely pathogenic, as less pathogenic, as contagious and as environmental (Afzal, 2015; El-Sayed *et al*., 2017), a few might indeed cause acute clinical and mild subclinical mastitis.

2.2.1 Contagious bacteria

The most common etiology of contagious mastitis are *S. agalactiae, S. aureus* and *Mycoplasma spp.* Typically present on infected cow's mammary gland or teat surface and usually during milking are the main source between infected and uninfected quarters of the mammary gland (Pieterse & Todorov, 2010).

2.2.2 Environmental bacteria

The environmental organisms (*Arcanobacterium pyogenes*, *Corynebacterium bovis, S. parauberis, S. zooepidemicus, E. durans, E. faecium, E. faecalis, Bacillus cereus, E. saccharoluticus* and *B. subtilis* can compromise host's immune system and reaction to anti-microbial treatment or quick cure can potentially vary by pathogen if hygiene and sanitation are not adequately practiced (Schukken *et al.,* 2005 and Pieterse & Todorov, 2010). These pathogens are found in the habitat of the cow, (pastures, dry-lot, bedding of cattle and the soil, sawdust and manure of housed cows) are transferred to the udder, although transmission via the milking machine is also possible (Lundberg, 2015).

2.2.3 Opportunistic organisms

Opportunistic bacteria contribute to moderate types of mastitis plus coagulase-negative staphylococci. The coagulase test is associated with pathogenicity, and coagulase-negative strains are considered non-pathogenic (Quinn *et al.,* 1999). They happen commensally and may be secluded from milk. They include *S.*

chromogenes, S. simulans, S. epidermidis (De Vliegher *et al.,* 2003) and *S. saprophyticus* (Dos Santos Nascimento *et al.,* 2005).

2.2.4 Other organisms

The source of infection is increased when cows are exposed to flies (during the rainy season with high humidity) infected by *A. Pyogenes* and *Peptostreptococcus indolicus* strains. This is commonly observed in non-lactating animals (Sol, 1984).

2.3 Classifications of mastitis

Mastitis can display itself in a variety range of symptom severity. The severity of the disease and the displayed symptoms are attributed to factors, such as the immune or nutritional status of the animal, a causative agent of the infection and habitat factors. When the balance between host immune response and causative pathogens cause a noticeable inflammatory response, symptoms become clearly visible (Kong *et al.,* 2015). The types of mastitis should be to recognise which prophylactic measures or curative regimen treatments to use. Based on their causative agents, mastitis is classified as contagious, environmental and summer mastitis (Kibebew, 2017).

2.3.1 Clinical mastitis

Clinical mastitis is an inflammatory reaction to infection characterized by the occurrence of apparent atypical milk (e.g., fibrin like clots, colour); at certain degree there are changes in the udder (inflamed, hotness, pain, redness) (Kibebew, 2017). Clinical cases including only local symptoms are considered moderate or mild. If the inflammatory reaction involves arise of the temperature, loss of appetite and life-threatening condition, the case is serious. When the onset is exceedingly quick, it is said to be an acute case of severe mastitis (Abebe *et al*., 2016). In seriously affected quarters, cows appear to have more secretions, and treatment should immediate and aggressive.

2.3.2 Sub-clinical mastitis

According to Abebe *et al.* (2016), sub-clinical mastitis is the absence of visible symptoms of infection, such as localised inflammation or systemic implication. In most cases coagulase negative staphylococci is the principal source of the infection (Waage, 1999). When an infection lasts for at least 2 months, it is called chronic mastitis. The definition of subclinical mastitis varies from one animal species to another. According to Contreras & Rodriguez (2011), if somatic cell count (SCC) is greater than 200 000 cells/mL, it is defined as subclinical mastitis for dairy cows. Subclinical mastitis has no detectable effects on mammary gland or milk quality (De Graaf & Swinger, 1996; Karimuribo *et al.,* 2006). Yet it has major effects on the composition of milk due to SCC increase (Iraguha *et al.,* 2017) and huge losses to milk processing industry (Romero *et al.,* 2018). An inflammatory udder that lasts for a very long period and can continue between lactations (Thompson-Crispi *et al.,* 2014). can occur as subclinical type, but may display subacute or acute intermittent flare-ups, which last for a short period of time. The affected quarter may become hard due to fibrous indurations.

2.4 Diagnosis of mastitis

In order to prevent economic losses due to mastitis, it is very important to detect the disease at an early stage. Unlike the clinical form, neither milk nor mammary gland irregularities are visually observed in the subclinical form. Awareness of routine diagnostic screening tests for early diagnosis of mastitis is thus desirable in order to treat the disease and prevent potential economic losses (Galdhar & Roy, 2003). Clinical observation or physical examination of the udder, California Mastitis Test (CMT) and white side test were initially the main field diagnostic tests, while laboratory-based approaches were Somatic Cell Count, culture and isolation (Rossi *et al*., 2018). Physical examination of udder, California Mastitis, Somatic cell count and others such as electric conductivity, pH, biochemical changes and non-specific culture assessing total bacterial count detect physico-chemico-biology alterations (Schabauer *et al*., 2014).

2.4.1 Visually observation and palpation

Udder visual inspection and palpation of the udder prior to milking the cow is part of all milking routines. Mastitis causes swelling, reddening, hardness, heat, and pain in the udder, which can often be identified with a quick inspection but relying on udder inspection as the first line of mastitis diagnosis is that udder changes are detectable late in the process, so significant losses have already occurred by the time disease is identified. Later diagnosis requires later treatment, which is less successful than early treatment and raises the risk of disease transmission.

2.4.2 California mastitis test

The California mastitis test (CMT) is the decisive screening assessment for subclinical mastitis. It is a simple indicator of SCC of milk. The CMT tests milk from distinct quarters nevertheless it can be used on composite quarter milk samples and bulk milk samples (Duarte *et al.,* 2015).

2.4.3 Somatic cell count

Milk somatic cells are mainly white blood cells acting as a deterrent against infections and restore injured tissues (Pretorius, 2008). The milk SCC checks the level of inflammatory somatic cells and is a diagnostic tool for subclinical mastitis and is accepted internationally as a criterion for assessing mammary gland health of a cow (Cardozo *et al.,* 2015). The SCC from a healthy udder of a cow is between 70 000 to 100 000 cells/mL, depending on breed, age milk yield and stage of lactation (Sawa *et al*., 2015). The SCC in excess of 100 000 per mL, is a sign inflammation. It has been suggested that a cup with 200 000 cells/mL is sometimes used to differentiate subclinical mastitis from healthy udders but lower or higher cut off values are being used in some studies (Skoulikas *et al.,* 2018).

2.4.4 Bacteriological culture

Bacteriological culture is essential to determine the types of bacteria causing the infections. It is not possible to identify these bacteria simply by looking at the milk, udders or SCC. Isolation of infectious agents frequently requires specialized media

for bacteriological culture. Nonselective (non-inhibitory) media permit the growth of many microorganisms. Selective media contain inhibitory substances that permit the isolation of specific types of microorganisms (Washington, 1996), colony and cellular morphology may permit preliminary identification. Growth characteristics under various conditions, utilization of carbohydrates and other substrates, enzymatic activity, immunoassays, and genetic probes are also used (Washington, 1996). Samples usually are collected from each quarter. Dirt and water are removed from the teats and udder; Hands washed and dried. It cannot be used 'on-site' and the waiting time for results can be days (Viguier *et al.,* 2009).

Procedures:

- Strip out the foremilk; disinfect the teat ends with alcohol and/or teat dip (do the far teats first); remove the cap from the sterile sample tube taking care to prevent contamination inside the tube; collect sample with tube at 45 degree angle to teat (collect the near teats first); replace cap on the tube and dip the teats.
- Plating the samples requires special bacteriological medium, conditions of laboratory and personnel with good technique in microbiology. Agitate 0.01 ml milk/quarter of blood agar plate using a loop or pipette. For coliforms plate 0.1 ml milk/half of plates of Blood agar and MacConkey agar plates. Incubate at 37 C for 48 hours and the bacterial colonies that grow are identified.

2.4.5 Infra-red thermography

Infra-red thermography is a simple, effective, onsite and non-invasive type of diagnostic method that is based on heat or thermal difference of skin or udder surface (Higher temperature in clinical and lower in subclinical); hence reflecting in the form of images, which are helpful in diagnosis of inflammation of udder (Sathiyabarathi *et al*., 2016).

2.4.6 Physiochemical diagnostics

Numerous physical, biochemical and / or markers that are altered during (subclinical) mastitis in milk. The physical ones include general appearance of milk, electrical conductivity (Khatun *et al*., 2018), and pH (Ondiek *et al*., 2018) and the biochemicals include various metabolic substances such as lactose (Pyörälä, 2003; Qayyum *et al*., 2016), proteins (amyloid A) (Hussein *et al*., 2018), peptides (Mansor *et al*., 2013),

and enzymes (N-acetyl-b-D-glucosaminidase) (Kalmus *et al*., 2013), Lactate Dehydrogenase (LDH) (Afaf *et al*., 2016), Alkaline phosphatase (ALP) (Patil *et al*., 2015; Afaf *et al*., 2016), or milk arginase (Kandemir et al. 2013). Detection of these markers has evolved from conventional spectrophotometry to various novel diagnostics, like immunoassays (Addis *et al*., 2016; Hussein *et al*., 2018). These have high specificity and sensitivity but less field applicability.

2.5 Treatment overview

According to Modi *et al.* (2012), antimicrobial therapy treatment of mastitis assists with normalisation of milk production and its composition, mortality prevention, elimination of the causative pathogens and practices that may lead to drug compounds in edible tissues and milk of animals. The treatment of mastitis choices is centred on bacteriological culture and antimicrobial susceptibility testing (Lundberg, 2015). Furthermore, prognosis is set on by pathogen, antimicrobial exposure, the amount infection, cow age and number of affected quarters.

2.5.1 Antibiotics

The use of antibiotic is to destroy or slow down the growth of the mastitis - causing pathogens from the cow or at least in the affected quarter of udder. Successful therapy can be measured by evaluating the diverse cure levels such as bacteriological and disease-free survival of treated animals against those free from the disease. To maintain and/or optimize the designated cure rates by implementing scientific based evidence treatment notion is necessity (Trevisi *et al.,* 2014, Krömker & Leimbach, 2017). The proposed combined intervention scheme is based on regular assessment of animal wellbeing parameters, consistent clinical inspections and, and use predictive and reliable laboratory analyses.

According to Mansion-de Vries *et al*. (2015), treatment of mastitis should be targeted towards the causative bacteria whenever possible, but in acute situations, treatment is initiated based on herd data (Pyörälä, 2009). Rapid or on-farm bacteriological diagnosis would facilitate the selection of the most appropriate antimicrobial. The use of on-farm protocols for mastitis treatment can promote judicious use of antimicrobials (Passantino, 2007; Raymond *et al.*, 2006). Therapeutic response of

the cows can be monitored using individual somatic cell count data if available, or using the California Mastitis Test, and with bacteriological samples in herds with contagious mastitis.

In dairy cattle antimicrobial use happens in case of clinical mastitis by applying antibiotic ointments to the mammary of lactating cows and additional antibiotics are administered through the teat into mammary gland in case of acute mastitis (Gomes & Henriques, 2016; Oliveira & Ruegg, 2014). Second, local antibiotic treatment at the onset of dry period has reduced the occurrence of mastitis and recommended for all cows at dry-off (Krömker & Leimbach, 2017).

The antibiotics (cephalosporin, streptomycin, penicillin and tetracycline) are utilized for curative and prophylactic purposes in maladies caused by gram-positive and gram-negative bacteria in dairy cows (Oliver & Murinda, 2012). However, the usage of antibiotics to fight bovine mastitis resulted in diminished milk and dairy products quality with antibiotic residues and favoured the increase of multidrug resistant (Dhanabalan *et al.,* 2008). The cost effectiveness of treatment during lactation is driven by the interaction between the value of discarded milk for not being safe for human consumption and the potential benefits of treatment. Given the extensive usage of different antibiotics, antibacterial mastitis cure produced less desired results (Ziv, 1980). Antibiotic therapy cannot be relied on as the only anti-mastitis intervention to reduce the occurrence of mastitis (Vaibhav *et al.,* 2013). There is a need to develop alternative therapeutic approaches for the management of acute and subclinical mastitis. Researchers drawn attention to those alternative treatments such as homeopathy, clay therapy and phytotherapy (Baars & Hamre, 2017).

2.5.2 Homeopathy

Few homeopathy studies have been conducted in animals (Hektoen *et al.*, 2004). This therapy uses natural remedy to stimulate the body defences of recovery (Chandel *et al.*, 2010). Farmers use homeopathic remedies because they are cheaper compared to conventional treatments (Ruegg, 2008). The selection of specific homeopathic remedies is done based on symptoms, such as lumpy milk and oedema type (Duval, 1995). In E. Coli induced clinical mastitis, with active treatment of Wolfsbane (aconite), pokeweed (*Phytolacca decantra*), wild hops (bryonia) and

subsequent treatment of pokeweed, wild hops, Bushmaster (Merck *et al*., 1989) and M*ercurius solubilis* (Hanif *et al*., 2017), better results were obtained. In subclinical mastitis homoeopathy is reported to have a poor effect (Walkenhorst *et al.,* 2001). The effects of the treatment may be observed in cases of acute mastitis compared to a cow with chronic mastitis.

2.5.3 Clay therapy

Clay poultice has been shown to be effective against mastitis-related inflammation due to its adsorbent potency of certain toxins (Duval 1995). The white, green or grey clay is mixed with water, olive oil or a 50/50 mixture of the two ingredients. The final mixture should be liquid but sufficiently adherent and should be applied to the udder two to three times a day after milking. The treatment for mastitis will be said to have worked when the teats stay oily after the dry oil-clay mixture has been removed. In acute mastitis, the results will be seen after 4-6 hours. In chronic mastitis, treatment will take two to three days. If healing signs are not seen, seek alternative mastitis treatment.

2.5.4 Phytotherapy

Phytotherapy is a therapy where plants, plant parts and their products are used as remedies (Tamminen, 2018), in South Africa, a country with a strong history of traditional healing, hosts a variety of around 30,000 flowering plant species (Street & Prisnloo, 2013). Plant therapies are often used in the form of extracts, sap and powder. Several plants are boiled to create extracts that can be applied, while others are applied directly in the fresh form. Though phytotherapy is gaining momentum for both human and animal drug research, drug formulation, dosage and route of administration need to be standardised (Blanco-Penedo *et al.,* 2018). It is important to confirm and demonstrate the relationships of ethno medicinal usages, bioactive substances, biological and pharmacodynamic effects and their mechanisms of action (Atanasov *et al.,* 2015).

Pressure to discover new antibacterial molecules with different modus of action targeting non-essential cell processes led to plant sources being exploited to identify new and efficient antibacterial agents (Simões & Bennett, 2009). Phytochemicals'

mechanisms may differ from usual antibiotics. This feature could deal with resistant bacteria. Plants and extracts can serve as a novel and substitute source of antibiotics. Antibacterial agents in plants can target the virulence of the bacterial and this category is still under exploited (Mushtaq *et al.,* 2018).

The use of kelp and marine algae has preventive effects on mastitis and contains many beneficial minerals to the animals (Pérez *et al.,* 2016). According to Jost (1984), an ointment made from bacon and marigold flowers is beneficial for mastitis. *Aloe vera* is used to treat the wounds of the worst that often cause staphylococcal mastitis, but the milk should be destroyed after this treatment (Hekmatpou *et al.,* 2019). According to Moshidi. (2015) a mixture of solvent extracts from leaves of *V. infausta, and E. globules* exhibit antimicrobial properties against range of bacteria including *S. epidermis* and *S. lutentiensis*, which are two strains known to cause mastitis in dairy cows. These antimicrobial effects are believed to be associated with the presence of different secondary metabolites (Compean & Ynalvez, 2014), such as alkaloids, anthraquinones, flavonoids, glycosides, phenols, saponins and tannins, as described by Jigna & Sumitra (2006).

2.5.4.1 *Vangueria infausta*

Vangueria infausta grows as a shrub and to a small deciduous tree, in wooded grassland, bushveld and between land and sea (van Wyk & van Wyk, 1997). This tree can also be found in woodlands, scrub, on kopjes or in sandy valleys (Seigler, 2003). The plant species is widespread in southern Africa, the countries of East Africa and Madagascar (Orwa *et al.*, 2009) and its height is between 3 - 7 m based on the environment or habitat type. Its fruits are almost circular; when young, silky dark green and a light brown colour when mature (Bridson, 1998).

2.5.4.1.1 Medicinal uses

In eastern and southern Africa, *V. infausta*'s leaves, fruits, seeds, bark, shoots and roots are used for human medicinal purposes (Maroyi, 2018). Gastro-intestinal disorders, malaria, pneumonia and more sicknesses, are treated in humans using concoctions prepared from *V. infausta* (Munodawafa, 2012).

2.5.4.1.2 Phytochemical compositions

Phytochemicals from *V. infausta* include anthraquinones, alkaloids, coumarins, polyphenols, glycosides, steroids, tannins, saponins, secoiridoids and terpenoids (Munodawafa *et al.,* 2013). Several biological activities are linked to these phytochemicals. Chaves *et al*. (2016), showed that the antioxidant, anticholinesterase, anti-inflammatory, anxiolytic and antidepressant properties of alkaloids extracted from plants are their characteristic. The anthraquinones contain antimicrobial and anti-inflammatory properties (Malik & Muller, 2016; Maroyi, 2018). The coumarins have pharmacological actions such as anti-HIV, anti-inflammatory, antimicrobial, anticancer, anticonvulsant, anti-TB, anti-depressant, antioxidant and anti-coagulant (Srikrishna *et al*., 2016; Maroyi, 2018).

2.5.4.2 *Eucalyptus globulus*

Eucalyptus globulus is indigenous plant to Australia and is broadly introduced to other countries worldwide due to their rapid growth and the manufacture of paper and plyboard (Zhang *et al.*, 2009). Almost all eucalyptus trees are evergreen, but a few tropical species lose their greeneries toward the end of the drought season (Potts & Reid, 2003). The *E. globulus* extracts are used as an antibacterial; antifugal; antidiabetic; antitumor; antiplaques; antiviral; anti-inflammatory; antimalarial; anti-HIV; antioxidant; larvicidal; insecticidal; nerve blocker; in respiratory diseases and ayurvedic cleanser for oily skin; tea; tree oil eucalyptus soap (Vishin *&* Nitave, 2014).

2.5.4.2.1 Medicinal uses

Extracts of *E. globules* have an antimicrobial ability that are found to be effective against pathogenic microorganisms implicated in urinary tract infections, infections of the gastrointestinal tract and typhoid fever. Eucalyptus leaves are used to heal wounds and fungal infections and they show many activities such as antioxidant, antiseptic and anti-inflammatory (Amabye *et al*., 2016; Rai *et al.,* 2013). Besides antimicrobial activity, the essential oil and its components also display herbicidal, insecticidal, anthelmintics, anti-tumour and anti-leech activity (Shubhreet *et al*., 2019).

2.5.4.2.2 Phytochemical compositions

Eucalyptus extracts are approved as food additives and are currently used in various cosmetics formulation (Kaur *et al.,* 2019). Saponins, tannins, steroids and flavonoids have been found in the leaf extract of eucalyptus and possess antimicrobial activity (Sartorelli *et al.*, 2007).

CHAPTER 3: MATERIALS AND METHODS

3.1 Study area

The study area, ARC-API (Agriculture Research Council-Animal Production Institute), at Irene, Gauteng province, South Africa with E 25º 53' 59.6", S 28º 12' 51.6" latitude and altitude respectively.

3.2 Animals

Research Ethics Committees from both the University of South Africa (UNISA) and Agriculture Research Council-Animal Production Institute (ARC-API) approved the proposal prior to the commencement of data gathering and have not acted outside the approval conditions. Thirty-eight mid-lactating (150-250 average days in milk) Holstein cows were selected from the ARC herd of lactating cows. Samples were taken from quarter on the cow prior to milking but after odder examination and preparation, resulting in 152 milk samples that were collected and analysed at the laboratory Veterinary Science of the University of Pretoria.

3.3 Detection of mastitis

Four different steps (physical examination of udder, milk physical examination, CMT and SCC of milk) were adopted for the assessment of mastitis to differentiate the clinical and subclinical mastitis. The diagnostic methods used were prescribed by Elbably *et al.* (2013) and Girma *et al.* (2012).

3.3.1. Udder examination

Physical examination of udder of mammary glands is important for successful detection of mastitis. It is used to examine the shape, size, consistency and contour of the udder. Thorough inspection of the teat and teat orifices should be made to assess inflammation, hot painful swelling and loss of function (Varshney, 2000).

3.3.2 Milk collection

Udder and teats were prepared according to the method prescribed by Sirois (2017). Udder and teats were meticulously scrubbed using antiseptic soap and dried with a towel before the collection of milk sample while all dirt such as particles of soil and other filth was also removed from teats and udder surface using the soft brush. Teats were scrubbed with a 70% alcohol solution, first for the teats on the far flanks of the udder, and the teats at the closest side to avoid re-contamination. The milk samples were collected from each quarter and analysed using CMT before milking the cow.

3.3.3 Milk examination

Milk coming from each mammary quarter was examined for colour (against physiological and pathological discoloration), for viscosity (decreasing or increasing of milk consistency) appearance and for clots, flakes, blood, pus presence and watery discharges. Its odour (putrefied, faecal, acetone, rancid, and antiseptic) was also examined. All the milk physical changes were done using the strip cup test.

3.3.4 California mastitis test

The California mastitis test (CMT) was used as a screening test for sub-clinical mastitis. It was carried out according to the procedure described by Abebe *et al*. (2016) and Quinn *et al*. (1999). A squirt of milk, about 2 ml from each quarter was placed in each of four 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 mixtures in a horizontal plane for 15 s. Positive and non- positive samples were kept for a maximum of 24 hours at 4°C for bacteriological analysis.

Thirty-eight mid-lactating (150-250 average days in milk) Holstein cows were selected from the ARC herd of lactating cows and screened for CMT.

3.3.5 Somatic cell count

Somatic cells were counted by fluoro-opto-electronic means using a Fossomatic 5000 (Rhine Ruhr, P.O. Box 76167, Wendywood 2144, South Africa)**.** Radostits *et al.*, (1994) noted that somatic cell count more than 250 000/ml was indicative of inflammation, whereas counts less than 100 000/ml was indicative of normal udder and counts more than 500 000 cells/ml was indicative of infection.

3.4 Milk chemical composition

Composite of milk samples were prepared from the mornings and afternoons milking and was analysed for fat and crude protein (CP) (AOAC 984.13., 2000), lactose, milk urea nitrogen utilising infrared spectrometry (HRN EN ISO 9622:2001) as reported by (Bendelja *et al.*, 2009) and somatic cell counts (McFadden, 2011).

3.5 Bacteriological examination

Milk was plated out on bovine blood tryptose agar (BTA) (Oxoid, supplied by Quantum Biotechnologies [Pty] Ltd, Ferndale, South Africa). Inoculated agar plates were incubated at 37 °C ± 1 °C and plates were examined after 18–24 h and 48 h. In addition, all samples from clinical mastitis cases were enriched, using 0.5 mL of milk added to 5.0 mL Brain heart media and incubated at 37 °C for 18–24 h. Isolated bacteria were identified in accordance with standard laboratory milk culture methodology based on colony morphology, as described by the IDF Document 132 of 1981. Tests used included Staphylase, Strepkit, Catalase, DNase, KOH (Oxoid, supplied by Quantum Biotechnologies [Pty] Ltd, Ferndale, South Africa), Maltose (Merck NT Laboratory Supplies, Halfway House, South Africa) and API® 20E and Staph API® (bioMérieux South Africa [Pty] Ltd, Randburg, South Africa). The plates between 30 - 300 colonies were considered, since the healthy cows have standard plate count lower than 1 000 cfu/ml.

3.6 Determination of the antibiogram

The Kirby–Bauer disk diffusion method was used and adapted for testing antibiotics sensitivity and adapted to the study. Seven antibiotics were used to determine the antibiogram of isolated bacteria, which were tylosin, penicillin, ampicillin, cloxacillin, oxytetracyclines, cephalexin and cefuroxime. In this study, the sensitivity results were classified as high sensitive (HS), sensitive (S), mild sensitive (M) and resistant (R). Samples were examined using microbiological and cytological tests according to PAS/Manual 002, Appendix 5, at South African National Accreditation System (SANAS) accredited Milk laboratory of the Faculty of veterinary science department of production animal studies.

3.7 Plant materials

3.7.1 Origin and preparation of plant materials

The trees were systematically identified, and the leaves were collected from mature trees. The plant leaves were collected in Ga-Masemola Village, in Limpopo province, South Africa during early wet season. The leaves were then removed from the stems and dried for two weeks at room temperature. The dried leaves were ground into fine powder using a "new Dietz grinder," and the powder was packed in plastic bottles and kept at room temperature for further study.

3.7.2 Preparation of plants extracts.

Fresh collected leaf samples were washed with distilled water and air-dried for five days to constant weight. A household electric blender was then used to mix the dried leaves. Leaf powder was processed for further examination and sealed in labelled reagent bottles. Extraction methods of bioactive components was done as recommended by Akerele *et al.* (2008).

3.7.3 Antimicrobial sensitivity test

The efficiency of plant extract mixture as modifier is based on additive of individual properties. Hence different combinations were tested and the mixture having higher level of inhibiting effect on gram positive bacteria was selected for further evaluation. According to Salie *et al.* (1996), disc diffusion method was utilised to assess anti-microbial activities using a series of isolated strains of gram negative and positive bacteria.

3.7.4 Assay for antibacterial activity

The antibacterial assays of aqueous extracts from the leaves of *V. infausta* and *E. globulus* were performed on the two majors' pathogens (*S. uberis* and *E. faecalis)* isolated for the farm and on two other major clinical microbial strains (*S. aureus* and *S. dysgalactiae*) provided by microbiology laboratory. The assays were performed using the agar disc diffusion method according to Jamine *et al.,* (2007). According to antibacterial activity and classified plants with a zone of inhibition 0 - 0.3 mm having low activity, 0.3 -0.7 mm zone of inhibition medium activity and 0.7-1.0 mm zone of inhibition as high activity.

3.7.5 Minimum inhibitory concentration and minimum bactericidal concentration

The extract's minimum bactericidal concentration (MBC) value was determined as the lowest concentration which fully inhibited bacterial growth at 37 ºC after 48 hours of incubation. A 5 µl from each plate with growth was taken for the determination of MBC, and then for 24 hours incubation at 37 ºC. The lowest concentration with no noticeable growth of the bacteria after subculture was taken as MBC. Positive and negative cultures were also prepared. The accepted values were < 1 mg/ml for extracts or 0.1 mg/ml for isolated compounds (Rios & Recio, 2005).

3.8 Statistical analyses

The data sets of the SCS test results were divided into 15 separate SCC dimensions ranging from 1 560 to > 12 800 000 cells/mL. The limits were determined by logarithmic partition of the SCC scale according SCS = log2 (SCC/100 000) + 3 (Ali & Shook, 1980). The data subset was analysed using the SAS 9.4 (2018) MIXED protocol, using the following model:

$Yijk = \mu + Hi + Qj + eijk,$

Where Yijk = observed SCS in herd i and udder quarter j of cow k; μ = overall mean; Hi = random effect of herd i (i = 1 to 1,338); Qj = fixed effect of quarter j (j = 1 to 4); and eijk = random error term.

CHAPTER 4: RESULTS

4.1 Distribution of SCC

Results indicated that SCC of the milk samples ranged from 1 000 to 10 407 000 cells/ml (Figure 1). The distribution of all milk samples in the 12 SCC ranges and 62 quarters, representing 40.7% showed inflammatory reaction with SCC more than 100 000 cells/mL). The SCC of the most quarters (59.3%) were in a physiological range that showed no inflammatory reaction (≤100,000 cells/mL).

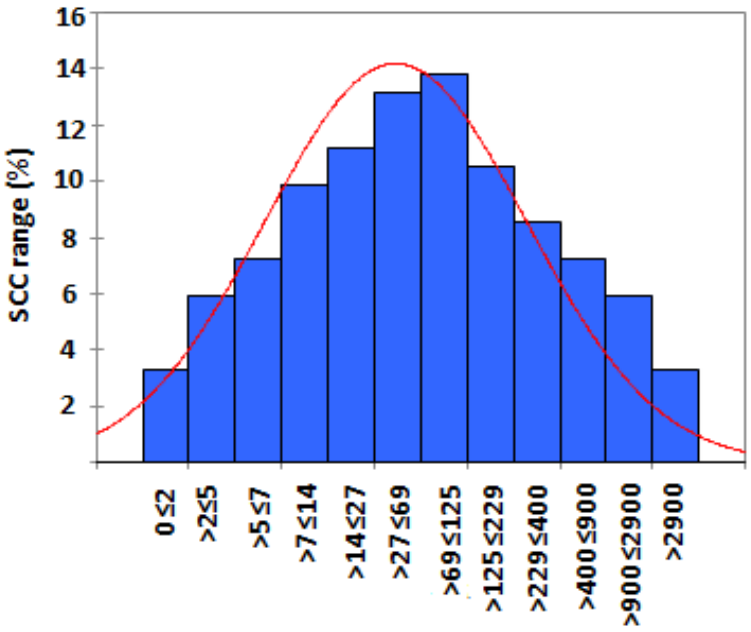

Figure 1: Distribution of SCC of all milk (n = 152) by classifying into 12 SCC ranges (n = 152)
The line shows the distribution of SCC estimated by the Gaussian (Figure 2).

Results indicated that majority (76.9 %) of the quarter milk samples analysed contained less than 250 000 cells/mL. This categorisation belonged to group 1(≤250 000) and only 24.1 % belonged to group 2 (>250 000 cells/mL).

4.2 Milk composition and correlation amongst milk parameters

The herd analysed for the presence of mastitis based on the SCS revealed the absence of the disease. From the 38 milking cows analysed, the milk SCS ranged from -5.6 to 2.1 (± 2.122) and milk yield ranged from 15.6 to 32.6 kg/d (±4.65). Milk fat, protein and lactose ranged from 2.99% to 4.11%, 2.78 % to 3.30 % and 4.17 to 4.95 %, respectively. A summary description of milk parameters (Table 1).

Table 1: Milk parameters per number of cows (n = 38)

Variable	n	Minimum	Maximum	Mean
Milk SCS	38	-5.6	2.1	-1.5 ± 2.12
Test-day milk yield, Kg	38	15.62	32.58	25.73 ± 4.65
Milk fat (%)	38	2.99	4.11	3.38 ± 0.24
Milk crude protein (%)	38	2.78	3.30	3.06 ± 0.10
Milk lactose (%)	38	4.17	4.95	4.59 ± 0.15

The correlation between SCS and lactose was negative and significant (-0.970). As can be observed in Table 2 a negative relationship was found between milk lactose concentration and SCS. No significant relationship was observed between all other parameters.

Table 2: Correlation coefficient obtained for SCS, test-day milk yield and fat (%), crude protein (%) and lactose (%)

Variables	SCS	Test-day milk yield, kg	Fat (%)	CP (%)	Lact (%)
SCS	**1**	-0.113	0.183	0.217	-**0.970***
Test-day milk yield, kg		**1**	0.100	-0.105	0.133
Fat (%)			**1**	0.246	-0.168
CP (%)				**1**	-0.259

*Significant at the 1% probability level

The relationship between milk lactose concentration and milk SCS were evaluated and presented in Figure 1. There was a strong relationship (R^2 = 0.94; P <0.001) between lactose concentration and milk SCS. The equation was milk lactose (%) = -0.0701 SCS + 4.8696 (R^2 = 0.94; P <0.001).

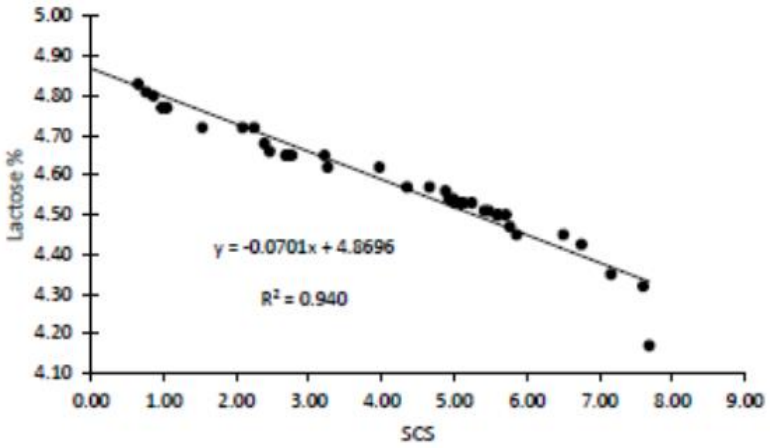

Figure 2: Linear regression of lactose on SCS

4.3 Major pathogens and prevalence in cow's quarter milk samples

The prevalence of pathogens in the 152 milk samples analysed is presented in Table 3. Among 152 samples, 122 (80.26%) were cultured negative and 30 (19.74%) cultured positive. The major pathogens isolated were coagulase-negative

staphylococci (CNS) (17 isolates, 56.66%) followed by *S. uberis* (5 isolates, 16.68%), *Enterococcus spp* (3 isolates, 9.98%), *E. faecalis* (3 isolates, 3.35%), *Micrococcus* (3 isolates, 3.35%) and culture considered as contaminated (3 isolates, 9.98%).

Table 3: Prevalence of pathogens in milk samples (n = 152)

Item	n	All quarters (%) [1]	Culture-positive (%) [2]
Quarters analysed bacteriologically	**152**		
Culture negatives	**122**	**80.26**	
Culture positives:	**30**	**19.74**	
Streptococcus uberis	5	3.29	16.68
Enterococcus faecalis	1	0.66	3.35
Enterococcus spp	3	1.97	9.98
Coagulant negative *Staphylococcus*	17	11.18	56.66
Micrococcus	1	0.66	3.35
Contaminated samples	3	1.97	9.98

[1]% of all quarters
[2]% of all culture-positive quarters

The percentage of prevalence and distribution of pathogens was isolated from quarter foremilk samples, collected and arranged in 12 different SCC ranges (Table 2). The results show that 63.15% pathogens are found in group 1 where SCC ranged from ≤ 2 ≤ 250[3] and 17.11% of pathogens in group 2 where SCC ranged from > 250 > 2900[3].

Table 4: Pathogens (%) isolated from milk collected from the dairy herd arranged in 12 different SCC ranges

Groups (x 1000)	Major			Minor		No pathogen	Contaminated
	Streptococcus uberis	*Enterococcus faecalis*	*Enterococcus spp*	CNS[2]	*Micrococcus*		
Group 1							
≤2	0.00	0.00	0.66	0.66	0.00	1.97	0.00
>2≤5	0.00	0.00	0.00	0.00	0.00	5.92	0.00
>5≤7	0.00	0.00	0.00	0.00	0.00	7.24	0.00
>7≤14	0.00	0.00	0.00	0.00	0.66	9.21	0.00
>14≤27	0.00	0.00	0.00	1.32	0.00	9.87	0.00
>27≤69	0.66	0.00	0.66	0.00	0.00	11.84	0.00
>69≤125	0.00	0.00	0.00	4.61	0.00	9.21	0.00
>125≤250	0.00	0.00	0.00	2.63	0.00	7.89	0.00
Group 2							
>250≤400	0.66	0.00	0.00	1.32	0.00	5.92	0.66
>400≤900	1.32	0.66	0.66	0.00	0.00	3.95	0.66
>900≤2900	0.66	0.00	0.00	0.66	0.00	3.95	0.66
>2900	0.00	0.00	0.00	0.00	0.00	3.29	0.00

[1]Somatic cell counts.
[2]CNS: Coagulase negative Staphylococcus.
[3]Contaminated samples
It shows that 63.15% pathogens are found in group 1 where SCC ranged from ≤ 2 ≤ 250[3] and 17.11% of pathogens in group 2 where SCC ranged from > 250 > 2900[3].

4.4 The effect of quarter positions on milk SCS

The influence of quarter position on milk SCS of sampled milk samples is presented in Table 5. The milk SCS for all quarters averaged 2.58 and did not significantly differ (P> 0.05) among all four quarters.

Table 5: Means of SCS in four udder quarter positions from quarter foremilk

Quarter Position	SCS	
	LSM	**SEM**
Front right	2.74	0.11
Rear right	2.58	0.11
Front left	2.52	0.12
Rear left	2.49	0.11

SCS: Somatic cell score; LSM: Least square means; SEM: Standard error of mean. LSM in the same column with no superscripts do not differ significantly (P> 0.05)

4.5 Antibiogram of mastitis causing pathogens.

Table 7. shows that, among bacteria, the coagulase negative *Staphylococcus* was highly sensitive to cloxacillin, oxytetracycline, cephalexin and cefuroxine. *Microccocus* were highly sensitive only to cefuroxine. *Enterococcus faecalis* and *S. uberis* were sensitive to penicillin, ampicillin, cephalexin and cefuroxine.

Table 6: Antibiogram/antibiotic sensitivity

Bacteria	Tylosin	Penicillin	Ampicillin	Cloxacillin	OxyTetracycline	Cephalexin	Cefuroxime
Coag. neg. staph	S	R	R	HS	HS	HS	HS
Micrococcus	M	R	R	M	R	S	HS
Enterococcus faecali	R	S	S	R	R	S	S
Streptococcus uberis	M	HS	S	R	R	HS	HS

Antibiotics sensitive: HS = highly sensitive, S = sensitive, M = mild sensitive, R = resistant.

4.6 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *V. infausta* and *E. globules*

The antimicrobial activities of *V. infausta* and *E. globulus* against the isolated organisms are in Table 6 (antimicrobials test). The *V. infausta* MIC ranges from 0.7mg/ml to 14.3 mg/ml, respectively of the isolated organism. The extracts of *V. infausta* did show activity against *Coag. Neg. staph, Micrococcus*, *E. faecalis* and *S. uberis* organisms at all varied concentrations.

Table 7: Inhibitory zones (mm) by *V. infausta* and *E. globulus* using different concentrations on four pathogens

	Vangueria infausta					***Eucalyptus globulus***				
Concentration MBC[1]	**0.010**	**0.015**	**0.020**	**0.025**	**0.030**	**0.010**	**0.015**	**0.020**	**0.025**	**0.030**
CNS[2]	0.7	9.1	11.4	13.2	13.2	9.1	11.4	12.3	15.4	15.3
Micrococcus	0.7	9.9	9.3	14.5	14.3	-	9.4	10.7	11.6	11.4
Enterococcus faecalis	2.4	-	1.3	10.8	11.2	2.4	1.7	10.5	13.3	12.3
Streptococcus uberis	-	0.7	9.5	10.6	10.13	-	-	9.7	10.0	10.2

MBC[1]: Minimum Bacteria Concentration. CNS[2]: Coagulase negative staphylococci.

CHAPTER 5: DISCUSSION

5.1 Distribution of SCC

Results indicated that large majority (76.9%) of the quarter foremilk samples contained less than 250 000 cells/ml. This is an indication that about ⅔ of all foremilk samples were within the physiological range of 250 000 cells/ml of SCC, when a cut-off value of bulk milk SCC currently used in South Africa was analysed (Petzer *et al.*, 2017). Thus, the quarter foremilk samples, with low somatic cells count in this study, are healthy quarter (Kivaria *et al.*, 2007).

High SCS is not only associated with udder health and milk loss (Talukder & Ahmed, 2017), but also may affect the milk composition and processing ability of milk, and shelf-life of the pasteurized milk (Barbano *et al*., 2006). However, several factors could contribute to the results of low number of SCS, such as age, breed, stage of lactation of the cow and environmental factors (Bhutto *et al.*, 2012). Our results show that, SCS of 2.58 are in support of Capuco & Akers, (1999) who stated that healthy cows maintaining low SCC in milk, are considered as uninfected cows with 0-3 SCS and clinically infected cows have 7-9 SCS with mastitis in general. Farmers should aim to have more cows in the 0-3 category and less in category the 7-9 (Oudah, 2009).

5.2 Milk composition and correlation amongst milk parameters

The mean contents of protein, milk yield and fat were close to those reported in previous studies (Toffanin *et al*., 2015; Costa *et al*., 2018; Silva *et al*., 2018). However, lactose and SCS values were not in accordance with the results of these authors, showing a strong relationship (R^2 = 0.94; P <0.001) between lactose concentration and milk SCS. The equation was milk lactose (%) = -0.0701 SCS + 4.8696 (R^2 = 0.94; P <0.001) by Toffanin *et al*., 2015 and Costa *et al*., 2018, respectively) with a lower SCS (Toffanin *at al.*, 2015) reported mean SCS of -1.5 ± 2.12). SCC in milk besides being a method to diagnose subclinical mastitis, is highly significant for the dairy industry because milk with high value of SCC is directly associated with decreased production of dairy products by reducing shelf-life (Andrade *et al*., 2007) changes in milk composition that can affect the quality of milk

products. (Le Roux *et al*., 2003). The value found for lactose (4.56) in our study was close to those values found by other authors that ranged from 4.28 (Gonzalez *et al.*, 2009) 4.56 (Silva *et al*., 2018), to 4.76 (Costa *et al*., 2018). These findings showed as the lactose concentration in milk can vary in different locations. Variation in lactose content in milk can attributed to the management conditions, feeding of dairy herds and health of the mammary glands (Allesio *et al.*, 2016). However, lactose levels found are more constant due to fewer problems of mammary glands and, consequently somatic cell counts are lower, in addition the cows are fed with appropriate nutrition (Jenkins & Mcguire, 2006).

The current study revealed also that nothing unusual was observed regarding to colour (against physiological and pathological discoloration), viscosity (decreasing or increasing of milk consistency) appearance and for the presence of flakes, clots, blood, pus and watery secretions. Judkins & Mack (1955) and Mubarack *et al*. (2010) agreed on these findings, normal milk has been reported to have a yellowish white colour indicating fat, casein and small amounts of colouring material. The colour differences may be caused by the differences of type feed consumption or the fat as well as solid contents of the milk or breed (Khan *et al.*, 2008, Afzal *et al.,* 2011; Gupta & Patiyal, 2013). All physical characteristics observed did not show any abnormalities.

5.3 Major pathogens and prevalence in cow's quarter milk samples

The first line of defence against intra-mammary infection are udder teats. It is known that there is a good relationship between resistance to mastitis and certain hereditary such as traits of udder-type, udder attachment, genetic and udder depth, to the cow's body and milk production. These factors are associated with mastitis incidence (Sorensen *et al.*, 2000; Klein *et al.*, 2005; Ptak *et al.*, 2011; Nakov *et al.*, 2014). The selection of cows with acceptable udder and teat morphology is promoted to minimise occurrence of mastitis and enhance the quality of milk (Juozaitiene *et al.*, 2006).

Organisms that have been identified and causing mastitis are many (Aarestrup *et al.*, 1995; Kuang *et al.*, 2009 and Karzis *et al*., 2018). Major pathogens isolated in our study were coagulase-negative staphylococci (CNS) 56.66% followed by *S. uberis* 16.68%, *Enterococcus spp* 9.98%, *E. faecalis* 3.35%, *Micrococcus* 3.35%. The findings in this study are in line with Petzer *et al*. (2009, 2017) and Karzis (2018), reported that *Staphylococcus aureus* and *Streptococcus agalactiae*, are termed major pathogens and are generally regarded as those commonly associated with mastitis in dairy cattle. The *Staphylococcus aureus* is the principal cause of mastitis in South Africa. A recent review by Motaung *et al*., (2017) on mastitis in the African context, mentioned that *S. aureus, S. agalactiae, Str. dysgalactiae, Str. uberis* and *E. coli* are the most reported pathogens in Africa.

The test of prevalence of pathogens in the 152 quarter foremilk samples showed that 80.26% of samples were cultured negative and 19.74% cultured positive. The ratio in percentage of negative and positive revealed that there can be a localised infection in teat (Mellenberger & Kirk, 2001). According to Kuehn *et al.,* (2013), culture negative samples, when associated with mastitis, could lead to tissue inflammation.
The results show that 63.15% pathogens are found in group 1 where SCC ranged from ≤ 2 ≤ 250. The 10^3 and 17.11% of pathogens in group 2 where SCC ranged from > 250 > 290. 10^3. Therefore, CNS differential cell counts could be used as a better indicator for an in-depth inflammation assessment than SCC, especially at SCC levels of 100 000 cells/mL (Schwarz, 2012).

Most samples were cultured negatives in this study, which can be attributed to clean milking environment and application of milking procedures. High prevalence of environmental pathogens is a sign of poor scrubbing and preparation of the udder before milking (Petzer *et al.*, 2009). Contaminated bedding, dirty environments, skin of teats colonisation and injuries are potential cause of environmental bacteria (Alekish, 2015). The udder position did not have effects on types of pathogens identified in milk samples. *Streptococcus uberis* were more in samples with higher SCC (400 000 and 900 000 cells/mL). *Micrococcus* were identified in the sample with SCC < 250 000 cells/mL. *Streptococcus uberis* is an environmental pathogen and its presence indicates hygiene and management systems.

5.4 The effect of quarter positions on milk SCS

Recording traits for udder quarters separately allows a more differentiated assessment of milk composition and udder health, which can be used for management and breeding purposes and as indicators of udder health (Kramer *et al*., 2013). The status of udder health, as measured by SCS, is known to be influenced by breed, production level, housing system and climate, but more especially by management (Ivemeyer *et al.*, 2009). Grootenhuis (1975), Flock and Zeidler (1965) and Pantoja *et al*. (2009), observed that evaluated interdependence of quarters with subclinical mastitis, in cows with no infected quarters or four infected quarters were found more frequently than expected based on independence of the quarters. In this study the milk SCS for all quarters averaged 2.58 and did not significantly differ (P> 0.05) among all four quarters. Our findings can be supported by Barkema *et al*., (1997) who reported that interdependence of quarters is based on exposure to similar risk factors of quarters within a cow or within a herd or to the same pathogens. When several quarters are infected in the proximity of an uninfected quarter, risk of intramammary infection in the uninfected quarter is high.

5.5 Antibiogram of mastitis causing pathogens.

Antibiotics sensitivity test is important to suggest suitable antibacterial treatment to prevent antibiotic resistance, potential health risk for humans. The results in Table-7 showed that CNS were resistant to Penicillin and Ampicillin. *Micrococcus* was resistant to Penicillin, Ampicillin and OxyTetracycline, while *Enterococcus faecalis* showed resistance to Tylosin, Cloxacillin and OxyTetracycline and *Streptococcus uberis* was resistant to Cloxacillin and oxtetracycline. This may be due to increased indiscriminate and frequent use of those antibiotic in dairy animals leading to develop of antibiotic resistance bacteria which necessitates develop and search for new sources as antimicrobial agents. Plant extracts and derived compounds have increased widespread interest in the search of alternative antibacterial agents. They are found to be safe and have a long history of use in traditional medicine for the treatment of infectious diseases (Ekor, 2014). According to the World Health Organization (WHO,2002), medicinal plants would be the best source to obtain a variety of drugs and active compounds. Therefore, plants should be investigated to

understand their properties, safety and efficiency (Wangensteen *et al.*, 2013). Both plant extracts of *V. infausta and E. globules* showed activity against (*Coag. neg. staph, Micrococcus*, *E. faecalis* and *S. uberis)* organisms at all varied concentrations (Table 7). According to Rios & Recio. (2005), the value below 100 g/ml for extracts and 10 g/ml for isolated compounds are active. This could be attributed to compounds they possess, and these phytochemicals agree with those reported in other studies (Nundkumar & Ojewole, 2002, Klancnik *et al.*, 2011). Food elements and extrinsic determinants like temperature or bacterial properties can affect bacterial sensitivity (Glass & Johnson, 2004). Factors such as pH, target microorganisms, processing, ambient temperature, ambient atmosphere, partition coefficients, and the interactions amongst these factors can affect the antimicrobial sensitivity test of pathogens (Klancnik *et al.*, 2011).

5.6 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *V. infausta* and *E. globules*

Results showed that *V. infausta* MIC range from 0.7mg/ml to 14.3 mg/ml, irrespectively of the isolated organism. According to Eloff (198) and Sánchez & Kouznetsov (2010), plant extracts with MIC levels lower than 0.1mg/ml are observed as active than those with MIC values >0.1 to 0.625mg/ml. They are moderately active, and if the MIC is >0.625mg/ml then the activity is weak. In this regard, extract of *V. infausta* showed activity against *Coag. neg. staph, Micrococcus*, *E. faecalis* and *S. uberis* organisms at all varied concentrations. Same conclusions have been applied to *E. globules,* where the MIC was between 2.4 mg/ml to 15.3 mg/ml. (Mekonnen *et al*., 2016). Current results of MIC *on V. infausta* and *E. globules* are supported by previous findings demonstrating that the activity of the plant extracts is higher regarding gram-positive organisms compared to gram-negative organisms (Valsaraj *et al.*, 1997, Bishnu *et al.*, 2015). This observation might be caused by the distinctive characteristics of cell wall between gram-positive and gram-negative organisms (Zampini *et al.*, 2009). However, efflux pump system of gram-negative organisms may facilitate for such difference (Li *et al.*, 2009; Bishnu *et al.*, 2015).

CHAPTER 6: CONCLUSION AND RECOMMENDATION

6.1 Conclusion

About 90% of all cases of mastitis are subclinical. This form of mastitis usually goes unnoticed at farm level, because the milk and udder appear normal and making it much more important than clinical mastitis. It is more prevalent, resulting in great loss of produced milk. The udders were less contaminated, than the teat canal, which showed high contamination with CNS, and potentially indicating the presence of mastitis. The CNS mastitis mostly remains subclinical and inducing continuous infections, leading to SCC increase in the milk affecting milk quality and can be related to reduced milk production. The level of SCC was less than 250 000 cells/mL in most of the milk samples tested. The quarter position on the udder had no effects on types of pathogens identified in samples. However, more *S. uberis* were found in samples with higher SCC (400 000 - 900 000 cells/mL) while *Micrococcus* were in sample with SCC less than 250 000 cells/mL. The presence of *Streptococcus uberis* indicates hygiene and management systems. In addition, *Micrococcus spp* are known as a normal and minor pathogenic teat flora. The rare prevalence of mastitis revealed by the current study could be attributed to the good management practices and measures to acquire general hygiene on regular basis in milking animals.

Many plant extracts have a good therapeutic potential and can be used against some pathogens in mastitis treatment. In our study, *V. infausta* and *E. globulus* showed various potential to inhibit pathogenic bacteria. In this regard, extract of *V. infausta* showed activity against *Coag. neg. staph, Micrococcus*, *E. faecalis* and *S. uberis* organisms at all varied concentrations. Same conclusions have been applied to *E. globules,* where the MIC was between 2.4 mg/ml to 15.3 mg/ml. More work is needed on both plants, *V. Infausta* and *E. globulus* as their bactericidal compounds promise potency and safety in suppressing pathogens. The nature of action of the bactericidal extracts against antibiotic resistant to clinical strains and bacteria can be researched further and with intensively scrutiny as use of antibiotics may be restricted in future. The effectiveness and sensitivity of many plant extracts on the test bacteria is not owed to the existence of a common poisonous substance but probably through another biochemical interaction.

6.2. Recommendations

Based on the conclusion it is recommended that regular monitoring of physical examination of udder and teat as well as applying of proper hygiene methods should be conducted. Inclusion of plant-based bactericides can be considered. Further investigation on bactericidal extracts of both plants (*V. infausta* and *E. globulus)* that has promising potency and safety should, however, be conducted to increase verifiable results.

REFERENCES

Aarestrup, F.M., Wegener, H.C. & Rosdahl, V.T. (1995). Evaluation of phenotypic and genotypic methods for epidemiological typing of Staphylococcus aureus isolates from bovine mastitis in Denmark. *Veterinary Microbiology*. 45: 139-150.

Abebe, R., Hatiya, H., Abera, M., Megersa, B. & Asmare, K. (2016). Bovine mastitis: Prevalence, risk factors and isolation of Staphylococcus aureus in dairy herds at Hawassa milk shed, South Ethiopia. *BMC Veterinary Research*. 12: 1-11.

Addis, M.F, Tedde, V., Dore, S., Pisanu, S., Puggioni, G.M.G., Roggio, A.M, Pagnozzi, D., Lollai, S., Cannas, E.A., Uzzau, S. (2016). Evaluation of milk cathelicidin for detection of dairy sheep mastitis. *Journal of Dairy Science*. 99(8):6446–6456.

Afaf, A.M.D., El Sheita W.A.M.A., Abdelwahab, M.G. (2016). Biochemical study of DNA markers for bacterial infection in bovine mastitis. *Benha Veterinary Medical Journal*. 31(2):93–100.

Afzal, A., Mahmood, M.S., Hussain, I. & Masood, A. (2011). Adulteration and Mircobiological quality of milk: A review. *Pakistan Journal of Nutrition.* 10: 1195-1202.

Akerele, J.O., Obasuyi, O., Ebomoyi, M.I., Oboh, I.E., & Uwumarongie, O.H. (2008). Antimicrobial activity of ethanol and fractions of the seeds of Garcinia kola Hackel (Guttiferae). *African Journal of Biotechnology.* 7(2): 169-172.

Alekish, M.O. (2015). The association between the somatic cell count and isolated microorganisms during subclinical mastitis in heifers in Jordan. *Veterinari Medicina*. 60(2): 1-76.

Allessio, D. R. M., Neto, A. T. J. P. Velho, I. B. Pereira, D. J. Miquelluti, D. A. Knob, and da Silva, C. G. (2016). Multivariate analysis of lactose content in milk of Holstein and Jersey cows. *Semina Ciencias Agrarias*. 37:2641–2652.

Ali, A.K.A. & Shook G.E. (1980). An optimum transformation for somatic cell concentration in milk. *Journal of Dairy Science*. 63: 487-490.

Andrade, L.M., El Faro, L., Cardoso, V.L., Albuquerque, L.G., Cassoli, L. D. & Machado, P.F. (2007). Efeitos genéticos e de ambiente sobre a produção de leite e a contagem de células somáticas em vacas holandesas. *Revista Brasileira de Zootecnia*. 36(2) : 343-349.

Amabye T.G., Bezabh A.M. & Mekonen, F. (2016). Phtochemical and antimicrobial potentials leaves extract of Eucalyptus globulus oil from Maichew Tigray Ethiopia. *International Journal of Complementary and Alternative Medicine.* 2(3): 1-4.

AOAC. (Official Methods of Analysis) (2000). 17th Ed., International, Gaithersburg, MD, Official Method.

Atanasov, A.C., Wiesenberger, B., Pferschy-Wenzing, E.M., Liner, T., Waarosch, C. & Uhrin, P. (2015). Discovery and resupply of pharmacologically active plant-derived natural products: A review. Biotechnology Advances. 33: 1582-1614.

Awale, M.M., Dudhatra, G.B., Kumar, A., Chauhan, B.N. & Kamani, D.R. (2012). Bovine mastitis: A threat to economy. *Science Reports*. 1: 295.doi:10.4172/scientificreports.295.

Baars, E.W. & Hamre, H.J. (2017). Whole medical systems versus the system of conventional biomedicine: A critical, narrative review of similarities, differences and factors that promote the integration process. *Evidences-Based Complementary and Alternative Medicine*. Volume 2017, Article ID 4904930, https://doi.org/10.1155/2017/4904930.

Bachaya, H.A., Iqbal, Z., Muhammad, G., Yousaf, A. & Ali, H.M. (2005). Subclinical mastitis in buffaloes in Attock district of Punjab, Pakistan. *Pakistan Veterinary Journal.* 25(3): 134-136.

Bachaya, H.A., Raza, M.A., Murtaza, S. & Akbar, I.U.R. (2011). Subclinical bovine mastitis in Muzaffar Garh district of Punjab, Pakistan. *Journal of Animal and Plant Sciences.* 21: 16–19.

Barbano, D.M., Ma, Y. & Santos, M.V. (2006). Influence of raw milk quality on fluid milk shelf life. *Journal of Dairy Science*. 89 E. Suppl:. E15-E19.

Barkema, H.W., Schukken, Y.H., Lam, T.J.G.M., Galligan, D.T., Beiboer, M.L. & Brand, A. (1997). Estimation of interdependence among quarters of the bovine udder with subclinical mastitis and implications for analysis. *Journal of Dairy Science*. 80: 1592–1599

Barkema, H.W., Schukken, Y.H. & Zadoks, R.N., (2006), The role of cow, pathogen, and treatment regimen in the therapeutic success of bovine S. aureus mastitis. *Journal of Dairy Science*. 89: 1877–1895.

Bendelja, D., Antunac, N., Mikulec, N., Vnučec, I., Mašek, T., Mikulec, Ž. & Havranek, J, (2009). Urea concentration in sheep's milk. *Mljekarstvo.* 59(1): 3-10.

Bhutto, A.L., Murray, R.D. & Woldehiwet, Z. (2012). California mastitis test scores as indicator of subclinical intramammary infections at the end of lactation in dairy cows. *Research in Veterinary Science.* 92(1): 13-17.

Bishnu, P.M., Pankaj, A., Kashi, R.G., Sanjiv, N., Nabaraj, D., Anjana, S., Laxman, G. & Kanti, S. (2015). Evaluation of antibacterial activity of some traditionally used medicine plants against human pathogenic bacteria. *BioMed Research International*. Doi: 10.1155/2015/265425.

Blanco-Penedo, I., Fernandez Gonzalez, C., Tamminen, L.M., Sundrum, A. & Emanuelson, U. (2018). Priorities and future actions for an effective used of phytotherapy in livestock- outputs from an expert workshop. *Frontiers in Veterinary Science*. 4: 248. Doi: 10.3389/fvets.2017.00248.

Blignaut, D., Thompson, P. & Petzer, I-M., (2018). Prevalence of mastitis pathogens in South African pasture-based and total mixed ration-based dairies during 2008 and 2013. *Onderstepoort Journal of Veterinary Research* 85(1), a1482.

Bridson, D.M. (1998). Rubiaceae (Tribe Vanguerieae). pp. 211–377. In G.V. Pope (editor), Flora Zambesica. Royal Botanic Gardens, Kew.

Capuco, A.V. & Akers, R.M (1999). Mammary involution in dairy animals. *Journal of Mammary Gland Biology and Neoplastic.* 4: 137-144.

Cardozo, L.L., Thaler Neto, A., Souza, G.N., Picinin, L.C.A., Felipus, N.C., Reche, N.L.M., Schmidt, F.A., Werncke, D. & Simon, E.E. (2015). Risk factors for the occurrence of new and chronic cases of subclinical mastitis in dairy herds in Southern Brazil. *Journal of Dairy Science.* 98: 7675-7685.

Castelani L., Arcaro, J.R.P., Braga, J.E.P., Bosso, A.S., Moura, Q., Esposito, F., Sauter, I.P, Cortez, M. & Lincopan, N. (2019). Short communication: Activity of nisin, lipid bilayer fragments and cationic nisin-lipid nanoparticles against multidrug-resistant Staphylococcus spp. isolated from bovine mastitis. *Journal of Dairy Science*. 102(1): 678–683

Chandel, B.S., Dadawala, A.I., Chauhan, H.C, Parsani, H.R. & Pankaj, K. (2010). Efficacity of a homeopathic complex and antibiotics in treatment of clinical mastitis cattle in North Gujarat. *Veterinary World.* 2(10): 383-384.

Chandra, H., Bisnoi, P., Yadav, A., Patni, B., Mishra, A.P. & Nautiyal, A.R. (2017). Antimicrobial resistance and the alternative resources with special emphasis on plant-based antimicrobials: A review. *Plants*. 16(6): 1-11.

Chaves, S.K.M., Feitosa, C.M. & Araújo, L.S. (2016). Alkaloids pharmacological activies: Prospects for the development of phytopharceuticals for neurodegenerative diseases. *Current Pharmaceutical Biotechnology.* 17: 629-635.

Compean, K.L. & Ynalvez, R.A. (2014). Antimicrobial activity of plant secondary metabolites: A review. *Research Journal of Medicinal Plants.* 8: 204-213.

Contreras, G.A. & Rodriguez, J.M. (2011). Mastitis: Comparative etiology and epidemiology. *Journal of Mammary Gland Biology and Neoplasia.* 16: 339-356.

Costa, A., Lopez-Villalobos, N., Visentin, De Marchi, G., Cas-sandro, M. M. and Penasa, M. (2018). Heritability and repeatability of milk lactose and its relationships with traditional milk traits, somatic cell score and freezing point in Holstein cows. *Animal* 13: 909–916.

Crowe, M.A., Hostens, M. & Opsomer G. (2018). Reproductive management in dairy cows - the future. *Irish Veterinary Journal*. 71(1): 1-13.

De Graaf, T. & Dwinger, R.H. (1996). Estimation of milk production losses due to subclinical mastitis in dairy cattle in Costa Rica. *Preventive Veterinary Medicine.* 26: 215-222.

De Vliegher, S., Laevens, H., Devriese, L.A., Opsomer, G., Leroy, J.L.M., Barkrama, H.W. & Kruif, A. (2003). Prepartum teat apex colonization with Staphylococcus chromogenes in dairy heifers is associated with low somatic cell count in early lactation. *Veterinary Microbiology*. 92: 245-252.

Dhanabalan, R., Doss, A., Jagadeeswari, M., Balachandar, S. & Kezia, E. (2008). *In vitro* phytochemical screening and antibacterial activity of aqueous and methanolic leaf of Tridax procumbens against bovine mastitis isolated Staphylococcus aureus. *Ethnobotany.* 12: 1090-1095.

Dos Santos Nascimento, J.S., Fagundes, P.C., Brito, M.A.V.P., Santos, K.R.N. & Bastos, M.C.F. (2005). Production of bacteriocins involved in bovine mastitis. *Veterinary Microbiology*. 106: 61-71.

Du Preez, J.H. (2000). Bovine mastitis therapy and why it fails. *Journal of the South African Veterinary Association.* 71(3): 201-208.

Duarte, C.M., Freitas, P.P. & Bexiga, R. (2015). Technological advances in bovine mastitis diagnosis: An overview. *Journal of Veterinary Diagnostic Investigation*. 27(6): 665-672.

Duval, J. (1995). Treating mastitis without antibiotics. Ecology agriculture projects, McGill University (Macdonald Campus). Ste-Anne-de-Bellevue, QC, H9X 3V9 Canada.

Ekor, M. (2014). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Frontiers in Pharmacology. 4(177):1-10.

El-Sayed, A., Awad, W., Abdou, N.E. & Vázquez, H.C. (2017). Molecular biological tools applied for identification of mastitis causing pathogens. *International Journal of Veterinary Science and Medicine*. 5: 89-97.

Elbably, M.A., Emeash, H.H. & Asmaa, N.M. (2013). Risk factors associated with mastitis occurrence in dairy herds in Benisuef, Egypt. *World's Veterinary Journal*. 3(1): 4-10.

Elisha, I.L., Dzoyem, J.P., Botha, F.S. & Eloff, J.N. (2016). The efficacy and safety of nine South African medicinal plants in controlling Bacillus anthracis Sterne vaccine strain. *BMC Complementary and Alternative Medicine*. 16(5): 1-7.

Eloff, J.N. (1998). Which extractant should be used for the screening and isolation of antimicrobial components from plants? *Journal of Ethnopharmacology*. 60: 1-8.

Eloff, J.N. & McGaw, L.J. (2006). Plants extracts used to treat bacterial, fungal and parasitic infections. In: Modern Phytomedicine-Turn medicinal plants into drugs. 1st Ed. Weinheim: Wiley-VCH. Germany. pp. 97-121.

FAO, GDP, IFCN. (2018). Dairy development's impact on poverty reduction. Chicago, Illinois, USA. Licence: CC BY-NC-SA 3.0 IGO.

Flock, D. & Zeidler, H. (1965). Research on the independence of the udder quarters in the mastitis process Z. Tierz. Zuechtungsbiol., 85 (1969), p. 193

Galdhar, C.N & Roy, S. (2003). Recent trends in therapeutic management of mastitis. *Journal of Remount Veterinary Corps*. 42 (1): 5-11.

Girma, S., Mammo, A., Bogele, K., Sori, T., Tadesse, F. & Jibat, T. (2012). Study on prevalence of bovine mastitis and its major causative agents in West Harerghe zone, Doba district, Ethiopia. *Journal of Veterinary Medicine and Animal Health*. 4(8): 116-123.

Glass, K.A. & Johnson, E.A. (2004). Antagonistic effect of fat on the antibotulinal activity of food preservatives and fatty acids. *Food Microbiology*. 21: 675-682.

Gomes, F. & Henriques, M. (2016). Control of bovine mastitis: Old and recent therapeutic approaches. *Current Microbiology*. 72(4): 377-382.

González, R.L., Mitchell, G., Gattuso, M., Diarra, M.S., Malouin, F., and Bouarab, K. (2009). Plant Antimicrobial Agents and Their Effects on Plant and Human Pathogens. *International Journal of Molecular Sciences*. 10: 3400-3419

Grootenhuis, G. (1975). Mastitis: a survey on the interdependence of the quarters of a cow Tijdschr. Diergeneeskd., 100, p. 745

Gupta, N. & Patiyal, A, (2014). Assessment of the quality of milk. *Journal of Veterinary Public Health*. 12(1): 45-48.

Hanif, K., Kumar, M., Singh, N. & Shukla R. (2017). Homoeopathic medicine Mercurius solubilis treatment improves cerebral blood flow and memory in experimentally induced Dementia in rats. *Indian Journal of Research in Homoeopathy.* 11: 58-63

Heikkilä, A.M.; Liski, E.; Pyörälä, S.; Taponen, S. (2018). Pathogen-specific production losses in bovine mastitis. *Journal of Dairy Science*. 101: 9493–9504.

Hekmatpou, D., Mehrabi, F., Rahzani, K. & Aminiyan, A. (2019). The effect of Aloe vera clinical trials on prevention and healing of skin wound: A systematic review. *Iran Journal of Medicinal Science.* 44(1): 1-9.

Hektoen, L., Larsen, S. S., Ødegaad, A. & Løken, T. (2004). Comparison of homeopathy, placebo and antibiotic treatment of clinical mastitis in dairy cows- Methodological issues and results from a randomized clinical trial. *Journal of Veterinary Medicine Series A.* 51: 439-446.

Hussein, H.A, Abd El-Razik, K.A, Gomaa, A.M., Elbayoumy, M.K., Abdelrahman, K.A., Hosein, H.I. (2018). Milk amyloid A as a biomarker for diagnosis of subclinical mastitis in cattle. Veterinary World. 11(1):34–41.

IDF. International Dairy Federation (1981) Laboratory Methods for Use in Mastitis Work. Document 132. Brussels.

Iraguha, B., Hamudikuwanda, H., Mushonga, B., Kandiwa, E. & Mpatswenumugabo, J.P. (2017). Comparison of cow side diagnostic tests for subclinical mastitis of dairy cows in Musanze district, Rwanda. *Journal of the South African Veterinary Association*. 88: e1-6. Doi:10.4102/jsava.v88i0.1464

Ivemeyer, S., Walkenhorst, M, Heil, F.C., Notz, C., Maeschli, A., Butter, G. & Klocke, P. (2009). Management factors affecting udder health and effects of a oneyear extension program in organic dairy herds. *Animal*. 3(11): 1596-1604.

Jadhav, P.V., Tarate, S.B., Bhuvana, M., Das, D.N. & Shome, B.R. (2016). Somatic cell count as a monitoring system for hygienic milk production in India: A review. *Asian Journal of Dairy and Food Research*. 35(4): 270-277.

Jamine, R., Daisy, P. & Selvekumar, B.N. (2007). In vitro efficacity of flavoids from Eugenia jambolana seeds against ESβL-producing multidrug-resistant enteric bacteria. *Research Journal of Microbiology*. 2(4): 369-374.

Jenkins, T.C. and McGuire, M.A. (2006) Major Advances in Nutrition Impact on Milk Composition. *Journal of Dairy Science*. 89: 1302-1310.

Jigna, P. & Sumitra, C. (2006). In vitro antimicrobial activities of extracts of *Launaea procumbns Roxb*. (*Labiataea*), Vitis vinifera L. (*Vitaceae*) and *Cyperus rotundus L.* (*Cyperaceae*). *African Journal of Biomedicinal Research* 9(2): 89-93.

Jost, M. (1984). Calendula as a healing plant for mastitis in dairy cows. *Biodynamics.* 152: 7-18.

Judkins, H.F. & Mack, M.J. (1955). The principle of dairying. 3rd Rev. Ed. John Wiley and Sons, Inc. New York.

Juozaitiene, V., Jouzaitis, A. & Micikeviciene, R. (2006). Relation between somatic cell count and milk production or morphological traits of udder in black and white cows. *Turkish Journal of Veterinary and Animal Science.* 30: 47-51.

Kalmus, P., Simojoki, H., Pyörälä, S., Taponen, S., Holopainen, J., Orro, T. (2013). Milk haptoglobin, milk amyloid A, and Nacetyl-b-D-glucosaminidase activity in bovines with naturally occurring clinical mastitis diagnosed with a quantitative PCR test. *Journal of Dairy Science*. 96(6): 3662–3670

Kandemir, F.M., Yuksel, M., Ozdemir, N, Deveci, H. (2013). A different approach to diagnosis of subclinical mastitis: milk arginase activity. *Veterinarski Arhiv*. 83(6): 603–610.

Karimuribo, E.D., Fitzpatric, J.L., Bell, C.E., Swai, E.S., Kambarage, D.M., Ogden, N.H., Bryant, M. & French, N.P. (2006). Clinical and subclinical mastitis in smallholders' dairy farms in Tanzania: Risk, intervention and knowledge transfer. *Preventive Veterinary Medicine.* 74: 84-98.

Karzis, J., Petzer, I.M., Donkin, E.F. & Naido, V. (2018). Proactive udder health management in South Africa and monitoring of antibiotic resistance of *Staphylococcus aureus* in dairy herds from 2001 to 2010. *Journal of the South African Veterinary Association.* 89: 1-8.

Kaur, S., Gupta, S. & Gautam, P.B. (2019). Phytochemical analysis of Eucalyptus leaves extract. *Journal of Pharmacognosy and Phytochemistry*. 8(1): 2442-2446.

Khan, M.T.G., Zinnah, M.A., Siddique, M.P., Rashid, M.H.A., Islam, M.A. & Choudhury, K.A. (2008). Physical and microbial qualities of raw milk collected from Bangladesh agricultural University dairy farm and the surrounding villages. *Bangladesh Journal of Veterinary Medicine*. 6: 217-221.

Khatun, M., Thomson, P.C., Kerrisk, K.L., Lyons, N.A., Clark, C.E.F, Molfino. J., Garcıa, S.C. (2018). Development of a new clinical mastitis detection method for automatic milking systems. *Journal of Dairy Science*. 101(10): .9385–9395

Kibebew, K. (2017). Bovine mastitis: A review of causes and epidemiological point of view. *Journal of Biology, Agriculture and Healthcare.* 7(2): 1-14.

Kivaria, K.M., Noordhuizen, J.T.P. & Nielen, M (2007). Interpretation of California mastitis test scores using Staphylococcus aureus culture results for screening of subclinical mastitis in low yielding smallholders' dairy cows in the Dar-es-Salaam region of Tanzania. *Preventive Veterinary Medicine.* 78: 274-285.

Klancnik, A., Piskernik, S., Mozina, S.S., Gasperlin, L. & Jersek, B. (2011). Investigation of some factors affecting the antibacterial activity of Rosemary extracts in food models by a food microdilution method. *International Journal of Food Science and Technology*. 46: 413-420.

Klein, D., Flock, M., Khol, J.L., Franz, S., Stuger, H.P. & Baumgarter, W. (2005). Ultrasonographic measurement of bovine teat: Breed differences and the significance of measurement for udder health. *Journal of Dairy Research*. 72: 296-302.

Kong, E. & Jabra-Rizk, M.A. (2015). The great escape: pathogen versus host. *PLoS Pathogens*.11: (3): 1-5.

Kramer, M., Erbe, M., Bapst, B., Bieber, A. and Simianer, H. (2013). Estimation of genetic parameters for individual udder quarter milk content traits in Brown Swiss cattle. *Journal of Dairy Science*. 96: 5965–5976.

Krömker, V. & Leimbach, S. (2017). Mastitis treatment-reduction in antibiotic usage in dairy cows. *Reproduction in Domestic Animals*. 52(3): 21-29.

Kuang, Y., Tani, K., Synnott, A.J., Ohshima, K., Higuchi, H., Nagahata, H. & Tanji, Y. (2009). Characterization of bacteria population of raw milk from bovine mastitis by culture-independent PCR-DGGE method. *Biochemical Engineering Journal*. 45: 78-81.

Kuehn, J.S., Gorden, P.J., Munro, D., Rong, R., Dong, Q., Plummer, P.J., Wang, C. & Phillips, G.J. (2013). Bacterial community profiling of milk samples as a means to understand culture-negative bovine clinical mastitis. (23634219). *PLoS One*: 8: e61959 10.1371/journal.pone.0061959

Lacto Data. (2015). A milk SA publication compiled by the milk producer's Organization. 18:1. Available at: https://www.mpo.co.za/en/information/lactodata. Accessed on May 2018.

Le Roux, Y., Laurent, F. & Moussaoui, F. (2003). Polymorphonuclear proteolytic activity and milk composition change. *Veterinary Research*: v.34(5): .629-645,

Li, X.Z. & Nikaido, H., (2009). Efflux-mediated drug resistance in bacteria: An update. *Drugs.* 69(12): 1555-1623.

Lopes, T.S., Fontoura, P.S., Oliviera, A., Rizzio F.S., Silveira, S. and Streck, A.F. (2018). Use of plant extracts and essential oils in the control of bovine mastitis. *Research in Veterinary Science*. 131: 186–193.

Lundberg, A. (2015). Mastitis in dairy cows: Genotypes, spread and infection outcome of the three important udder pathogens (Doctorate) Swedish University of Agriculture Sciences, Uppsala. Sweden.

Malik, E.M. & Muller, C.E. (2016). Anthrraquinones as pharmacological tools and drugs. *Medicinal Research Reviews*. 36: 705-748.

Mansion-de Vries, E.M., Hoedemaker, M. & Krömker, V. (2015). Evidence-based aspects of clinical mastitis treatment. *Tierärztliche Praxis Ausgabe G, Grosstiere Nutztiere*. 43(5): 287-295.

Mansor, R., Mullen, W., Albalat, A., Zerefos, P., Mischak, H., Barrett, D.C, Biggs, A., Eckersall, P.D. (2013). A peptidomic approach to biomarker discovery for bovine mastitis. *Journal of Proteomics*. 85: 89–98.

Maroyi, A. (2018). Nutraceutical and ethnopharmacological properties of Vangueria infausta subsp. *Molecules.* 23: 1-19.

McFadden, M. (2011). California mastitis test and milk quality. *Michigan Dairy Review*. 16: 1-3.

Mekonnen, A., Yitayew, B., Tesema, A. & Taddese, S. (2016). In Vitro Antimicrobial Activity of Essential Oil of Thymus schimperi, Matricaria chamomilla, Eucalyptus globulus, and Rosmarinus officinalis. *International Journal of Microbiology.* DOI: 10.1155/2016/9545693

Mellenberger, R. & Kirk, J. (2001). Mastitis control program for *Staphylococcus aureus* infected dairy cows, Department of Animal Studies, Michigan States University and Veterinary Medicine Extension, School of veterinary Medicine. University of California Davis, http://www.milkquality.wisc.edu/wp-content/uploads/2011/09/mastitis-controlprogram_staph.-aureus.pdf .

Mendonça-filho, R.R. (2006). Bioactive phytocompounds: New approaches in the phytosciences. In: I. Ahmad, F. Aqil, and M. (Eds.), Modern Phytomedicine. Turning medicinal plants into drugs. Wiley-VCH Verlag GmbH & Co. pp. 1-24.

Merck, C.C., Sonnenwald, B.M. & Rallwage, H. (1989). Untersuchungeen über den Einsatz homöopathisthischer arzeimittel zur Behandlung akuter Mastitiden beim Rind (Studies on the use of homeopathic medicines for the treatment of acute mastitis in cows). *Berliner und Münchener Tierärztliche Wochenzeitschrift.* 102: 266-272.

Modi, C.M., Hitesh, P.B., Patel, H.B. & Mody, S.K., (2012). `A comprehensive review on pharmaco-therapeutics of bovine mastitis. *Molecular Microbiology*. 2(1): 1-9.

MPO (Milk Producers Organisation) 2020 https://www.mpo.co.za/wp-content/uploads/2020/06/Dairy-Market-Trends-May-2020.pdf . Accessed on August 2020

Moshidi, P. (2015). Antibacterial activities of plant extracts and their effects on ruminal fermentation and protein degradability in the dairy cows. M-Tech dissertation. Tshwane University of Technology, South Africa.

Motaung, T.E., Petrovski, K.R., Petzer, I.M., Thekisoe, O. & Tsilo, T.J. (2017). Importance of bovine in Africa. *Animal Health Research Review*. 18(1): 58-69.

Mubarack, H.M., Doss, A., Dhanabalah, R. & Balachander, S. (2010). Microbial quality of raw milk samples collected from different villages of Coimbatore District, Tamilnadu, South India. *Indian Journal of Science and Technology*. 3(1): 61-63.

Munita, J.M., Arias, C.A. (2016). Mechanisms of antibiotic resistance. Microbiology Spectrum. 4: 1-24.

Munodawafa, T. (2012). Screening of some traditional medicinal plants from Zimbabwe for biological and anti-microbial activity. Master's Thesis, University of Zimbabwe, Harare, Zimbabwe.

Munodawafa, T. Chagonda, L.S. & Moyo, S.R. (2013). Antimicrobial and phytochemical screening of some Zimbabwean medicinal plants. *Journal of Biologically Active Products from Nature*. 3(5-6): 323-330.

Mushtaq, S., Shah, A.M., Shah, A. Lone, S.A., Hussain, Q.P. & Ali Md, N. (2018). Bovine mastitis: An appraisal of its alternative herbal cure. *Microbial Pathogenesis*. 114: 357-361.

Nakov, D., Hristov, S., Andonov, S. & Trajchev, M. (2014). Udder-related risk factors for clinical mastitis in dairy cows. *Veterinarski Arhive*. 84: 111-127.

NMC. (National Mastitis Council). (2016). Current concepts of bovine mastitis, 5th Ed. The National Mastitis Council, New Prague, MN.

Nundkumar, N. & Ojewole, J.A.O. (2002). Studies on the antiplasmodial properties of some South African medicinal plants used as antimalarial remedies in Zulu flock medicine. *Methods and Findings in Experimental and Clinical Pharmacology*. 24: 397-401.

O'Rourke, D. (2009). Nutrition and udder health in dairy cows: A review. *Irish Veterinary Journal*. 62(Supplement): 15-20.

Oliver, S.P. & Murinda, S.E. (2012). Antimicrobial resistance of mastitis pathogens. Veterinary Clinics of North America: *Food Animal Practice*. 28(2): 165-185.

Oliveira, L., & Ruegg, P.L. (2014). Treatment of clinical mastitis occurring in cows on 51 large dairy herds in Wisconsin. *Journal of Dairy Science*. 97(9): 5426-5436.

Ondiek, J.O, Ogore, P.B, Kemboi, F. (2018). Clinical mastitis gives off-flavor and reduces quality of milk in smallholder goat farms. International *Journal of Current Microbiology and Applied Sciences*. 7(1): 2387–2396.

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R. & Anthony, S. (2009). Agroforestry database: A tree reference and selection guide version4.0. World Agroforestry Centre, Kenya.

Osumi, T., Kishimoto, Y., Kano, R., Muruyama, H., Onozaki, K., Ito, T., Matsubara, K. & Hasegawa, A. (2008). Prototheca Zopfii genotypes isolated from cow barns and bovine mastitis. *Japan Veterinary Microbiology.* 131: 419-423.

Oudah, E.Z.M. (2009). Non-genetic factors affecting somatic cell count, milk urea content, test-day milk yield and milk protein per cent in dairy cattle of the Czech Republic using individual test-day records. *Livestock Research for Rural Development*. Volume 21, Article #71. Retrieved September 1, 2020, from http://www.lrrd.org/lrrd21/5/ouda21071.htm

Pantoja, J.C.F., Hulland, C. Ruegg, P.L. (2009). Dynamics of somatic cell counts and intramammary infections across the dry period. *Preventive Veterinary Medicine.* 90: 43–54

Passantino, A. (2007). Ethical aspects for veterinarians regarding antimicrobial drug use in Italy. *International Journal of Antimicrobial Agents*; 29: 240–244

Patil, M.P., Nagvekar, A.S., Ingole, S.D., Bharucha, S.V., Palve, V.T. (2015). Somatic cell count and alkaline phosphatase activity in milk for evaluation of mastitis in Buffalo. *Veterinary World*. 8: 363–366.

Pereira, P.C. (2014). Milk nutritional composition and its role in human health. *Nutrition*. 30: 619-627.

Pérez, M.J., Falgué, E. & Dominguez, H. (2016). Antimicrobial action of compounds from marine seaweed. *Marine Drug*. 14(52): 1-38.

Petzer, I.M., Karzis, J., Watermeyer, J.C., van der Schans, T.J. & van Reenen, R. (2009). Present trends in udder health and emerging mastitogenic pathogens in South Africa dairy herds. *Journal of the South African Veterinary Association*. 80: 17-22.

Petzer, I.M., Karzis, J., Donkin, E.F., Edward, C.W. & Etter, E.M.C. (2017). Somatic cell count thresholds in composite and quarter milk samples as indicator of bovine intramammary infection status. *Onderstepoort Journal of Veterinary Research*. 84(1): 1-10.

Pieterse, R. & Todorov, S.D. (2010). Bacteriocins-exploring alternatives to antibiotics in mastitis treatment. *Brazilian Journal of Microbiology*. 41: 542-562.

Potts, B.M & Reid, J.B. (2003). Tasmania's eucalypts: Their place in science. Papers and Proceedings of the royal Society of Tasmania. 137:21-37. ISSN 00804703. Tasmania 7001. Australia.

Pretorius, C. (2008). The effect of coryne bacterium cutis lysate to control somatic cell count in dairy cows. Dissertation submitted in accordance with the requirement for the degree of Magister Science Agriculture to the Department of Animal, Wildlife and Grassland Sciences. University of the Free State, Bloemfontein. South Africa.

Ptak, E., Jagusiak, W., Żamecki, A. & Otwinowska-Mindur, A. (2011). Heritabilities and genetic correlations of lactational and daily somatic cell score with conformation traits in Polish Holstein cattle. *Czech Journal of Animal Science*. 56: 205-212.

Pyörälä, S. (2003). Indicators of inflammation in the diagnosis of mastitis. *Veterinary Research*. 34: 565–578

Qayyum, A., Khan, J.A., Hussain, R., Khan, A., Avais, M., Ahmad, N. & Hassan, F. (2016). Molecular characterization of staphylococcus aureus isolates recovered from natural cases of subclinical mastitis in cholistani cattle and their antibacterial susceptibility. *Pakistan Journal of Agricultural Sciences*. Vol. 53(4): 1-8.

Quinn, P.J., Carter, M.E., Markey, B. & Carter, G.R. (1999). Clinical veterinary microbiology. Harcourt Publishers Ltd. London.

Radostits, O.M.; Gay, C.C.; Hinchcliff, K.W.; Constable, P.D. (2007). Veterinary medicine: A textbook of diseases of cattle, horses, sheep, pigs and goats. *Canadian Veterinary Journal***.** 10: 673–762.

Rai, A.N., Vargas, M.L., Wang, L., Andersen, E.F., Miller, E.L & Simon, J.A. (2013). Elements of the polycomb repressor SU (Z) 12 needed for Histon H3-K27 methylation, the interface with E(Z), and in vivo function. *Molecular and Cellular Biology*. 33(24): 4844-4856.

Rates, S.M.K. (2001). Plants as source of drugs. *Toxicon.* 39: 603-613.

Raymond, M.J, Wohlre, R.D, Call, D.R. (2006). Assessment and Promotion of Judicious Antibiotic Use on Dairy Farms in Washington State. *Journal of Dairy Science.* 89: 3228–3240.

Rios, J.L & Recio, M.C. (2005). Medicinal plants and antimicrobial activity. *Journal of Ethnopharmacology.* 100: 80-84.

Romero, J., Benavides, E. & Meza, C. (2018). Assessing financial impacts of subclinical mastitis on Colombian dairy farms. *Frontiers in Veterinary Science*. 5(273): 1-11.

Rossi, R.S., Amarante, A.F., Correiria, L.B.N., Guerre, S.T., Nobrega, D.B, Latosinsnki, G.S., Rossi, B.F., Rall, V.L.M & Pantoja, J. (2018). Diagnostic accuracy of Somatic, California mastitis test and microbiological examination of composite milk to detect Streptococcus agalactiae intramammary infections. *Journal of Dairy Science*. 101(11):10220-10229

Ruegg, P.L. (2008). Management of mastitis on organic and conventional dairy farms. *Journal of Animal Science*. 87: 43-55.

Sánchez, J.G. & Kouznetsov, V.V. (2010). Antimycobacterial susceptibility testing methods for natural products research. *Brazilian Journal of Microbiology*. 41(2): 270-277.

Salie, F., Eagles, P.F.K. & Lens, H.M.J. (1996). Preliminary antimicrobial screening of four South African Asteraceae species. *Journal of Ethnopharmacology*. 52(1): 27-33

Sartorelli, P., Marquioreto, A.D., Amaral- Baroli, A., Lima, M.E. & Moreno, P.R. (2007). Chemical composition and antimicrobial activity of the essential oils from two species of Eucalyptus. *Phytotherapy Research*. 21(3): 231-233.

SAS Institute Inc. 2004. SAS/ STAT®9.1User's Guide. Cary, NC: SAS Institute Inc.

Sathiyabarathi, M., Jeyakumar. S., Manimaran, A., Jayaprakash, G., Pushpadass, H.A, Sivaram, M, Ramesha, K.P, Das, D.N., Kataktalware, M.A, Prakash, M.A, Kumar, R.D. (2016). Infrared thermography: a potential non-invasive tool to monitor udder health status in dairy cows. *Veterinary World*. 9(10): 1075–1081.

Sawa, A., Krezel-Czopek, S. & Bogucki, M (2015). Dry period length as related to milk yield and SCC during the first month of subsequent lactation. *Annals of Animal Science*. 15(1): 155-163.

Schabauer, L., Wenning, M., Huber, I. & Ehling-Schulz, M. (2014). Novel physico-chemical diagnostic tools for high throughput identification of bovine mastitis associated gram-positive, catalase-negative cocci, *BMC Veterinary Research*, 10 (156): 1-11.

Schukken, Y.H., Tikofsky, L.L. & Zadocks, R.N. (2005). Environmental control for mastitis prevention, milk quality and food safety. In: Hogeveen, H. (ed.), Mastitis in dairy production current knowledge and future solutions, Wagenigen Academic Publishers. The Netherlands, pp. 109-114.

Schwarz, D. (2012). Evaluation of the immunological status in clinically healthy and subclinically infected bovine mammary glands. (PhD), Georg-August-University Göttingen, Göttingen, Germany.

Seegers, H., Fourichon, C. & Beaudeau, F. (2003). Production effects related to mastitis and mastitis economics in dairy cattle herds. *Veterinary Research*. 34: 475-491.

Seigler, D.S. (2003). Phytochemistry of *Senegalia-sensu lato*. *Biochemical Systematic and Ecology*. 31: 845-873

Sharma, N., Rho, G.J., Hong, Y.H., Kang, T.Y., Lee, H.K., Hur, T.Y. & Jeong, D.K. (2012). Bovine mastitis: An Asian perspective. *Asian Journal of Animal and Veterinary Advances.* 7: 454-476

Shubhreet, K., Gupta, S. & Gautam, P.B. (2019). Phytochemical analysis of Eucalyptus leaves extract. *Journal of Pharmacology and phytochemistry*. 8(1): 2442-2446.

Simões, M.R.N., & Bennet, E.A.S. (2009). Understanding antimicrobial activities of phytochemicals against multidrug resistant bacteria and biofilms. *Natural Products Reports*. 26: 746-757.

Silva, J.E., Barbosa, S.B.P., Abreu, B.S., Santoro, K.R., Silva, E.C., Batista, Â.M.V. & Martinez, R. L.V. (2018). Effect of somatic cell count on milk yield and milk components in Holstein cows in a semi-arid climate in Brazil. *Revista Brasileira de Saúde e Produção Animal., Salvador* : 19(.4) : 391-402.

Sirois, M. (2017). Principles and Practices of veterinary Technology (Fourth ed.) St Louis, Mississouri: Elsevier.

Skoulikas, S., Dufour, S., Haine, D. & Perreau, J.Y. & Roy, J.P. (2018). Early-lactation extended Pirlimycin therapy against naturally acquired Staphylococcus aureus intramammary infections in heifers: A randomized controlled trial. *Journal of Dairy Science*. 101:1419-1427.

Sol, J. (1984). Control methods in summer mastitis: The importance of fly control. In proceedings of the XIIIth. World Congress on Diseases of cattle, Durban, 17-21st September 1984. South Africa Veterinary Association, Pretoria, pp. 236-242.

Sorensen, M.K., Jensen, J. & Christensen, L.G. (2000). Udder conformation and mastitis resistance in Danish first-lactating cows: Heritabilities, genetic and environmental correlations. *Acta Agriculturæ Scandinavica.* 50: 72-82.

Srikrishna, D., Godugu, C. & Dubey, P.K. (2016). A review on pharmacological properties of coumarins. *Mini-Review in Medicinal Chemistry*. 18: 113-141.

Street, R. A. and Prinsloo, G. (2013). Commercially Important Medicinal Plants of South Africa: A Review. *Journal of Chemistry.*DOI:10.1155/2013/205048 Sunder, J., De, A.K., Jeyakumar, S. & Kundu, A. (2013). Effect of feeding of *Morinda citrifolia* fruit juice on the biophysical parameters of health as well as mastitis – affected cow milk. *Journal of Applied Animal Research*. 41(1): 29-33.

Talukder, M. & Ahmaed, H.M. (2017). Review effect of somatic cell count on dairy products: a review. *Asian Journal of Medicinal and Biology Research*. 3(1): 1-9.

Tamminen, L.M., Emanuelson, U. & Blanco-Penedo, I. (2018). Systematic review of phytotherapeutic treatments of different farm animal under European conditions. *Frontiers in Veterinary Science*. 5(140): 1-11.

Thompson-Crispi, K., Atalla, H., Miglior, F. & Mallard, B.A. (2014). Bovine mastitis: Frontier in immunogenetics. *Frontiers in Immunology*. 5(493): 1-10

Tilahun, A. & Aylate, A. (2015). Prevalence of bovine mastitis in lactating cows and its public health implications in selected commercial dairy farms of Addis Ababa*. Global Journal of Medicinal Research: G. Veterinary Science and Veterinary Medicine.* 15(2): 1-9.

Toffanin, V., Penasa, M., Mcparland, S., Berry, D.P., Cassandro, M., De Marchi, M. (2015). Genetic parameters for milk mineral content and acidity predicted by mid-infrared spectroscopy in Holstein-Friesian cows. *Animal.* 9(.5) : 775–780.

Trevisi, E., Zecconi, A., Cogrossi, S., Razzuoli, E., Grossi, P. & Amadori, M. (2014). Strategies for reduced antibiotic usage in dairy cattle farms. *Research in Veterinary Science.* 96(2): 229-233.

USAD. (2010). United States of America dairy industry. IUF Dairy Industry Research. www.usda.mannlib.cornell.edu.(Accessed on 20 June 2018.

Vaibhav, D.B., Bhavyata, B.P., Chaitanya, G.J. & Anju, P.K. (2013). Curcuma longa: An alternative to antibiotics to combat mastitis in cattle. *Wayamba Journal of Animal Science*. 2013:.582-589

Valsaraj, R., Pushpangadan, P., Smitt, U.W., Adsersen, A. & Nyman, U. (1997). Antimicrobial screening of selected medicinal plants from India. *Journal of Ethnopharmacology*. 58(3): 75-83.

Van Wyk, B. & Van Wyk, P. (1997). Field guide to trees of Southern Africa. Cape Town: Struik Publishers, South Africa.

Varshney, J.P. (2000). Proceedings of India Veterinary Congress, Roundtable conference on mastitis. Indian Veterinary Research Institute. February 18-19, pp. 1-5.

Viguier, C., Arora, S., Gilmartin, N., Welbeck, K. & O'Kennedy, R. (2009). Mastitis detection: current trends and future perspectives. *Trends Biotechnology.* 27: 486–493.

Vishin, P.A., Nitave, S.A. (2014). A review on *Eucalyptus globulus*: A divine medicinal herb. *World Journal of Pharmacy Science*. 3(6): 559-567

Waage, S., Mørk, T., Røros, A., Aasland, D., Hunshamar, A., Ødegaard, S.A. (1999). Bacteria associated with clinical mastitis in dairy heifers. *Journal of Dairy Science.* 82: 712-719.

Walkenhorst, M., Garbe, S., Klocle, P., Merck, C., Notz, C., Spranger, J. (2001). Strategies for prophylaxis and therapy of bovine mastitis. In Fifth Network for Animal health and welfare workshop (pp.33-41). Rødding, Denmark.

Wangensteen, H., Klarpås, L., Alamgir, M., Samuelsen, A.B.C., Malterud, K.E. (2013). Can Scientific Evidence Support Using Bangladeshi Traditional Medicinal Plants in the Treatment of Diarrhoea? A Review on Seven Plants. *Nutrients.* 5(5): 1757-1800.

Washington, J.A. (1996). Principles of Diagnosis. In: Baron S, editor. Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston; Chapter 10. Available from: https://www.ncbi.nlm.nih.gov/books/NBK8014/ Accessed on 17 January 2019

WHO. World Health Organization; Geneva, Switzerland: (2002). Traditional Medicine Growing Needs and Potential – WHO Policy Perspectives on Medicines, No. 002, May

Wu, J., Hu, S., Cao, L. (2007). Therapeutic effect of nisin z on subclinical mastitis in lactating cows. *Antimicrobial Agents and Chemotherapy* 51(9): 3131-3135.

Zadocks, R.N., Middleton, J.R., McDougall, S., Katholm., J., & Schukken, Y.H. (2011). Molecular epidemiology of mastitis pathogens of dairy cattle and

comparative relevance to humans. *Journal of Mammary Gland Biology and Neoplasia.* 16: 357-372.

Zampini, I.C., Cuelllo, S., Alberto, M.R., Almeida, R.D., Solorzano, E., & Isla, M.I. (2009). Antimicrobial activity of selected plant species from the argentine puna' against sensitive and multi-resistant bacteria. *Journal of Ethnopharmacology*. 124(3): 499-505.

Zhang, H.Y., Yang, Y.M., Liu, X.Z., Lin, Z., Long, T., & Mo, X.Y. (2009). AFLP analysis of somaclonal variations in Eucalyptus globulus. *Biologia Plantarum*. 53 (4): 741-744.

Ziv, G. (1980). Drug selection and use in mastitis: systemic vs local therapy. *Journal of the American Veterinary Medical Association*. 176: 1109-1115.