Pro-active dairy herd udder health management decisions based on micro-biology and cytology of milk samples

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by

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DECLARATION

I, Inge-Marié Petzer declare that the thesis, which I hereby submit for the PhD degree at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institute.

Signature: ……

Date: …….

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LIST OF ABBREVIATIONS

ACR	Automatic cluster removers
AUC	Area under the curve
BCS	Body condition score
BMSCC	Bulk milk somatic cell count
BVD	Bovine viral diarrhoea
CI	Confidence intervals
Cum	Cumulative
CNS	Coagulase negative staphylococci / Non aureus staphylococci
DCAD	Dietary cation anion difference
DIM	Days in milk
EBL	Enzootic bovine leukosis
REPEC	Enteropathogenic
ETEC	Enterotoxigenic
EIEC	Enteroinvasive
EHEC	Enterohaemorragic
EAEC	Enteroadherent
E. canis	*Enterococcus canis*
E. coli	*Escherichia coli*
E. faecalis	*Enterococcus faecalis*
FN	False negative
FP	False positive
h	Hours
HP	High prevalence

HPHT	High prevalence - high transmission risk
HPLT	High prevalence - low transmission risk
LPHT	Low prevalence - high transmission risk
LPLT	Low prevalence - low transmission risk
IDF	International Dairy Federation
IMC	International Mastitis Council
IMI	Intramammary infections
ISVEE	International Symposium of Veterinary Epidemiology & Economics
KOH	Potassium hydroxide
LR	Likelihood Ratios
LCI	Lower confidence interval
M	Mastitis
MSD	Milk Sample Diagnostic program
NAGase	N-acetyl-beta-D-glucosaminidase
NMC	National Mastitis Council of the USA
NSD	Non-specific disturbance
PMN	Polymorpho-nuclear neutrophils
ROC	Receiver operating characteristics
Rx	Treatment
SA	South African
SCC	Somatic cell count
SD	Standard deviation
S. aureus	*Staphylococcus aureus*
S. chromogenes	*Staphylococcus chromogenes*
S. pseudintermedius	*Staphylococcus pseudintermedius*

Str. agalactiae	*Streptococcus agalactiae*
Str. dysgalactiae	*Streptococcus dysgalactiae*
S. hyicus	*Staphylococcus hyicus*
Str. pyogenes	*Streptococcus pyogenes*
Str. uberis	*Streptococcus uberis*
TCS	Teat canal scores
TCI	Teat canal infection
THI	Temperature Humidity Index
TMR	Total mix ration
TN	True negative
TP	True positive
TR	High transmission ratio
T. pyogenes	*Trueperella pyogenes*
UCI	Upper confidence interval
ZAR	South African Rands

MAIN AIMS AND OUTCOMES ON THE THESIS

Chapter 1

Aim: To develop a good understanding of udder health and to use this to the advantage of the dairy producers.

Outcome: Knowledge and practical experience gained in the field of udder health lead to the development of a computer program that is used to analyse laboratory results. Reports were developed to provide information on herd and individual udder health status, to help identifying the origin of problems and to monitor progress.

Chapter 2

Aim: To develop a semi-intelligent computer program that would assist in analysing herd udder health data generated by a milk laboratory. The reports generated should support a pro-active herd udder health approach that focus on prevention rather than cure.

Outcome: The MSD program was developed and tested over a period of 15 years under field conditions. Reports provide information in a format that can be used to identify problems and their origin and is used to monitor progress. The current dataset holds information of more than 1000 South African dairy herds. Dairy producers find the information and practical assistance beneficial.

Chapters 3 and 4

Aim: To learn more regarding the relation between IMI (aerobic udder pathogens) and somatic cell count (SCC) levels (chapter 3) as well as that of specific pathogens and SCC levels (Chapter 4) in both quarter milk and composite milk samples from South African dairy herds.

Outcome: The threshold for SCC of 200 000 cell/ml used to detect only IMI in quarter and 150 000 cells/ml milk in composite samples were find to be optimal as selection criteria for culturing milk sample. The level of SCC differed considerably according to the various udder pathogens investigated in both quarter and composite milk samples. It was further noticed that 20.5% and 30.8% *S. aureus* IMI might remain undetected in a herd, when SCC thresholds of 200 000 and 150 000 cell/mL (in quarter and composite milk respectively) were used to select samples for culturing. The knowledge gained can assist with more efficient goal-orientated decision-making at farm level.

Chapter 5

Aim: To develop models that could be used by dairy consultants, veterinarians or producers to help with decision making. These models should utilize current farm information and be able to accurately predict future events. The second aim was to predict cost effectiveness of

management changes in combination with an indication of epidemiological outcome in the *Staphylococcus aureus* population in herds.

Outcome: Models providing both financial and epidemiological outcomes of *Staphylococcus aureus* intramammary herd infections were developed and can be used under field conditions. They utilize current farm information such as initial prevalence of this bacterium in the herd combine with the level of management (transmission ratio) and compare the outcome of various treatment scenario. Various time frames can be used. When both financial and epidemiological outcomes of scenario are calculated informed decisions can be made.

THESIS ABSTRACT

Pro-active dairy herd udder health management decisions based on micro-biology and cytology of milk samples.

South Africa is a country that has gone through a period of many changes, also in the agricultural arena. The number of dairy herds shrank from 7077 in 1997 to 1600 in 2016 (Lactodata 2017, Personal communication with Milk SA, January 2017). Many herds amalgamated and cows were bought in from different herds. The movement of cattle between herds increased risks of new infections to spread from herd to herd as sound biosecurity practices were and are still lacking in the South African dairy industry. Diseases such as Bovine Viral Diarrhoea (BVD) and Bovine Leukosis spread through the country and though *Staphylococcus aureus* (*S. aureus*) and *Streptococcus agalactiae* (*Str. agalactiae*) intramammary infections (IMI) were introduced into many herds. The magnitude and consequences of BVD or EBL on the somatic cell count (SCC) have not been taken into consideration in this study. We are currently unsure as to what extent of EPL and dairy producers are reluctant to test for this disease. Producers soon realized that an increase in herd size required much better planning and management. With an increasing herd size the risk of stress may increase and this in itself may have a negative effect on the immune status of cows. In addition to new infections risk due to cattle movement, it is known that the bacterial population that is isolated from milk of dairy cows do not remain static but changes with time as many factors may contribute to the shift in bacterial population. The role that anaerobic bacteria play in udder health was not investigated in this study.

The Milk Laboratory (Faculty of Veterinary Science at Onderstepoort, Department of Production Animal Studies) took the initiative to assist farmers with mastitis outbreaks and the implementation of better management practices by starting to analyse milk samples of all lactating cows in herds for both microbiology and cytology. This provided knowledge on pathogen specific herd challenges. A computer programme (Milk Sample Diagnostic – the MSD program) was developed to assist in data manipulation. The laboratory not only provided the producers with mere microbiological and SCC results but all data was analysed by an experienced veterinarian at the laboratory. The data analysed was firstly aimed at the herd level, helping to identify root problems in management and infection status. The study does not address individual stressor but only evaluated overall responses of a large data set over time and seasons. Once the principle microorganisms or SCC cause were identified in herds the immediate emphasis was to change inappropriate management practices or target causes of high herd SCC including the milking machine settings, use or maintenance. Actions could be directed to first addressing the cause, and once this was achieved the emphasis shifted to monitor progress in the herd udder health. Monitoring of herds focused on detection of probable problems early in order to eliminate them in order to prevent major

losses in herds and damage to udder parenchyma of cows. Herd trends such as levels of new pathogen specific IMI, persistent infections on cow or quarter level, cure of mastitis cases (bacterial cure and not only clinical cure or a decreased SCC beyond a chosen level) and the shifts in SCC ranges were monitored and analysed.

Teams worked together involving the producer, his local veterinarian, the laboratory and other specialists. An important aim was to become more pro-active in identifying potential problems at the start in order to prevent mastitis as far as possible. The data obtained from investigating the milk samples was used as basis, but a more holistic approached towards udder health was used.

Many success stories could be told. Herds positive for *S. aureus* tested were tested monthly to 3 monthly. Where good management was maintained few new *S. aureus* IMI occurred, positive cases were separated and chronic cases were culled. All milk samples in those herds become *S. aureus* free (bacterial negative) by following testing regimes that were followed by effective management. Herds continued to participate in routine testing and remained S. aureus negative (bacteriology of milk samples). Data to confirm results available in the MSD program. No teat canal swabs were used in this study.

Testing of milk samples and mainly the identification of microorganisms is becoming expensive, mainly in large herds with more than 1 000 lactating cows. Although it is still done in South Africa it is a huge task to sample and analyse samples of all individual cows. Due to the increase in herd size in South African dairies, composite cow milk samples are now preferred to quarter milk samples. Little current data is available on the SCC threshold that indicate IMI in composite samples. The information available on SCC thresholds in quarter milk samples are more readily available but practical levels used in the field differ from country to country, indicating that these aspects require further research. The aim of the study was to contribute additional knowledge to the interaction between IMI and SCC levels as well as specific pathogens and SCC levels in both quarter milk and composite milk samples from dairy cows. The determination of SCC is a more cost effective and a less cumbersome test to perform. Once appropriate SCC threshold levels could be determined per pathogen, the result of using the selected threshold was used in a model to test both the cost / benefit and the epidemiological outcome after a period of 255 days for various scenarios. The scenarios not only simulated the percentage of infected animals treated but also the level of risk of transmission of *S. aureus* in these scenarios.

Despite decades of intensive research and management strategies, bovine mastitis still remains an immense challenge. While the occurrence of clinical mastitis has been reported to have decreased there has been almost no reduction in the prevalence of subclinical mastitis (Pyörälä, 2002). One can only speculate on what the reason for the phenomenon can be. Is the immune system of the modern cow challenged beyond its natural abilities due to the increased demands on the genetically changed animal for high milk yield? Are we now dealing

with more pathogenic bacteria than in the past that can also enter with ease through a damaged teat canal caused by our quest to remove milk as fast as possible because time is so precious and enter into an udder of a cow with an inferior immune system? The focus of milking machines was not always on preserving the first line of the natural defence mechanism of the udder, notably the teat canal. New teat liners are emerging with different shapes (triangular, or square), sizes, harness, made of silicon, impulse lines, quarter milkers and liner compression (over pressure) is taken into account.

Papers in perspective and into context

In the introduction (Chapter 1) the basic information regarding mastitis and background on cytology and different microbiological infections in the udder are discussed.

In Chapter 2 some results and reports of the milk Sample diagnostic (MSD) program are discussed and the proactive usage of the data in this program under field conditions. The advantage of being able to identify most infected animals in consecutive samplings, even in *S. aureus* herds where the bacteria is an intermitted shedder. We were able to determine bacterial cure post treatment and did not only base cure on clinical symptoms vanishing or SCC lowering but could indicate that bacteria were no longer present.

In Chapters 3 and 4 we used a part of the large dataset generated in the laboratory over years to investigate the effect that limited culturing, using SCC with cut-off point may have on the efficacy of identification of IMI. The use of SCC as monitoring tool is generally accepted but the level indicating IMI or bacterial positive cultures is not. This SCC threshold remained controversial through many years. The first objective was to compare IMI with SCC levels in the two sample types: quarter and composite cow milk samples. Although composite cow milk samples combine milk of the 4 quarters of a cow, these milk samples became very valuable in identifying cows with IMI. They are more practical for monitoring udder health in large herds and are less expensive to analyse. Recommendation on the level of SCC level for milk with a greater risk of being infected are available from the NMC Guideline, (2001) for quarter milk samples. The same is not true regarding composite cow milk samples. We used composite milk samples for many years and analyses indicated the value of these samples in practise despite their limitation. An objective was to determine in this large dataset under South African conditions a SCC threshold for composite cow milk samples that could mimic the 200 000 cells/ml milk recommended for quarter milk samples that could be used in the field. In Chapter 4 we took another step and compared bacterial groups (Major Gram positive and negative and minor pathogens) and individual bacterial species with SCC thresholds. The study investigated SCC levels of 15 different bacterial species. The investigation compared findings of quarter and composite milk samples. The aim of this study was to be able to change the SCC threshold level in individual herds depending on what the principle mastitogenic bacteria are in managing udder health and when sampling had to be selected for microbiological determination.

In Chapter 5 the knowledge obtained on the SCC threshold values for *S. aureus* was used. In this study models were developed to predict the cost benefit of various scenarios. Two treatment regimens were compare (treatment duration varied) when subclinical *S. aureus* were all identified *S. aureus* IMI were treated, compared to no treatment and treatment of only those cases with a SCC exceeding 200 000 cells/ml in quarter and 150 000 cells/ml in composite cow milk samples. The stochastic models compared these treatments in herds with low and high initial *S. aureus* herd prevalence and in good and poor management situations. In addition to the partial cost benefit models epidemiological models were developed. They were used to predict the probable number of persistent, new and cured cases of *S. aureus* IMI for the same scenarios as in the partial cost benefit study over a period of 255 days. The number of cases that would need treatment were calculated and compared for the scenario. Different combinations of the cost benefit and epidemiological models were compared to find the most effective (financially, number of treatments and *S. aureus* IMI status of the herd at the end of a chosen time period) combination for practical use by a producer for his circumstances. This was done to assist managers on farms to regularly upgrade relevant management protocols using current information to make more effective decisions for better future outcomes.

In the last chapter (6) findings were summarised, limitations and weakness in the research were discussed and the need for future research were identified.

Relevance and importance of this study

Research is a crucial element for development and finding solutions to problems. In South Africa we were able to assist more than 900 commercial dairy farmers over the past 15 years with udder health challenges. The experience that was gained by evaluating the herd udder health results was partly captured in the MSD laboratory program that is a semi-intelligent program assisting with problem identification and measurements. Program reports were developed and tested in practice under field condition. Microbiology and cytology were performed on milk samples of all lactation cows in herds over the years. These results were used to investigate less costly but still effective methods of udder health determination. The aim was to help assure that the udder health status of the individual cow could still be monitored on a subclinical level even though lactating cow numbers in herds in South Africa increased substantially. Once the udder health status is known decisions need to be made that always have cost implications. A further aim was therefore to assist decision makers by providing them with a tool to help predict financial and epidemiological implications. Various scenarios were compared in herds with either high or low prevalence of *Staphylococcus aureus* intramammary infection, different hygiene management levels and different treatment scenarios. With the models that were developed in this study the decision makers are assisted to take both financial and epidemiological outcomes into consideration when planning ahead.

An interesting and perhaps unexpected finding was that by prudent treatment of subclinical *Staphylococcus aureus* intramammary infections less treatments were needed than when only clinical cases were treated. Although this was not one of the objectives of the study, this information can be helpful in coping with the current prudent antimicrobial use to maintain sensitivity to antimicrobial remedies.

CHAPTER 1

Pro-active dairy herd udder health management decisions based on micro-biology and cytology of milk samples.

INTRODUCTION TO MASTITIS

Mastitis is defined as an inflammation of the udder. This inflammation is quantified in lactating cows using somatic cell count (SCC) of milk. Milk normally contains somatic cells but the concentration is generally considered to be less than 100,000 cells/ml milk when the udder quarters are uninfected or uninflamed. Once the udder becomes inflamed, the somatic cell concentration in the milk increases.

Abnormal milk is defined by the National Mastitis Council of the USA (NMC) Guidelines (2001, 2015) as milk with a SCC of ≥200 000 cells/ml whether clinical changes to the milk are present or not. According to the NMC Guidelines (2001, 2015) quarters with a SCC of ≥200 000 cells/ml are likely to be infected or are recovering from infections. Increased SCC is further associated with a reduced suitability of the milk for human consumption. Clinical mastitis is defined as having the appearance of milk changes and flakes or clots are present, and in this situation milk is generally accepted almost always to have a SCC in excess of 200 000 cells/ml. Udder infections are often as result of inferior or inadequate hygiene in housing or during milk harvesting. The higher the SCC the greater the likelihood of the presence of pathogens in the milk. Researchers are unsure, based on current knowledge, whether milk with a SCC of between 100 000 cells/ml and 199 000 cells/ml is normal or not (NMC Guidelines 2001, 2015)

Mastitis is the most costly disease of dairy cattle in first world countries and is also regarded as an animal welfare and food safety problem (Sharma et al. 2011). Sub-clinical mastitis which is not effectively diagnosed in the parlour is responsible for most losses. This disease is characterised by physical, chemical and bacteriological changes in the milk and pathological changes in the udder. Mastitis is responsible for a reduced efficiency of production, reduced suitability of products for processing and it can affect human wellbeing directly through zoonosis. This amounts to a great reduction in the total value a society gains from livestock (Giesecke et al., 1994, NMC Guidelines 2001, 2005, Huijps et al., 2010, Sharma et al. 2011). Mastitis affects the dairy producer directly through lower milk yields, milk that is discarded, and a lower milk price. Modern dairy cows are bred to reach their highest production during the 4^{th} and 6^{th} lactation. Mastitis is however often the reason for a shortened productive life and a lower lifetime milk yield both due to lower milk yields and increased culling rate (Huijps et al., 2010). The cost of replacement of mature dairy cows decreases proportionally to the number of years that the cow has been productive in a herd (Giesecke et al. 1994, Sharma et

al. 2011). The International Dairy Federation (IDF) recommendation for dairy cows is to complete at least six lactations. According to figures provided by the South African Studbook (personal communication, Jan 2018) the average age of 14 266 active lactating Holstein cows on total mix rations systems is 5.7 (±2.3) years and that of 14 252 active Holsteins on pasture systems, 7.5 (±3.0) years. Studies in South Africa have shown that within a herd 7% of cows can be responsible for 40% of clinical mastitis cases and 6% of cows for 50% of all discarded milk (Giesecke et al. 1994). The frequency of subclinical mastitis varies, but has been estimated to be responsible for 93-97 % of the total incidence of mastitis (Giesecke et al. 1994).

Veterinary practitioners, dairy workers and producers are changing their herd udder health management system from a reactive approach which involved only the treatment of clinical cases to a pro-active approach which focuses on preventing both clinical and subclinical mastitis. The latter concept is based on identifying critical points that with regular monitoring would show early signs of deterioration of management and identify changes in the level of sub-clinical mastitis within a herd.

Current literature causes confusion regarding criteria considered as a cure of mastitis. Some authors refer to cure as clinical cure only, some to a reduction in SCC to below 200 000cell/ml, and others refer to a true cure involving clinical cure, reduction of SCC and the absence of bacteria (bacterial cure) (Harmon 2001, NMC Guideline 2001, 2015).

The primary defence mechanism of the udder is the teat canal. It forms a physical barrier preventing bacteria from entering. When damaged or dilated after milking, the chance of ascending infection is high (Kehrli & Harp 2001). Epithelial desquamation and milk flow during milking helps to prevent bacterial colonisation in the teat canal. The keratin layer contains antibacterial proteins and fatty acids to combat bacteria. Lymphocytes and plasma cells accumulate beneath and between the epithelial cells of the teat canal and particularly at Fürstenberg's rosette to combat bacteria that have gained entrance (Sordill et al. 1988).

When pathogens pass through the teat canal, the secondary (innate and adaptive) immune responses of the udder are activated. The innate immunity, a combination of chemical secretions (lactoperoxidases, lactoferrin, complement) and a cellular immune response is activated immediately after pathogen invasion (Kehrli & Harp 2001, Tizard 2013). This is followed by a response of the adaptive immune system (Tizard 2013). The adaptive immune system recruits antigen-specific T and B lymphocytes responsible for an antibody-mediated response and activates a cell-mediated immune response directed against intracellular pathogens (Tizard 2013, Morin 2015). The cellular response is measured in practice utilizing the SCC (Morin 2015).

1.1 Somatic cells in milk

Somatic cells in this study, were counted by fluoro-opto-electronic means using a Fossomatic 5000 and Fossomatic FC (Rhine Ruhr).

Although numerous factors can influence the SCC at individual cow and quarter level, such as parity, stage of lactation, incorrect milking machine settings, stress and other factors including genetics, the most important cause remains the infection status of the udder (Schepers et al. 1997).

Milk somatic cells are primarily leukocytes (macrophages, lymphocytes and polymorpho-nuclear neutrophils (PMN)) and the SCC in cow milk may include 0 to 7% epithelial cells originating from the udder (Harmon 2001, Morin 2015). Blood monocytes become macrophages when they enter the tissue and are the predominant cell type in normal milk and constitute to 30 to 74% of the total white blood cells in the milk (Burvenich et al. 2003).

During bacterial infection, macrophages serve to facilitate the immune response and numerous inflammatory mediators may be directly involved (Gallin et al. 1992, Zeconni & Smith 2000, Sharma et al. 2011). Mediators include components such as prostaglandins, leukotriene, histamine, serotonin, interleukins, interferon and other cytokines (Shuster *et al.* 1997, Kehrli & Harp 2001). Inflammation causes increased vascular permeability, vasodilatation, oedema, increased blood flow to the affected area, neutrophil migration, decreased mammary secretion and it is responsible for pain and fever (Harmon 2001). Neutrophils that undergo programmed cell death through apoptosis are phagocytised by macrophages present at the site to help to reduce damage to the epithelium cells (Biggs, 2009).

Lymphocytes are the only immune cells that recognize a variety of antigenic structures through membrane receptors, which define their memory characters (Oviedo-Boyso et al. 2007). One of the initial major defence responses is the influx of leucocytes into the mammary tissue (Nickerson & Pankey 1984). The PMN pass through the larger blood vessels in the udder, adhere to the endothelium of small blood vessels and pass between cell linings into the tissue (Kehrli & Harp, 2001). Chemical chemotactic agents released from leukocytes attract PMN into the milk in large numbers (Akers & Nickerson, 2011). Up to 90% of the cells present in the milk during early inflammation may be PMN (Nickerson & Pankey, 1984). Speed of migration is believed to be a key factor in response to infections and severity of the disease (Burvenich et al. 2000) and this depends on the effectiveness of the immune system of the animal. The PMN engulf and digest the bacteria; however the fat globules and casein that can also be engulfed, decrease their efficacy in milk. Casein and the milk fat globule are produced long after the immune system has matured. They thus remain foreign to the immune system. During lactation the PMNs therefore may phagocytize and ingest of these unrecognised casein micelles and milk fat globules in the normal milk, and this is believed to diminish the

function of the PMN (Paape et al. 2003). Mammary epithelial cells digest phagocytised bacteria and can produce inflammatory mediators (Sharma et al. 2011). Akers and Nickerson, (2011) reported that damage to the milk secretory cells caused by an influx of PMN may likely be the most damaging during mammary development in heifers and may therefore cause damage during the peripartum period when the udder is developing in preparation of the first lactation.

Increased SCC is not only an accepted indicator of intramammary infection (IMI), but very low SCC has also been associated with a reduced immune response and an increased susceptibility to clinical mastitis increasing the complexity of the measurement. This suggests that somatic cells may provide protection from IMI as well as act as markers of infection (Green et al. 2006).

In practice one might need to consider a trade-off between low cell counts with higher production versus low cell counts and a reduced ability to combat future IMI (Fox, 2013) because somatic cells are an important part of the immune system. A question that is asked from time to time is whether SCC can become too low for a sufficient number of leucocytes to fight future IMI. Primiparous cows have lower SCC than multiparous cows (Laevens et al. 1997). The most important cause of increased SCC remains IMI in all lactating cows and especially first lactating cows (Laevens et al. 1997). Heritability of resistance to mastitis has been found to be low and estimated to range from .04 to .14 depending on the test used (Mrode et al. 2012). Shook & Schutz (1994) evaluated genetic control of the udder, as well as physiology, anatomy and immunology and found that the incidence of mastitis was mainly caused by the cow's environment and management.

Schuster et al. (1996) found that the most important immune factor to resist IMI was the ability to recruit leukocytes to the udder and not the pre-challenge numbers of SCC in milk. The expression of L-selectin, a cell membrane protein that is shed during diapedesis, was found to be highly associated with the ability of a pathogen to replicate in the udder (Schuster et al. 1996). Another factor associated with resistance to pathogens is the level of phagocytosis. It appears that the primary immune factors associated with combating IMI is not only the number of cells present in the milk prior to pathogen entry, but mostly the speed at which these cells can be mobilized to the udder (Fox, 2013).

The current generally agreed SCC threshold does not distinguish between principal udder pathogens although pathogens are known to differ in their pathogenicity (Barkema et al. 2006, Petzer et al. 2009, Petzer et al. 2012). Little is currently known regarding SCC thresholds indicating IMI of species-specific udder infections. Current information about these specific pathogens is required because of changes that may occur in bacterial infection in dairy herds over time (Petzer et al. 2009, Zadoks and Fitzpatrick 2009, Petzer et al. 2012).

Any dairy producer with a herd infected with contagious bacteria (*S. aureus* and *Str. agalactiae*) and who wants to eliminate these infections from the herd can benefit from a herd survey where microbiology and cytology is performed. Such a herd survey will identify

the prevalence of specific pathogens and their distribution patterns within that herd. The success of such a programme will be dependent upon accurate identification of infected cows and a fast processing time in the laboratory in order to be able to segregate and treat positive cows, but even more so to prevent new IMI. As microbiology on a herd basis can be costly, alternative methods should be sought for indicating IMI in general. Debate continues about which SCC thresholds are adequate for indicating IMI in general, or IMI with major or minor udder pathogens, the presence of mastitis or even the cure of mastitis. The threshold recommended for SCC in quarter milk samples has been documented by the National Mastitis Council (2001) but no such threshold for composite cow milk samples appear to have been proposed. Although a composite milk sample has the disadvantage that it combines samples from the four quarters and therefore has a dilution effect, taking these samples is much less time consuming, it costs less to analyse them and they are practical to use in large herds. This has been proven to be true under South African conditions. Confusion regarding the apparent cure of mastitis may sometimes exist when researchers have not explained the criteria used to measure this and only refer to "mastitis cure". This assumption may be made either when clinical signs of mastitis disappear, or when a reduction is measured in the SCC from above 200 000 to below 200 000 cells/ml, or when there is actual elimination of bacteria (Hoogeveen and Lam, 2011; Swinkels et al. 2012). This research was intended to also investigate the validity of the assumption that the cure of IMI was associated with a decrease in SCC to below 200 000 cells/ml.

1.2 Introduction to micro-organisms causing udder infections

1.2.1 Contagious udder pathogens (Host adapted)

Contagious udder pathogens spread from cow to cow mainly but not exclusively during milking. Contagious udder pathogens are mostly intra-cellular invaders of udder parenchyma or they may possess a mechanism that enables them to adhere to the epithelial udder cells in order to protect themselves against the local immune system and antimicrobial agents. The main contagious bacteria causing IMI in South Africa have been identified as *Staphylococcus aureus (S. aureus)*, *Streptococcus agalactiae (Str. agalactiae)* and *Streptococcus dysgalactiae* (*Str. dysgalactiae*) of which *S. aureus* is the most difficult to eradicate (Petzer et al. 2009). *Mycoplasma spp*. have not yet been isolated from milk samples in South African herds although this organism is present in cases of respiratory disease. Other bacteria such as *Enterococcus canis* (*E. canis*) (Tikofsky and Zadoks, 2005) and *Streptococcus uberis* (*Str. uberis*) (Zadoks, 2007b) are not classified as contagious, but are thought to have the potential also to spread in a contagious manner. Mastitis caused by contagious bacteria often becomes chronic and SCC levels remain elevated. These organisms are transmitted within the herd from cow to cow mainly at milking, and adhere to hands of milkers, teat liners or udder cloths. It is possible, but not easy, to eradicate contagious mastitis pathogens from a herd by aggressive

antimicrobial therapy, culling, maintaining good biosecurity and pro-active management (Hovinen and Pyorala, 2011, Petzer, et al 2016).

Staphylococcus aureus

Staphylococcus aureus remains a major challenge in South African dairy herds and has been isolated from 10.99% of intramammary infections (IMI) (n=201 062) for the period from 2006 to 2012 (Petzer & Karzis, 2012). *Staphylococcus aureus* IMI has been said to cause a decrease of up to 45% in milk production per infected quarter or 15% in the infected cow (NMC, 2001, 2015). Cows with *S. aureus* IMI with often do not recover their yield potential due to its chronic and destructive nature. Irregular shedding patterns of many *S. aureus* strains complicate the diagnosis (Sears et al. 1990), and the control and management of these udder infections (Piccinini et al. 1999). The ability and the severity of *S. aureus* to cause IMI depends on bacterial characteristics as well as on the susceptibility of the udder of the cow to this organism (Piccinini et al. 1999). The *S. aureus* bacteria possess the ability to produce biofilm resulting in a structured community of bacterial cells enclosed in a self-produced matrix that adhere to a living surface (Costerton et al. 1999). Biofilm provides a protected environment allowing bacteria to resist antimicrobial therapy and host immunity response. This resistance is related to factors such as the increased difficulty of the antimicrobials to penetrate through the extracellular matrix, a lower rate of cell division (detrimental for β-lactam action) and a greater resistance to phagocytosis (Bronzo et al. 2012; Costerton et al. 1999). The exopolysaccharide poly-N-acetyl-β-1, 6 glucosamine (PNAG) enzyme responsible for the production of biofilm was found to be present in 94.36% to 100% (Vasudevan et al. 2003) of *S. aureus* strains and in 75% of CNS isolated from mastitis cases. The protective effect of the biofilm in CNS is currently still being research. Middleton et al. (2002) made use of SCC and N-acetyl-beta-D-glucosaminidase (NAGase) activity as indicators of udder parenchyma injury. They found no significant differences between *S. aureus* strains and suggested that the degree of parenchyma injury during IMI with *S. aureus* was not due to the strain type.

Conventional intramammary and systemic antibiotic therapy used in the treatment of IMI is often unsuccessful in preventing and eliminating chronic *S. aureus* (Barkema et al. 2006; Zadoks, 2007a). Some *S. aureus* strains can produce β-lactamase which inactivates penicillin. In recent years *S. aureus* isolated from animals (pigs, horses, calves and pets) and cow milk has shown resistance to methicillin and has become an important public health concern. Antibiogram results are no guarantee for bacterial cure but only provide knowledge that an antimicrobial possesses the ability to inhibit bacterial growth in a laboratory situation. Strain-specific resistance cannot be routinely diagnosed and is therefore not yet of practical value. Treatment duration is for now the most important factor promoting better cure (Sol et al. 2000, Deluyker et al. 2005).

Genetic resistance to mastitis in cows has been studied. Although favourable resistant alleles have been found against *S. aureus* (Aarestrup et al. 1995), there were also negative

correlations with milk yield and a lack of cross resistance against other pathogens. Cure rates of *S. aureus* IMI have been shown to decrease with increasing cow age, the number of infected quarters per udder, infections in hind quarters, increased SCC, increased duration of infection, and the number of bacterial colonies isolated before treatment (Sol et al. 1994, Barkema et al. 2006). Palpation of udder parenchyma after milking is a valuable tool to determine the severity of chronic udder damage. Before considering treatment of sub-clinical *S aureus* mastitis, the probability of success of such treatment should be estimated (Sol et al. 1994). Extended treatment may or may not be justified economically, but indirect effects such as prevention of shedding and new infections should also be taken into consideration (Swinkels et al. 2005).

Streptococcus agalactiae

Streptococcus agalactiae is an obligate udder pathogen and is found in early infections in the lactiferous ducts of the udder parenchyma. During a *Str. agalactiae* outbreak in a herd the bulk milk SCC (BMSCC) can increase within days to become more than 1,000 000 cells/ml. *Streptococcus agalactiae* IMI is usually introduced into a herd with purchased cows, and the likelihood is high that the infection will spread, especially when parlour hygiene is lacking or stress levels of cows are high. Treatment success (bacterial cure) of over 80% can be regularly achieved in early outbreaks, but once udders are chronically infected and the parenchyma damage is evident, this success rate drops significantly. Eradication of *Str. agalactiae* IMI in South African herds is based on repeated sampling and identification of all *Str.* a*galactiae* infected cows. This is followed by immediate segregation and treatment of all identified cases. Sampling and follow-up actions continue till the herd is negative in more than one successive examination. Eradication is only achieved when there is strict adherence to milking hygiene and parlour management (disinfection of milkers' hands and liners) to prevent new IMI and dry cows are included in the investigation (Loeffler et al. 1995).

Managing and identifying intramammary infections with contagious udder pathogens

The focus of udder health management should be pro-active and preventative. The NMC, 2001 published a 10-point plan for dealing with mastitis pathogens (Reneau, 2001). This plan includes correct milking procedures, machine hygiene and use, the use of post-milking teat disinfectants, dry cow treatment of all cows, and treatment of clinical mastitis cases, culling of chronically infected animals, ensuring biosecurity is in place, and effective record keeping and planning. Although routine dry cow treatment with antimicrobials has been criticized due to concern over the potential development of antimicrobial resistance, Robert et al. (2006) and Karzis et al. (2016) showed that this is not always the case and under effective management this situation sensitivity to antimicrobials increased.

Fast identification methods are needed for successful implementation of a mastitis control programme for *S. aureus* and *Str. agalactiae* IMI, in order that the opportunity for spread of the pathogen in the herd is reduced (Zadoks et al. 2002). Polymerase chain reaction (PCR) analysis of bulk milk can be used to indicate the presence of pathogens such as *Str. agalactiae* and *S. aureus* in a herd. The bulk milk test should be followed by monitoring of pathogen specific identifications of individual animals (Fox et al. 2005). An effective system of diagnosis will play a key role in the successful elimination of these IMI from the herd. *S. aureus* and *Str. agalactiae* positive cows should all be segregated and milked last immediately after identification. Decisions regarding treatment should be based on an individual cow risk assesment (Sol et al. 1994). Alternative actions include quarter inactivation, early drying-off, or culling of cows with a bad prognosis for cure. Any new dairy animals purchased, and cows and heifers that calve down need to be tested for the presence or these bacteria before entering the parlour. Calves in herds where *S. aureus* and *Str. agalactiae* have been positively identified should only be fed fresh milk that has been pasteurized. Introducing new cows and even heifers into a herd increases the risk of new IMI (Fox 2009; Petzer et al. 2012).

1.2.2 Environmental mastitis pathogens

Coliforms is a large group of non-spore forming Gram-negative bacteria capable of fermenting lactose with production of acid and gas at 32°C. They include bacteria of faecal and non-faecal origin and some are pathogenic and some not. A high coliform count in bulk milk indicates contamination during production, storage processing or handing of the milk.

Environmental mastitis bacteria can either be Gram-negative species such as *E. coli, Klebsiella* spp*., Enterobacter* spp. and *Serratia* spp. or Gram-positive species such as *Streptococcus uberis* (*Str. uberis*) and *Enterococcus faecalis (E. faecalis).* The prevalence of both *Str. uberis* and *E. coli,* although still low, has increased over the past 10 years in South African dairy herds (Petzer et al. 2009). *Escherichia coli* mastitis increased mostly in free stall barns in the Western Cape province of South Africa during winter rainfall. Although most *E. coli* strains are harmless commensals that live in the intestinal track. Five *E. coli* strains are known to cause diarrhoea; enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enterohaemorragic (EHEC) and enteroadherent (EAEC). An enterohaemorragic *E. coli* 0157:H7 has been associated with outbreaks of haemorragic colitis in humans associated with consumption of raw milk and dairy products.

Streptococcus uberis appears to be increasing in pasture based herds according to Blignaut (2016). Gröhn et al. (2004) found that milk loss due to mastitis caused by streptococci, *Klebsiella spp.* and *Trueperella pyogenes* (*T. pyogenes*) can persist for up to 70 days after diagnosis.

Escherichia coli

Escherichia coli mastitis affects mainly high producing cows around the time of parturition and during the first trimester of lactation in herds with low bulk milk somatic cell count (BMSCC). Wet and unhygienic bedding poses a high risk environment for *E. coli* IMI challenge (Bradley and Green, 1998). The environmental challenge includes an increase in the herd size, confinement of cows (freestall barns), and high milk yields (with subsequent increase in dry matter intake and manure production). These are dairy management realities that all promote the risk of environmental mastitis. Although only 10% to 30% of clinical coliform cases have been reported to be acute (Hogan et al. 1989) such an acute infection is presented as causing severe local udder damage accompanied by an acute febrile disease that can end in the death of the cow.

Escherichia coli are present in the environment of the cow and invade the udder via the teat canal. It is generally accepted that the *E. coli* strain itself does not play a major role in the severity of mastitis but rather the lipopolysaccharide (LPS) endotoxin produced (Burvenich et al. 2003) and the failure of the immune system of the cow. *Escherichia coli* multiplies rapidly once in the udder and can reach peak numbers within 5 to 16 hours (Berning et al. 1993). The LPS endotoxin induces defence mechanisms in the udder and an influx of neutrophils. Much of the inflammatory changes are due to the release of the LPS endotoxin following phagocytosis and killing of *E. coli* by neutrophils. To reduce the severity of acute coliform mastitis either bacterial growth must be slowed down or the effect of the LPS endotoxin needs to be neutralized (Burvenich et al. 2003). Treatment only commences when clinical symptoms are seen, and in the case of coliform mastitis this may be at the peak of bacterial growth. When antimicrobial remedies with bacteriocidal action are used and bacteria are killed in large numbers, high levels of endotoxin may be released that may cause the death of the cow. Antimicrobial remedies with bacteriostatic action should therefore rather be used when *E. coli* mastitis is suspected.

The release of neutrophils is beneficial to the udder following *E. coli* infection but the response needs to be rapid and it is essential they arrive at the lumen of the udder in order to prevent the coliform bacteria from reaching high numbers (Burvenich et al. 2003). According to Burvenich et al. (2003) it seems that at parturition and during early lactation, high producing cows are especially sensitive. In the second and third trimester of the lactation, clinical signs of coliform mastitis tend to be moderate and subside very rapidly, and the cows may cure themselves (Berning et al. 1993).

In summary, environmental conditions that increase the risk of coliform mastitis are over-crowding as seen in zero-grazing systems, poorly designed housing, wet unhygienic bedding, housing systems that lead to teat injuries, poor udder preparation before milking, milking of wet udders and milking machine malfunctions. High stock densities on pastures may result

in increasing manure contamination with a greater risk of environmental pathogens affecting the udder (Burvenich et al. 2003).

Streptococcus uberis

Streptococcus uberis (*Str. uberis*) is currently still categorised as an environmental udder pathogen but with the knowledge derived from strain typing it is becoming clear that some stains can be contagious in nature and can survive inside the udder of the cows (Bradley, 2002, Charman et al. 2012, McDougall, 1998). *Streptococcus uberis* is one of the important cultured mastitogenic pathogens. It has been isolated from cows' bedding, pastures, water, and from their feet, teat and udder skin. *Streptococcus uberis* is also thought to colonize the intestine of 10 - 35% of cows where it contaminates the environment and pastures via faecal excretion. The proportion of faecal samples containing *Str. uberis* was found to be highest during the winter grazing season in New Zealand where low levels of solar radiation, low temperatures, high moisture and high stocking density enhanced their survival on the pasture (Lopez-Benavides et al. 2005).

Although transmission of *Str. uberis* IMI from cow to cow is not of primary importance, herd surveys can be a valuable management tool to identify cows with persistent IMI and to monitor the success of control programme. As IMI with environmental streptococci and coliforms are more likely to occur during the dry period than during lactation, it can be beneficial to sample cows shortly after calving in order to identify cows at risk of having these IMI (Smith et al. 1985).

Tamilselvam et al. (2006) showed that *Str. uberis* has the potential to survive within mammary epithelial cells for up to 120 hours. Other studies (Zadoks et al. 2003, Zadoks, 2007b, Abureema et al. 2014) suggest that *Str. uberis* has the epidemiological characteristics similar to that of contagious pathogens regularly found in dairies. Zadoks et al. (2003) showed that a predominant random amplified polymorphic DNA fingerprinting (RAPD) strain of *Str. uberis* was isolated from milk samples from multiple quarters of a cow and from within a group of cows. This indicated the possibility that multiple cows became infected from a common environmental source or alternatively it could have been the result of between-cow transmission of the pathogen.

1.2.3 Minor udder pathogens

Coagulase negative staphylococci (CNS) have been identified increasingly as mastitis pathogens in many countries with intensive dairy production, including South Africa (Petzer et al. 2009; Taponen et al. 2010,). According to Zadoks (2007a) CNS strains vary in their ability to cause mastitis and have been classified as opportunistic, environmental or contagious. Based on strain-typing studies of *Staphylococcus chromogenes*, *Staphylococcus hyicus* and *Staphylococcus epidermidis,* contagious transmission of CNS appears to be relatively rare

(Gillespie et al. 2009). Increasing numbers of CNS have been identified which are thought to be capable of causing both subclinical and clinical mastitis (Myllys, 1995). This may be due to an increased pathogenicity of this group or to a reduced ability of the udders' immune system in the cows or a combination of both of these factors (Myllys, 1995).

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CHAPTER 2

A pathogen specific approach towards udder health management in dairy herds - using micro-cytology from routine herd investigations.

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SIGNIFICANCE OF THE WORK

A dedicated udder health diagnostic program was developed and used over a 15 year period in South Africa. This programme is used to analyse milk samples of herds based on microbiological and cytological results of individual cows. This is a fresh approach as very few countries worldwide can perform micro-cytology on a complete herd. This programme with a current dataset of 1.5 million samples makes a detailed analysis possible and helps to identify and provide insight into possible sources of udder health problems. The aim of this manuscript is to share the information and experience that was acquired over the years with veterinarians, udder health consultants and animal scientists. This knowledge enables a detailed pro-active udder health approach based on knowledge of pathogens isolated from individual cows or quarters in the complete lactation herd. Results are presented as various group reports indicating trends within herds, cases studies and also providing analysis of the individual cow or quarters.

ABSTRACT

A dedicated udder health diagnostic programme was developed and used over a 15 year period in South Africa to analyse milk samples based on microbiological and cytological patterns within various groups and for individual cows and udder quarters in dairy herds. These pathogen specific analyses are utilized for pro-active improvement and management of udder health in South African commercial dairy herds. The programme acts as a monitoring tool and identifies management areas at risk and individual cows with udder disease and utilizes both quarter and composite milk samples. Intramammary infection (IMI) is a dynamic situation and depending on the time a milk sample is taken, false negative results may be obtained. A new IMI and an infection that is curing (after treatment or spontaneous cure) may both have low somatic cell counts (SCC), masking the true bacterial status. Somatic cell count in individual infected udder quarters may differ greatly depending on the causative bacterial species, its pathogenicity, the host immune status and environmental factors involved. A pathogen-specific udder health approach was followed with repeated herd tests to take account of these udder health dynamics. The results of the herd IMI investigation is applied

in practice to assist veterinarians, udder health consultants and managers to make informed and specific detailed decisions at both herd basis and on an individual cow basis regarding udder health.

Key words: pathogen-specific, herd udder heath, management programme, dairy cows, udder quarter and composite samples

INTRODUCTION

Mastitis is an endemic disease and is considered the most frequent and costly disease in the dairy industry responsible for the highest financial losses, which effects both the animal and the quality of the product (Halasa et al. 2007; Hoogeveen et al. 2010).

Mastitis is complex and multi-factorial in nature and generally results from an interaction between a variety of microbial infections, host factors, environment and management factors, and with a generally poor treatment success. It is defined by the National Mastitis Council (NMC) as an inflammation of the mammary gland mainly due to bacterial infection (NMC Guidelines 2001, 2015). Dealing with clinical mastitis cases remains important but damage to the udder parenchyma may have lowered a cow's lifetime production potential and she may have already shed pathogens (DeGraves & Fetrow, 1993; White, 2010). Optimal management practices are considered to be the most effective way to control the disease. Research conducted during the 1960s set the stage for our current understanding of mastitis and established standards for contagious mastitis control (Davidson, 1961; Dodd et al. 1969; Neave et al. 1969; Neave et al. 1966). From this work a five-point mastitis control programme was developed and was later upgraded to the NMC 10-point mastitis control plan (Smith and Hogan, 2001). Utilization of this program has led to a reduction in the prevalence and elimination of contagious mastitis from many South African farms. However, *Staphylococcus aureus* (*S. aureus*) and *Streptococcus agalactiae* (*Str. agalactiae*) remain a challenge on individual farms on South Africa and abroad (Petzer et al. 2009).

In a paper published by Middleton (2013) he questioned what we have learned regarding *S. aureus* mastitis in the last 50 years. His response summarised the global udder health dilemma. We have gained much knowledge but the basic fact that milking time hygiene is the main critical control point has not changed. Decisions regarding the control of mastitis in a given herd will depend on the contagiousness, persistence and inflammatory nature of the main infecting species and strains. The use of historical data to evaluate true new IMI rates, the extent of chronic carriers within the herd, the bacterial cure rates in combination with somatic cell count (SCC) levels are all valuable measures to identify mastitis and stress-related causes of high SCC.

Knowledge of the bacterial species present in the udders of cows in a herd and the ability to identify cows with and without intramammary infections (IMI) can be used as a tool for in-

depth management decisions. The rate of new IMI can be more effectively controlled by the separation of cows with IMI in the case of contagious pathogens, and despite the best efforts bacteria will be transmitted from cow to cow. Cows infected with known contagious IMI can be milked last, thereby limiting the risk of spreading this infection to the healthy cows. Parlour supervision of the cows infected with contagious pathogens should then be intensified. It has been well established that the probability of IMI cure depends on cow, pathogen and treatment factors (Barkema et al. 2006). Although treatment success is influenced by choice and use of an appropriate product and also the lactation stage of the cow, the main factor influencing treatment success has been found to be treatment duration (Barkema et al. 2006). In addition the bacterial species or strains involved, their pathogenicity and possible antimicrobial resistance needs to be considered, as well as the immune response of the cow. Cure rates for *S. aureus* mastitis have found to range from 3-74%. Cure is lower in older cows, and in those with high SCC, increased chronic IMI and increasing numbers of mammary quarters infected (Barkema *et al*. 2006). Spontaneous cure of new *S. aureus* IMI may be as high as 21%, while in chronic cases this figure can be as low as 3% (Swinkels et al. 2012). Only a few udder pathogens such as *Str. agalactiae* are known for a high treatment success during lactation (Keefe, 1997). *Staphylococcus aureus* and *Str. agalactiae* IMI are still present in dairy herds in South Africa (Petzer et al. 2013) and warrant a more efficient testing system that could identification truly infected quarters and enable producers to eliminate both of these pathogens from their herds.

The SCC measure has been used worldwide for several decades as a primary indicator of udder health in dairy herds (Hillerton, 1999; Reneau 2001; Heeschen, 2010). More than 95% of the cells in milk are leucocytes consisting of variable proportions of macrophages that recruit the neutrophils and lymphocytes in the event of invading pathogens by releasing chemo-attractants. The lymphocytes act mostly as memory cells for the immune system while neutrophils phagocytise and destroy the pathogens. Less than 5% of SCC consists of epithelial cells originating from the mammary gland. The level of SCC in milk depends on various factors of which IMI is the most important. Factors include parity, stage of lactation, milking frequency, stress (environmental, nutritional, systemic disease, and day to day changes in management) and non-specific disturbance (NSD) where an increase in SCC is not cause by pathogens.

Traditionally bulk milk tank somatic cell count (BMSCC) is used as a primary index when analysing herd udder health and is used as one of the quality criteria for payment by the secondary milk industry (Ruegg and Pantoja 2013). High BMSCC could indicate udder health problems, whereas a too low level (below 100 000 cells/ml) could act as a warning to become pro-active in preventing mastitis. However, the BMSCC provides only an estimation of the prevalence of infection and irritation of the udders of the cows. This is not a true reflection of the herd udder health status as milk from problem cows should have been excluded (cows with clinical mastitis, fresh in milk cows, cows under treatment, and sometimes those with high SCC). The BMSCC can also be influenced by herd dynamics such as the average parity of

cows, and the distribution of lactation stage seen in seasonality calving herds compared to herds with an all year round calving pattern. The impact of any individual cow on BMSCC will further depend on its SCC and the milk yield of the cow and the dilution factor within the tank. It has been estimated that there is likely to be a 10% increase in IMI prevalence in a herd for every 100 000 cells per ml increase in BMSCC (Bradley, 2007). In the year 2014 an estimated 145 million dairy farms operated globally and the largest average dairy herds were found in Saudi Arabia (8125 cows) followed by New Zealand (393 cows) and South Africa (238 cows) (Lactodata, 2015). The larger the herd the more critical the interpretation of the BMSCC needs to be and the less valuable it becomes as a pro-active udder health monitoring tool due to the dilution effect of the milk masking high SCC of an individual cow.

To evaluate changes in udder health status the somatic cell counts of individual cows are often analysed together with herd clinical mastitis statistics (Bradley and Green, 2001). A SCC threshold is used as a guideline to estimate new IMI, chronic IMI, cure rates and those cows or udder quarters that remain without IMI. There has never been absolute consensus regarding the correct threshold level to indicate IMI, but 200 000 cells/ml for udder quarter milk samples has been adopted to indicate IMI by most in the field (Heeschen, 2010). The reliability of records of clinical mastitis cases depends on accurate detection of mastitis on a day to day basis on farms and these records are often questionable.

The aim of this article is to outline a fresh approach to herd udder health monitoring that facilitates an in-depth analysis of clinical and subclinical mastitis using software that has been developed and tested over the past 15 years at the Faculty of Veterinary Science, University of Pretoria. Currently more than 930 commercial dairy herds from South Africa and neighbouring countries are benefiting from its application. The Milk Sample Diagnostic (MSD) program that was developed provides an overview of the current and historical herd udder health situation and allows for analysis related to parity, days in milk, and other herd groupings, and facilitates decision making on an individual cow level based on current and historic species-specific and udder pathology information. It is used as a practical tool to identify challenges at herd, cow and udder quarter level, and guides decision making at an operational level. Only by addressing the particular cause can the problem be eliminated. This udder health approach differs from the conventional approaches that use primarily SCC and data of clinical mastitis cases as an aid to decision making.

MATERIALS AND METHODS

Milk samples and data

Udder quarter and composite milk samples (one milk sample taken from all four quarters) are taken in most cases from all lactating cows in a herd to establish the presence and prevalence of udder pathogens. Professional milk samplers assist milk producers in South Africa to take

samples in an aseptic manner, ensuring that the sample quality is good, and that the cold chain is maintained until the samples reach the milk laboratory. Most milk samples reach the laboratory within 24 hours. On arrival at the Milk Laboratory (Milk Laboratory, Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, South Africa) the batch temperature of the milk samples is recorded. Milk is then plated out on Bovine Tryptose agar, and cultures are read after 18-24 and 48hours (Petzer et al. 2012b). Samples are not preserved but stored in a walk-in fridge at 4̊C for at most 48 hours till cell counting. Somatic cell counts were performed using a Fossomatic 5000 (Petzer et al. 2012b). Identification of mastitis pathogens is generally completed within 48 to 72 hours after the samples have been received at the laboratory.

In addition to the micro-cytology and clinical appearance of milk, general cow information is added to the MSD program. This includes information regarding calving dates, parity, milk yield, stage of pregnancy, status of the udder parenchyma (assessed by palpation) and teat canal scores.

Results of quarter milk and composite milk samples are analysed separately and the results are summarised in individual cow reports and in various group reports.

Some of the various reports that will be discussed

The Serial Herd Udder Health Report used for evaluation of composite cow milk samples provides an overview of the current and historical udder health status of the herd based on microbiological and cytology results. The report also provides a perspective on the level of new, persistent and cured cases for each bacterial species isolated from the herds, and indicates the SCC distribution within the herd.

A Current Herd Udder Health Report for analysis of quarter milk samples summarises the micro-cytology results of the current test of the herd as percentage of quarters with mastitis, or with NSD, IMI with low SCC, and the percentage of normal quarters.

Group Reports focus on species identification, early post-partum reports (for the first 30 days in lactation), a lactation stage report (5-90 days, 91-180 and 180+day groups) also differentiating between parities.

The Economic Report provides financial information on probable loss in revenue as a result of milk not being produced due to an udder that is not completely healthy, indicated by the SCC level.

Criteria used for diagnosis

Microbiology and cytology results are available for each quarter milk sample. In this program quarters that tested bacteria negative with a SCC of below 300 000 cells/ml milk were

regarded as being normal (N); quarters that tested bacteria positive with a SCC of equal or above 300 000 cells/ml milk were regarded as having mastitis (M); quarters that tested bacteria positive with a SCC of below 300 000 cells/ml were identified as having a "teat canal infection" (TCI) or sub clinical mastitis; and quarters that tested bacteria negative with a SCC of equal or above 300 000 cells/ml were identified as having a non-specific disturbance (NSD). The desired herd values aimed for are: less than 5% of quarters with mastitis; less than 5% quarters with TCI; less than 3% quarters with NSD and more than 50% normal quarters.

RESULTS AND DISCUSSIONS

2.1 Serial Udder Health Reports (Composite milk samples)

The Serial Herd Udder Health report is presented in two parts. The first part provides a summary overview of 1 to 4 consecutive herd micro-cytology examinations based on results of composite milk samples, while the second part deals with the SCC. The current herd udder health status is analysed and compared to results of previous examinations. Positive progress or negative developments in the herd in terms of IMI and SCC trends is measured and evaluated. The various bacterial species isolated from individual cows are indicated as numbers and percentages of cows sampled (see Table 2.1) while the SCC are summarised according to six threshold levels (Table 2.2).

2.1.1 Part 1 – Serial Herd Microbiological report

Each bacterial species isolated from individual composite milk samples in the herd is indicated as a number and as a percentage of the total samples examined. Of each bacterial species isolated the number of new, repeat or persistent cases, and cases cured are indicated. In the first examination of a herd all cases are indicated as new infections. In consecutive herd examinations, new infections are indicated when a specific bacterial species has not been isolated from the same cow at the previous examination; persistent cases are those with identical bacterial isolations as shown previously; while "cases cured" (those that are culture negative) in the current examination for the specific bacterial species that was isolated at the previous examination (Table 2.1).

Prevalence of species-specific herd intramammary infections

Depending on the principal bacteria present and its prevalence in the herd, management strategies should be planned in collaboration with the herd manager. A policy of zero tolerance is followed in most cases where *Str. agalactiae* and *S. aureus* are isolated, aiming at the eradication of these bacteria from all udders in these herds. This approach has proved to

be practical and successful in South African herds over the past fifteen years. The prevalence of *S. aureus* IMI in more than 930 South African commercial dairy herds decreased from 14.08% in 2008 to 7.77% in April 2012 (Petzer and Karzis, 2012a) and to 5.14% in December 2014 (Petzer, unpublished data). Bacteriological results as shown in Table 2.1 provide the veterinarian, udder health consultant and dairy manager and owner with detailed results to assist informed decision making regarding udder health management at the herd level.

New intramammary infections

Depending on the species of bacteria and the level of new intramammary infections (IMI) in the herd deductions can be made regarding parlour hygiene and bedding management (Table 2.1). Management may prove to be inadequate and protocols may need to be revised and upgraded.

The level of new IMI can be a valuable measurement of effective parlour hygiene and of milker education, dedication and health, especially in the case of contagious mastitis bacteria such as *S. aureus* and *Str. agalactiae*. It can also indicate ineffective separation of *S. aureus* and *Str. agalactiae* positive cows or an incorrect milking order. The level of new infections may increase if there is a lack of biosecurity when new cows or heifers are introduced into a dairy herd without determining the status of their IMI prior to allowing them on the farm, in the parlour and mixing them with the local herd. Bacteria such as *S. aureus* and *Streptococcus pyogenes* (*Str. pyogenes*) are known to be able to cause reverse zoonosis (Messenger et al. 2014). When reverse zoonosis is suspected in a herd milkers and people in close contact with the cows should be tested for the presence of these bacteria by requesting throat swabs.

When most new IMI are predominantly environmental bacteria such as the coliforms (*E. coli, Klebsiella spp*. and *Serratia spp.*) or streptococci other than *Str. agalactiae,* then udder, feet and flank hygiene scores (Cook and Reinemann, 2007) can be performed to quantify the challenge and identify areas of risk. Sources may include inadequate management of bedding which can cause increased levels of loose faeces on bedding surfaces (Reneau et al. 2003), or water pollution (mineral or microbial), or over-crowding.

Repeat (persistent) cases and cases cured

When the same bacterial species is isolated from the same udder (composite milk samples) or the same quarter (quarter milk samples) on two consecutive examinations within a reasonable time period it is regarded as a repeat or persistent IMI. The percentage of cases that repeat and those cured (bacterial cure) indicate the level of chronicity and the problematic bacterial species or strains present in the herd. It may be an indication of ineffective mastitis treatment. Udders of cows that repeat are palpated to identify possible parenchyma pathology because unsuccessful treatment may be as a result of fibrosis, nodules or atrophy of the udder parenchyma, and not necessarily due to bacteria that are resistant to

antimicrobials. "Fibrosis" (hardening udder quarter) is used as an indication of a more recent chronic case compared to "atrophy" (shrinking udder quarter).

Table 2.1. Serial Herd Udder Health Report. Case study Part 1: Bacteriology history report for four consecutive herd examinations of the same herd using composite cow milk samples.

Bacteria isolated	Dates	Oct 28, 2014	Nov 17, 2014	Jun 4, 2015	Jul 2, 2015
	Numbers examined	1 290 (*)	1 304 (*)	1366 (*)	1 297 (*)
Staphylococcus aureus	Total	78(6.05)	214 (16.41)	72 (5.27)	58 (4.47)
	New	78(6.05)	159 (12.19)	22 (1.61)	12 (0.93)
	Repeat	0 (0.00)	44 (3.37)	32 (2.34)	27 (2.08)
	Cured	0 (0.00)	11 (0.84)	18 (1.32)	19 (1.47)
Streptococcus agalactiae	Total	24 (1.86)	0 (0.00)	73 (5.34)	45 (3.47)
	New	24 (1.86)	0 (0.00)	72 (5.27)	40 (3.08)
	Repeat	0 (0.00)	0 (0.00)	1 (0.07)	5 (0.39)
	Cured	0 (0.00)	1 (0.80)	8 (0.59)	33 (2.54)
Streptococcus dysgalactiae	Total	4 (0.31)	0 (0.00)	2 (0.15)	2 (0.15)
	New	4 (0.31)	0 (0.00)	2 (0.15)	1 (0.80)
	Repeat	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.80)
	Cured	0 (0.00)	3 (0.23)	1 (0.70)	1 (0.80)

(*) Percentage of samples examined

Application of results in a *Streptococcus agalactiae* positive herd

The herd indicated in Table 2.1 had 3.47% of cows infected with *Str. agalactiae* at the examination dated 2 July 2015. Although this percentage decreased from the previous examination on 4 June 2015 (from 5.34% to 3.47%) too many (40 of 45) of these infections were new *Str. agalactiae* IMI possibly indicating a relaxed parlour hygiene. Five cases of *Str. agalactiae* IMI persisted and 33 had been cured since the June examination. The producer was asked to provide information on the whereabouts of 35 cows that were infected with *Str. agalactiae* in June 2015 and which were not tested in July. These cows might have been dried off, removed from the herd or were merely not sampled. The targets should be < 5% for of *Str. agalactiae* should be new IMI and < 5% persistent cases. A revised management plan should include better prevention and follow-up of cases that did not cure.

In this case where *Str. agalactiae* IMI has been isolated from a herd a partial "blitz therapy" can be implemented because in this case positive cows have been identified. They should immediately, after conformation of their infections status, be separated from the rest of the cows for the treatment period and until they have been resampled and found to be cured (bacteria free). Retesting of both the lactating herd and treated cows is essential for the

successful elimination of *Str. agalactiae* from the herd in a relative short period of time. A percentage of *Str. agalactiae* infected cows may have been missed during the laboratory examination due to the small volume of milk plated out; or because of sub-minimal concentrations of bacteria present in milk samples; or due to the presence of coagulase negative staphylococci (CNS) in udders with high SCC initially masking the presence *Str. agalactiae* (personal experience). A short laboratory turnover time in the case of *Str. agalactiae* IMI is crucial to the success of eliminating these bacteria from the herd (Keefe, 1997).

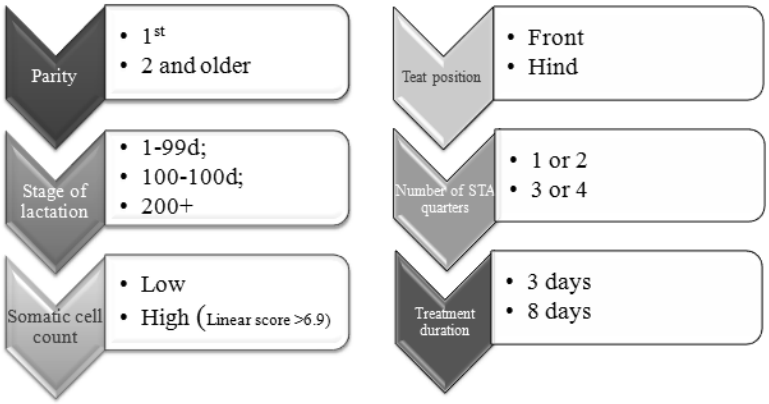
Case study: Application of results in a *Staphylococcus aureus* positive herd

In herds where a low prevalence of *S. aureus* is identified, the few positive animals should be culled as soon as possible, and management should focus on sound parlour and milking procedure hygiene. The lactating herd should be retested and quarter udder secretion samples of dry cows in late gestation should be included. When a herd is identified with a medium to high prevalence of *S. aureus* a longer term strategy is adopted rather than that of culling all positive animals, although culling will form part of the action taken. An important action will be to upgrade the protocol and application of parlour management and the milking routine, the hygiene and monitoring strategies. Other factors such as the within-herd prevalence, the contagiousness of the bacteria, the milk price, and the current percentage of cows culled because of mastitis, and the number of replacement heifers available must also be considered (Bradley, 2007).

The herd indicated in Table 2.1 was diagnosed with 78 (6.05%) *S. aureus* cows in October 2014 and was regarded as herd with a moderate level of infection prevalence. The producer would be advised to separate the *S. aureus* positive cows, if possible for life, and to keep them in a *S. aureus* group. The results from November 2014 (Table 2.1) showed an increased in the number of *S. aureus* cases to 214 (16.41%). Of these, 159 cows (12.19%) cows had new *S. aureus* infections indicating that the preventative measures were inadequate; 44 cows (3.37%) showed persisted infection from the previous test, indicating a in these cases a high possibility of chronic cases, and only 11 cows (0.84%) were apparently “cured”.

Though the choice and duration of treatment should be discussed with the farmer the probability of cure could be calculated for each *S. aureus* positive cow using the system developed by Sol *et al*. (1997). This formula incorporates parity (1st lactation and higher), stage of lactation (early, mid or late), level of SCC (above or below a linear score of 6.9 or approximately 800 000 cells/ml), the number of quarters per udder positive for *S. aureus* (less or more than 3) and quarter position (front or hind) of individual cows, as well as the treatment duration. It can be used to calculate the probability of *S. aureus* cure (Swinkels *et al.* 2012) (Figure 2.1). Information for individual cows on parity, lactation stage, pregnancy status, milk yield, mastitis and SCC history is available in the MSD program to aid in the decision making. Udders of *S. aureus* cows should be palpated to identify gross parenchyma

damage such as fibrosis, nodules and atrophy, which usually would make treatment ineffective. An informed decision could then be made regarding actions to be taken in case of each individual *S. aureus* cow, and a detailed action list for individual cows can be formulated for the manager. This might include intramammary therapy with or without an extended duration, early drying off with therapy, inactivation of a quarter, or culling of the cow.



Adapted from Sol et al. 1997

Figure 2.1. The criteria used in the calculation of the probability of cure for a cow with *Staphylococcus aureus* intramammary infection of the individual cow.

In this particular herd (Table 2.1) at the next examination date on 4 June 2015 the number of new cases had decreased to 22 cows (1.61%) of samples, while persistent infections remained relative low at 32 (2.34%), as does the numbers with bacterial cure (1.32%). *Staphylococcus aureus* "cure cases" however needs to be retested because of the nature of *S. aureus* to shed intermittently. This is the reason why these cows should remain separate from the rest of the herd for life.

South African dairy managers and veterinarians are aware of the existence of reverse zoonosis where infections carried by people may pose a threat to udder health of cows. A noteworthy number of the work force (Statistics South Africa, 2013) has immune systems compromised because of HIV with a consequence of an increased risk for disease. Milkers with upper respiratory infections caused by *S. aureus* may pose a risk to the udder health of dairy cows if hygienic principles are not adhered to in the parlour (Petzer et al. 2009). Precautions such as the wearing of facial masks, and placing milkers that have tested positive for *S. aureus* infections into strategic milking positions where they do not need to touch udders (like teat dipping) have been helpful (personal experience).

Application of results in herds with mainly gram negative IMI.

When IMI with bacteria of environmental origin is predominantly in a herd the management focus should shift to camps, pasture, bedding and the parlour as sources and areas of risk. The specific bacterial species may provide information on the probable source. *Pseudomonas spp*. for instance is often found in a water source, and *Listeria* spp. is mostly present in silage (Hogan and Smith 2003). In South African pasture based dairy herds the prevalence of *Streptococcus uberis* (*Str. uberis*) is increasing (Petzer et al. 2009).

2.1.2 Part 2 – Serial Herd Somatic Cell Count Report

In the second part of the Serial Herd Udder Health Report six SCC thresholds are calculated from the herd test (percentages and cumulative percentages). The SCC increments are 125 000 cells/ml up to 500 000 cells/ml and become larger for higher SCC levels. The herd target is to have more than 80% of lactating cows with SCC of less than 250 000 cells/ml, while less than 5% should have a SCC in excess of 750 000 cell/ml. Herds with a high percentage (>90%) of cows with low SCC may be at higher risk of contracting *E. coli* mastitis. In such a situation management practices such as teatdip prior to milking and feeding immediately after milking to prevent cows from lying down would be advised to allow enough time for the teat canal to close. This is especially true of high yielding herds where the immune system of cows is more likely to be weakened during the first trimester of their lactation (Hogan and Smith, 2003).

Table 2.2. Serial Herd Udder Health Report. Part 2: Somatic cell count (SCC) history report for four consecutive herd examination of the same herd based on results of composite cow milk samples.

SCC x 10^3 cells/ml	**Dates x Values (% of lactating cows)**							
	Oct 28, 2014		**Nov 17, 2014**		**Jun 4, 2015**		**July 2, 2015**	
	1 290 cows		**1 304 cows**		**1 366 cows**		**1 297 cows**	
	%	**Cum* %**	**%**	**Cum %**	**%**	**Cum %**	**%**	**Cum %**
1-125	35.71	35.71	25.14	25.14	36.85	36.85	59.46	59.46
126-250	13.75	49.46	15.45	40.62	17.61	54.46	9.27	68.73
251-375	9.53	58.99	11.48	52.09	10.56	65.02	5.66	74.39
376-500	6.39	65.38	7.29	59.38	5.87	70.89	3.99	78.38
501-750	8.32	73.7	9.49	68.87	7.51	78.4	4.89	83.27
750+	26.3	100	31.15	100	21.6	100	16.73	100

*Cum= Cumulative

Somatic cell count dynamics of four consecutive herd investigations from 26 October 2014 to 2 July 2015 are compared in Table 2.2. The percentage of cows with SCC below 250 000 cells/ml although still low at 68.13% in July 2015 has improved from 40.62% in November 2014. Similarly, the percentage of cows with SCC above 750 000 cells/ml decreased from 31.15% to 16.73% for the same period.

In herds with a high proportion of cows with high SCC, a distinction is made between those with and without IMI. In cases where most samples that showed high SCC also had IMI, the sources of these infections should be identified and eliminated or managed. When samples from which no bacteria were isolated form a significant portion of samples with high SCC, possible stressors and causes of udder irritation need to be investigated. Composite milk samples from cows identified with high SCC can be tested on farm with the California Milk Cell test to gain insight into the inter-quarter SCC relation. Heat stress, mud stress, nutritional stress and social or handling stress are examples of stressors that can be responsible for increased SCC (Du Preez, 2000). Differences in SCC between quarters of the same udder are used. Physiological changes and stressors to the cow will be more prone to cause an elevated SCC in 3 or 4 quarters, while udder irritation is more often seen in only 1 or 2 quarters of an udder. The latter can be caused by incorrect milking techniques, incorrect milking machine settings, or lack of adequate machine maintenance. Cows that have recently been treated with intra-mammary antimicrobials could also test culture negative with a high SCC.

The probability of incorrect milking machine settings and incorrect use causing irritation can be investigated by using teat canal scoring (Neijenhuis et al. 2001) on first lactating cows, 1 to 3 months in lactation. The teat canal is the first line of udder defence and a very important barrier preventing IMI when damaged. Pulsator function should be tested by performing the static test on all milking units followed by dynamic testing to check vacuum stability and level at the teat end during milking. Milking machines with high milk lines as well as swing-over parlour systems are still used in South African dairies. Teat canal damage is more likely occur in high milk lines systems than in systems with low milk lines and due to over milking and the increased risk of the higher system vacuum. Swing-over systems with automatic cluster removers (ACR) are now installed in South African dairies. Flow meters that measure the milk flow per unit and initiate cluster take-off are installed in these systems far above the level of the udder, and the milk lines transporting milk from the cluster to the flow meters are often more than 1.5 to 2 metres long. There is therefore too long a delay in the time from when the take-off flow rate is reached until the cluster is actually removed, increasing the risk of overmilking.

Milking routine should be monitored on site; and a System Lactocorder (WMB AG, Balgach) can provide measurements of the milk let down time, rate of milk flow and the timing of cluster removal. Automated inline monitoring systems such as Afimilk program (Afimilk Ltd, Kibbutz Afikim, Israel) are available to identify cows that are in incorrect groups, and to identify current and past trends in the milking routine. These can include the time from touch

to attachment of clusters, milking speed, cluster fall-off, re-attachment and early detachments. Reasons for insufficient stimulation or delay in take-off can be identified and rectified, whether by training or notifying the milkers or by correcting a milking machine fault.

Nutritional stress can contribute to high SCC and practical methods such as bunker score and space (Bolsen and Pollard, 2004), rumen filling (Burfeind et al, 2010), faecal score and percentage cows ruminating may be used as a starting point for the investigation in total mixed ration herds, body condition scoring (Wildman et al. 1982) and locomotion scoring (Manson and Leaver, 1988) followed by an in depth analysis of the feed when indicated. Cows on pastures and in paddocks may suffer from stress because of mud during the rainy season. In South Africa the high Temperature Humidity Index (THI) may often have a negative effect on the SCC levels, milk yield and reproduction efficiency during the hot summer months (Giesecke et al. 1988, Du Preez et al. 1990).

2.2 Current Herd Udder Health Report (Quarter milk samples)

A summary of species-specific IMI and somatic cell counts for herds are shown in Table 2.3. The Report is divided in two sections with the first part indicating results from lactating cows and the second part from non-lactating cows. The Milk sample Diagnostic (MSD) program currently uses a somatic cell count level of 300 000 cells/ml as the lower threshold to diagnose mastitis in quarter milk samples when a pathogen is present, and classifies these findings classified and NSD in the absence of IMI. Quarters with a SCC level of below 300 000 cells/ml and without IMI are identified as “normal”, while those with a low SCC where and IMI is present are diagnosed as “teat canal infections”. Dry cow secretions are only examined for the presence of bacteria and no SCC is done.

Table 2.3. Current Herd Udder Health Report based on somatic cell count and culture results of quarter milk samples from lactating and dry cows.

Cows	Diagnosis	Micro-organisms	Right front quarters (%)	Right hind quarters (%)	Left front quarters (%)	Left hind quarters (%)	Total (%)
Lactating	Mastitis*	*S. aureus*	0	1.1	1.1	1.1	3.3
	Mastitis	CNS	0	2.1	0	0	2.1
	Mastitis	*Str. uberis*	0	0	0	1.1	1.1
	Normal	none	14.7	10.1	9.8	10.9	45.5
	NSD**	none	5.9	8.4	6.4	8.6	29.3
	TCI***	*S. aureus*	3.3	1.8	2.2	0	7.3
	TCI	CNS	1.1	1.5	4.4	3.3	10.3
	TCI	*Str. agalactiae*	0	0	1.1	0	1.1
Dry	Dry Normal	none	8	12	8	17	45
	Dry IMI	*S. aureus*	5	3	3	1	12
	Dry IMI	CNS	10	8	11	5	34
	Dry IMI	***Str. uberis***	2	2	3	2	9

*Mastitis criteria: (SCC ≥ 300 000 cells/ml and culture positive), **NSD: Non-specific disturbance or udder irritation (SCC ≥ 300 000 cells/ml and culture negative), ***TCI: teat canal infections (SCC < 300 000cells/ml and culture positive), Normal criteria: (SCC < 300 000 cells/ml and culture negative), CNS: coagulase negative staphylococci, IMI: Intramammary infection.

Two major concerns can be identified regarding the udder health status in the lactating cows in the herd indicated in Table 2.3, namely the large percentage of quarters with high SCC, and the presence of *S. aureus* IMI in the herd. *Staphylococcus aureus* was isolated from 10.6% of quarters, coagulase negative staphylococci (CNS) from 12.4%, and *Str. uberis* and *Str. agalactiae* each from 1.1%. Of the 35.9% quarters with SCC of 300 000 cells/ml and above (mastitis and NSD cases) only 6.6% had IMI, indicating that something besides IMI was prime reason causing high SCC in the herd, even though 25.3% of all quarters had IMI. More hind than front quarters were diagnosed with NSD and might indicate incorrect removal of clusters.

Of the dry cows sampled 4 weeks post intra-mammary treatment, 12% were infected with *S. aureus*, 34% with CNS and 9% with *Str. uberis*. This could indicate a poor cure rate for *S. aureus* and a possible high new IMI occurring during the dry period for both CNS and *Str. uberis* when compare to the results of the lactating cows (Table 2.3).

An action list could be complied for the producer which may include:

Staphylococcus aureus cows to be followed up by evaluating their mastitis history, performing udder palpation, and evaluating criteria of each cow for her probability of cure (Fig 2.1). The milking machine should be checked, milk routine evaluated and other possible stressors investigated.

2.3 GROUP REPORTS

Calving dates, pregnancy status, level of milk yield and status of the udder parenchyma can be entered into the MSD program as information additional to the laboratory results for individual cows.

Pathogen Specific Group Report

Reports of cows currently infected with specific udder pathogens are generated to use as on-farm action lists. As explained above cows should be selected and separated, to deal with contagious IMI such as *S. aureus* or *Str. agalactiae* IMI in a herd. Cows that have had *Str. agalactiae* IMI and were cured (bacterial cure) may be returned to their previous groups, but *S. aureus* cases should remain in a separate group for life. Reports of consecutive examinations provide information on the dynamics of IMI in the individual cow and identify cows with persistent or chronic IMI.

Application in herds during a *Str. agalactiae* mastitis outbreak

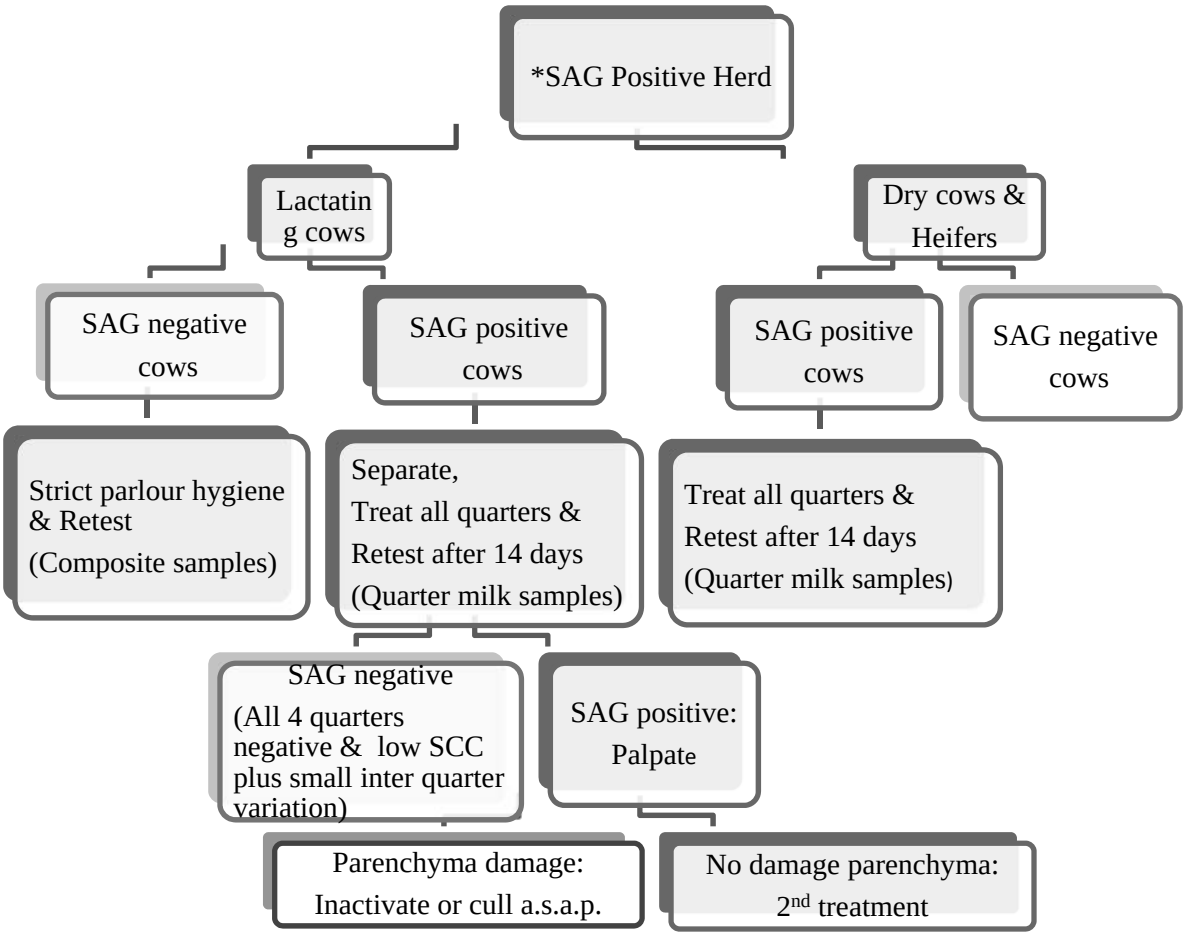
During a *Str. agalactiae* IMI outbreak in a dairy herd composite milk samples are taken from all lactating cows and quarter secretion samples from the dry cows not currently under antibiotic treatment. When the heifers presently in late gestation had been reared on fresh milk as calves are they are also sampled due to a risk of them having *Str. agalactiae* IMI (Petzer and Karzis, 2012).

The initial list of cows that tested positive for *Str. agalactiae* is emailed to the producer within 24 hours after receiving the samples at the laboratory. Managers should then immediately separate cows that tested positive for *Str. agalactiae*, treat all quarters with of those cows with intramammary antimicrobials, and milk these cows last under strict hygiene conditions. Udders of *Str. agalactiae* cows should be palpated to identify gross pathology, in which case the cure rate may be low. Any dry cow or heifers positive for *Str. agalactiae* IMI are also treated.

Ten to fourteen days after the last intra-mammary treatment the *Str. agalactiae* positive cow group is resampled (quarter milk samples) for micro-cytological analysis, and composite samples are collected from the rest of the lactating herd. The percentages of *Str. agalactiae*

cases that are cured, those that persisted and the number of new IMI are analysed, and a management plan is formulated based on this information. Cows with *Str. agalactiae* IMI are only regarded as being cured when no *Str. agalactiae* can be isolated from any of their quarters, when the SCC in all quarters is below 200 000 cells/ml and when there is only a small variation in the SCC between quarters.

Monitoring of the herd should be continued on a regular basis, and intervals may be monthly or longer until all lactating cows have tested negative for *Str. agalactiae* at least two or more consecutive tests. This may take 3 to 6 months depending on the initial *Str. agalactiae* prevalence as well as the motivation and dedication of the producer and milkers (Figure 2).



*SAG: *Streptococcus agalactiae*

Figure 2.2. A flow chart indicating events during the management of a *Streptococcus agalactiae* IMI outbreak in a dairy herd.

For most major udder pathogens different protocols are required. In the case of a *Str. uberis* IMI different strains were identified (Zadoks, 2007). Some strains were found to be more likely

to cause chronic IMI while others cured spontaneously after a period of only days. Strain typing of *Str. uberis* from individual cow samples on a herd basis has not been shown to be cost effective. When *Str. uberis* is isolated repeatedly from the same cows the herd manager is advised to increase the duration of intramammary treatment only in the event of clinical mastitis in those cows, and to use intramammary dry cow therapy at drying-off in those specific cows.

Stage of lactation and parity

The immune system of the lactating cow is known to be weakened during the peripartum period due to hormonal changes and many stressors. These can include calving and onset of lactation stress, social stress, and stress due to the adaption to a new diet, and the onset of a negative energy balance in the cow that can last up to day 100 of lactation (Collard et al. 2000). Due to vulnerability of the cow during this period, this stage of the production cycle is critical for monitoring udder health.

Udder health up to 30 days post-partum (multiparous and primiparous cows)

The number of new IMI and cases with elevated SCC that occur from days 5 - 30 post-partum provides insight into the udder health during the dry period and calving hygiene. It is also an indication of bacterial cure rate during the dry period. There is however also a risk of new IMI post-partum when milking commences. Knowledge about the prominent bacterial species causing IMI in a herd enables advisors and managers to identify and deal with the sources and causes of infection timeously and effectively. The report that summarises the species-specific IMI information including total, cured, persistent and new IMI for cows between 5 to 30 days can be used to evaluate udder health during the dry period. The current species-specific IMI and SCC results of individual multiparous cows should be compared to their udder health in their previous late lactation. Results for primiparous cows are indicated in a separate report as total numbers (and percentages) of species-specific IMI that have been isolated. Managers should aim to have less than 10% of cows with IMI and SCC in excess of 200 000 cells/ml during this period, and an incidence of less than 5% of clinical mastitis.

Heifers are more prone than cows to develop severe udder oedema prior to calving and recently calved primiparous cows are said to have a greater prevalence of mastitis than older cows, despite having less mastitis later in lactation (Barkema et al. 1998). Heifers that are close to calving and primiparous cows should receive a diet with an adequate energy balance and without excessive sodium and potassium therefore a negative dietary cation anion difference (DCAD) balance (Goff et al. 2004). Stress around calving should be limited by stimulating heifers to exercise, and by avoiding overcrowding in order to reduce negative social interactions (Hutjens & Aalseth 2005). The IMI profile of first lactation cows shortly post-partum is an indication of udder health challenges that have occurred mostly during

late pregnancy (environmental bacteria) but can in some cases be traced back to the way they were reared as calves when fed infected milk and kept in groups (Petzer and Karzis, 2012).

When bacteria isolated from milk samples shortly post-partum are predominantly contagious, this may indicate treatment failure, the presence of chronic udder damage or new infections contracted during early lactation. When few cases are cured treatment protocol needs to be revisited to indicate whether the correct anti-microbial product was used for the correct duration of time.

Udders of treated cows should be palpated to determine if there is chronic udder parenchyma damage (fibrosis, nodules or atrophy). A high rate of new IMI caused by contagious bacteria could be an indication of poor milking hygiene of newly calved cows. Trends of total, cured, new and persistent IMI in a herd can be followed over time to improve management decisions.

In the event where most IMI are caused by environmental bacteria shortly post-partum, caused can be chronic infections or treatment failure but new environmental IMI are more likely to have occurred during the dry period or at calving. Most new IMI caused by environmental bacteria are known to occur just after drying off and shortly before and after calving (Oliver and Sordillo, 1988). In these situations daily pre-calving teat dipping during the high risk periods in the dry period should be added to the management routine. No teat seal is currently registered on the South African market to assist in the prevention of new IMI during the dry period of cows.

Udder Health in early, mid and late lactation (90 days, 180 days and later in lactation)

Bacteriological and SCC results of milk samples during early, mid and late lactation are compared to evaluate progress or failure of udder health management during lactation (Table 2.4). Important events occur during the first 90 days of lactation, which includes peak milk production and re-breeding.

Early lactation is also a high risk period for metabolic diseases and multifactorial stress together with other diseases such as metritis and mastitis. The status of IMI in cows at calving will determine udder health in that whole lactation. If recently calved cows have a high incidence of IMI, little progress will be made in lowering the BMSCC, as each animal cured during lactation would be replaced by another infected cow that has recently calved. The management aim should be to have less than 15% cows with SCC in excess of 200 000 cells/ml up to 90 days in lactation (Green and Bradley, 2012).

Mid-lactation is generally a lower risk period and a less eventful time for dairy cows than early lactation. During late lactation the optimum body condition of around 3.5 for cows should be

achieved, foetus growth accelerates and cows are prepared for drying-off. The timing of drying-off usually depends on the expected calving date and level of milk yield to allow for a sufficient dry period. Concentrates may need to be reduced when milk yields are still high close to the date of drying-off to prevent excess udder oedema. While there may be a small rise in a cow's SCC in late lactation, sharp increases that are seen at this stage may be as a result of udder infection or irritation.

Table 2.4. Herd Udder Health status correlated with different stages of lactation using quarter milk samples.

Diagnosis	**Bacteria isolated**	**% In Early lactation (5-90days)**	**% In Mid lactation (91-180days)**	**% In Late lactation (180+ days)**
	Portion of herd	34.78	27.53	37.67
Normal	none	66.68	78.97	70.22
Aseptic mastitis (NSD)	none	14.58	6.57	12.50
Mastitis	*Str. dysgalactiae*	1.04	1.31	0.00
Mastitis	CNS	7.30	2.62	2.87
Mastitis	*Str. uberis*	3.13	1.31	0.96
Mastitis	S. aureus	2.07	0.00	0.96
Total % mastitis (M)		13.54	5.23	4.78
Teat canal infection	CNS	4.17	9.23	12.50
Teat canal infection	*Str. uberis*	1.04	0.00	0.00
Total % Teat canal infection (TCI)		5.20	9.23	12.50
Total % with intramammary infections (M + TCI)		18.75	14.46	17.28
Total % with high somatic cell count (M + NSD)		28.12	11.81	17.28

CNS: coagulase negative staphylococci, *Str. dysgalactiae: Streptococcus dysgalactiae*; *Str. uberis: Streptococcus uberis*; *S. aureus: Staphylococcus aureus,* Mastitis criteria: (SCC ≥ 300 000 cells/ml and culture positive), NSD criteria: (SCC ≥ 300 000 cells/ml and culture negative), TCI criteria: (SCC < 300 000cells/ml and culture positive), Normal criteria: (SCC < 300 000 cells/ml and culture negative).

General udder health of the herd

In a herd used as an example 71.38% of quarters were diagnosed as being normal, 11.6% with NSD, 7.95% with mastitis and 9.06% with TCI. Although the percentage of normal quarters in this herd is satisfactory, the 19.55% of quarters with a SCC above 300 000 cells/ml milk is unacceptable high and so are the 17.01% cases of IMI. The quarters with high SCC in the herd originated from 7.95% mastitis and 11.6% NSD cases. When no bacteria is isolated from a large percentage of milk samples with high SCC, the milking machine and stress factors (high temperature humidity index, overcrowding, mud, nutritional shortcomings and inadequate management) may be indicative as causes.

The bacteria responsible for IMI in this herd are CNS (13.04%), *Str. uberis* (2.14%), *S. aureus* (1.08%) and *Streptococcus dysgalactiae* (0.72%). It was noted that 72.24% of intramammary infections in early lactation were due to mastitic quarters while only 27.65% of IMI identified in quarters in late lactation were mastitic.

Lactation stages – intramammary infections

As the percentage of cows in this herd in early (34.78%), mid (27.53%) and late lactation (37.67%) differ the percentage IMI and high SCC quarters were calculated per lactation stage (Table 2.4). There were IMI (mastitis plus TCI cases) in 18.75% of quarters taken from cows in early, 14.46% in mid and 17.28% in late lactation respectively (Table 2.4). The percentage IMI detected in early lactation cows was high. This would warrant further investigation into the dry period and calving management, persistent of chronic cases, stress during early lactation and supressed immunity of cows early in lactation. When the IMI increases with days in milk, a distinction should be made between failure to cure of existing IMI that (increased persistent cases) and an increase of new IMI. Persistent cases may be due to udder parenchyma damage, treatment failure or virulent or resistant pathogen strains, and a supressed host immune system. Depending on the bacterial species involved, new infections may originate from the environment (bedding, pastures, water contamination and inadequate milking machine hygiene), the parlour hygiene during the milking routine; or when biosecurity is lacking.

Lactation stages – somatic cell counts

In the example in (Table 2.4) cows in early lactation had the highest percentage (28.10%) quarters with increased SCC compared to mid (11.80%) and late lactation (17.30%) cows although cows less than 5 days in milk are excluded. Quarters identified with mastitis (13.54%) and NSD (14.58%) contributed almost equally to the high percentage SCC found in early lactation (Table 2.4). In this herd both suspected causes responsible for IMI and NSD in early lactation cows needed therefore to be investigated.

Economic Report

Estimations are made on the whole herd and do not take into account variations between cows. The MSD program is used to estimate milk production losses based on quarter milk sample with elevated somatic cell counts (Giesecke et al. 1994; Hortet and Seegers, 1998; Sharma et al. 2011). The herd used as an example in Table 2.5a had a daily milk production of 2400 litres. IN the calculation used losses with SCC less than 125 000 cells/ml are disregarded and a loss of 100% would indicate an inactive quarter (Table 2a). The daily milk loss in this herd due to elevated SCC was estimated to be 162.3 litres. This loss represents an estimated loss of 4 936 litres per month and 59 235 litres annually. This represented a 14.81% loss in potential milk production for this herd.

When making a management decision regarding a *Str. agalactiae* IMI in a herd it is helpful to have an indication of the current production loss due to *Str. agalactiae*. In Table 2.5b the daily milk production loss in the *Str. agalactiae* infected quarters was estimated to be 48.2 litres amounting to a just over 1% production loss. Although this case may not warrant blitz therapy to eradicate *Str. agalactiae,* the focus should be on improving the milking routine. This calculation is only based prevalence at the time of sampling and does not incorporate risks of new infection, shedding, cure rate and number of persistent cases.

Table 2.5a: Estimated milk production losses in a herd associated with elevated quarter milk somatic cell counts of all lactating cows in the herd.

Producer Code: 840			Daily Milk Production: 2650 litres	
Producer: Farmer A			Milk price per litre : ZAR 4.45	
Dairy: Dairy A			Selection criteria: All samples tested	
SCC levels x 1000 cells/ml	**% Losses based on the SCC level of individual quarters**	**Number of quarters per SCC level**	**Loss in litres**	**Lost Revenue**
< 125	0	183	0.00	R 0.00
125 - 350	3.7	33	12.26	R 54.54
351 - 500	11.3	14	15.88	R 70.67
501 - 750	16.3	8	13.09	R 58.25
> 750	25	34	85.32	R 379.68
Inactive quarters	100	0	0.00	R 0.00

Estimated daily milk loss: 126.55 liters values at ZAR 563.14
Estimated monthly milk loss: 3 796.43 liters values at ZAR 16 894.10
Estimated annual milk loss: 46 190 liters values at ZAR 205 544.84

Table 2.5b: Estimated milk production losses in a herd associated with quarters infected with *Streptococcus agalactiae*.

Producer Code: 840			Daily Milk Production: 2650 litres	
Producer: Farmer A			Milk price per litre: ZAR 4.54	
Dairy: Dairy A			Selection criteria: Quarters positive for *Str. agalactiae*	
SCC levels x 1000 cells/ml *	**% Losses based on the SCC level of individual quarters**	**Number of quarters per SCC level**	**Loss in litres**	**Lost Revenue**
< 125	0	1	0	R 0.00
125 - 350	3.7	6	2.23	R 10.12
351 - 500	11.3	4	4.54	R 20.60
501 - 750	16.3	2	3.27	R 14.86
> 750	25	11	27.60	R 125.32
Inactive quarters	100	0	0	R 0.00

Estimated daily milk loss: 37.64 liters values at ZAR 170.90
Estimated monthly milk loss: 1 129 liters values at ZAR 5 127.00
Estimated annual milk loss: 13 739 liters values at ZAR 62 378.50

CONCLUSION

There are many advantages of having species specific IMI information about udder health in the current MSD system. It allows early detection of IMI, rapid follow-up on information from tests; there is a short turnaround time after the receipt of milk samples, and prompt communication of results to herd managers and owners. The program firstly allows evaluation of the herd udder health situation enabling the consultant to identify the main causes of udder health problems in detail. This will assist in identifying and eliminating the sources of the problems timeously. At the same time it provides information on each cow on parity, lactation stage, pregnancy status, production level, and mastitis and SCC history, to enable informed decisions for individual cows and even individual udder quarters. Management decisions can be based on sound information and cows that are cured, or have persistent IMI and new IMI can be identified, based on actual bacterial identification. This improves the accuracy of decisions made. This approach has proved to be practical and contributed to building the confidence of dairy farm managers (personal experience).

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ADDENDUM 2.1 (Case study – Herd Udder Health Report)

The goal is to eventually rid your herd completely from *Staphylococcus aureus* (*S. aureus*). The source of the *S. aureus* infection in a herd is the udders of the *S. aureus* cows, people with upper respiratory *S. aureus* infections, open wounds and cows that are bought into the herd without testing them first.

This addendum act only as an example of the type of report that is generated for dairy producer when a herd visit and consultation is done. Consultations follow when laboratory data identified an udder health herd problem. More detail reports are then generated to help in pin pointing the probable causes of this problem. During the farm consultation that follows milking routine and milker performance are evaluated. The milking machine is tested (dry and wet testing) and udders are palpated, either the whole herd or only selected groups such as cows that tested positive for *S. aureus* IMI. The latter is done to identify nodules, fibroses and atrophy in quarters. When the parenchyma is damaged treatment success is low.

The MSD program was developed to assist in data mining after every herd examination. Milk from 5 to 20 herds are tested weekly for 11 months of the year. Laboratory herd data is interpreted for dairy producers and reports included the overall udder health herd status and the current most probable udder health challenges in the herd visible from the data are identified. Individual problem groups are identified for instance *S. aureus* positive cows as well as advice regarding individual cows. When the root of the udder health problem cannot be identified in a problem herds, an udder health herd visit is scheduled.

An example of a letter to a dairy producer

You have culled a number of chronically infected *S. aureus* cows lowering the risk of shedding of this bacteria significantly. We should focus now on farming the rest out. Keep the *S. aureus* cows separate but your main focus should be on preventing new infections in the herd. The latter will require excellent parlour hygiene at every milking! Do not underestimate the value of basic parlour management and hygiene.

Also take note that the teat canal is the first and core part of the immune system of the udder in the dairy cow. Once the teat canal has been damaged the immune system of the udder is compromised and the cow can no longer protect herself against new IMI. Teat canal damage occur as a result of incorrect milking machine settings, maintenance or use.

The immediate way forward I want to suggest*:*

- You will need to resample your herd from time to time (maybe 6 monthly) to make sure that the negative cows remain negative and if we do find a *S. aureus* cow to move her to the *S. aureus* group a.s.a.p.
- Parlour hygiene is the number one key to success – hands of milkers, teat liners and communal papers/cloths from udder to udder.

- Ensure that people are not a source
- Biosecurity when buying new animals into the herd
- Heifers that calve down (small risk) and dry cows that were not tested before
- Take care of the teat canals of the cows and their immune system and they can take care of themselves
- Keep the functioning of the milking machine sound.

Findings with the herd visit - cows

It was such a pleasure to work with your tame cows. The animals are handled with care on a constant basis. It does promote good hygiene when the bottom part of the tail hair is cut at calving and again later during lactation.

Udder conformation of cows was generally very good with some excellent hind quarter development.

The teat canal scores were overall good (mostly1 and 2) but what did worry me was that a large number of cows had hard teats and hardened rings at the teat base just after the clusters came off.

Milking Routine

- The waiting area in front of the parlour is well designed (size, surface and slope).
- Some hosepipes in the parlour are just too long and touch the floor – this may contaminate the hands of the milkers when handled. The part that the milkers touch should be off the floor and kept clean.
- The management of your liner plugs was good.

Pre-milking

- Time from touch (of teats) to attachment (of clusters) varied but seemed to be a bit too long. However, this is not a major problem. The reason for doing this measurement is to be sure that sufficient time is allowed for the milk let-down (your teat dip containing oxytocin is also assisting), and to be done with milking within 8 min. max after attachment in most of the cows. The Afimilk report indicated that there were only a few cows (this might have been those fresh in milk / colostrum cows) that milked for too long. The average milking time per cow should be more or less 5 minutes when they are producing 10 litres.
- The pre-teatdip application needs improvement as many of the teats were not well covered. You are using the pre-teatdip for 2 reasons – the first is to lower the bacterial load on the teat skin prior to milking (including the STA) and the second is to disinfect the hands of the milkers. The latter remains one of the 3 crucial actions during the milking routine to prevent the spread of STA from cow to cow.

Milking

- It would be beneficial to test milkers, yourself (all those in close contact) with the cows at least on an annual basis (throat swabs at your local clinic) just to ensure that new infections do not occur via human contact.
- Clusters are attached from time to time with some vacuum lost, but it is not easy to attach a cluster to Jerseys with deep udders without losing vacuum. Again this is not a major problem. Where there were liner slips milkers reacted fast to rectify the situation. Afimilk could be used to monitor trends in liner slips, re-attachments and kick-offs.

Post-milking

- As mentioned earlier in this report many cows showed increased hardness (swelling) of teats and teat base rings. This may be an indication that clusters are attached for too long. As discussed – it is a long time after milk flow has decreased at teat end that the flowmeter registers it because it is lifted about 1.8m above the udder and the long milk pipes are 2.5m long. These settings need to be changed. Could Piet from Afimilk please provide us with your various machine settings.
- Post teat dip application should also improve – to cover at least 2 thirds of the bottom part of the teats.
- Backflush of units should be done every time after each cow has been milked, as this is the other crucial action in the prevention of spread of *S. aureus* in the parlour.
- One can milk the average residual milk - the ideal is 150 to 200ml per udder. Emptying and filling time of the parlour was good.
- The graph on Afimilk indicating the % low-flow-time of a milking is valuable. Not only does it indicate the preparation efficacy for milking but it also indicates the risk of teat canal damage due to the milking machine. It mainly indicate for how long is the test being milked at a high vacuum (when little milk in flowing) – the ideal is 15%.

The Milking Machine (30 point swing-over Waikato system with ACR)

- The overall setting of the pulsator ratio is within the norms (but will cause milking to be relatively slow). Your machine helps to prevent teat canal damage in your herd.

- The work to rest ratio is 59:41 with long A and D phases. The pulsation is average at 55. The high milk line needs a high system vacuum and again can promote damage:

  A phase: 10 – 20% (Vacuum is created in the pulsation chamber)

  B phase: > 35% (Liner totally open)

  C phase: 10 – 20% (Air is let into the pulsation chamber and the liners close)

  D phase: > 15% (Liner totally closed or collapsed)

Summary of the Pulsograph results

- System vacuum was approximately 46.7 kPa at all units.
- The variation within (2x2) milking units is too much. In most of the cases the 2x2 values within units differ more than 1% point. The most serious problems are where the limping is above 1% point as indicated. The latter creates a situation where one side of the udder is milked out before the other side and is then over milked.
- The following pulsators are not functioning optimally:
- 4 (units 7 & 8); 7 (units 13 & 14); 9 (units 17 & 18); 10 (units 19 & 20); 11 (units 21 & 22) and 15 (units 29 & 30) plus units 1, 10, 11 and 28.

Teat end vacuum at maximum milk flow (2nd minute)

- Two of the second minute teat end vacuums were over 40kPa. Besides some cases of high teat end vacuum there where indentations of the teat base and hardness of teat after milking.
- The teat end vacuum of the average producers should be at 36-38 kPa while those of the high producers should be at 33 to 34 kPa. The teat end vacuum while milking low producing cows should preferably not be above 40 kPa. This monitoring should be an ongoing process. The sooner you know about a problem, the less damage is done to your cows.
- The timing of the take-off units should be investigated and most likely changed.

Results of the second minute teat end vacuums

Milk production	Average vacuum (kPa)	Variation in vacuum (ideal < 5 kPa)
Low	40.5	3.8
High	32.6	4.0
Average	36.8	4.8
Low	42.2	3.6
Average	34.8	3.6
Average	34.9	2.4

- The normal usage time of a teat liner is usually 2500 milkings. Please ensure that you always adhere to this liner life. In your parlour with 30 units and 2 milkings per day for 330 cows the liners should be replaced every 114 days.
- Be sure that the vacuum control valves and the filters in the filter lines are clean at all times.
- Automatic cluster removals (ACR) settings

The Afimilk program dictates when the ACR will be taken off. It is based on more than one setting in the program but the 2 most important ones are those that indicate at what volume (formula that relates time and volume) the ACR should be taken off and the second one is the

setting that is set for the delay time (when that volume is reached – how long must the machine wait before taking the unit off). Could you kindly obtain these values from the program? They are taking off too late. The flow meter in your system is far from the udder of the cow and high above it with the result that there is a delay time before the machine measures the end of a milking. We therefore have to set them to take off earlier that the setting where flow meters are close and below the level of the udders. I believe that the incorrect setting is the major reason for the swollen teats after milking.

Another setting that should be checked is the min. time on. If this is too long - cows may be over-milked when units are reattached.

Clinical examination of udders

- Lesions in front of the udder increased from 9 to 27 cases. They are: 2084; 9711; 3052; 1045; 9990; 1255; 1124; 9785; 1043; 9778; 9950; 1086; 97154; 30411; 1009; 3032; 1165; 1063; 1160; 2103; 96120; 3026; 3087; 1108; 9978; 1215 & 9922 (Those printed in bold were also positive with the previous visit). Any wounds can harvest bacteria, especially *S. aureus.*
- There were again 5 cows with clinical mastitis on the day of our visit (cows: 1028(RA), 9714 (LA); 1010(RA); 9960(RA) and 2030(LA).
- There were 5 cows with old or new abscesses on their udders: 1068 (puss dripped from teat); 97119; 3002(RA); 1102 and 9960.
- Cow 1310 had blood in her quarter. It is contra-indicated to treat these cows with intramammary drugs. This increases the tissue irritation in the affected udder. She should however, always be treated systemically and the quarter should be milked very gently by hand. Hose the udder down for several minutes a day with cool water.
- Two rudimentary teats that produce milk were found: 1289 and 3042.
- Warts were found on teats of 12 cows.
- 79 cows (29%) had asymmetric udders while the overall teat canal scores (TCS) were as follows:
- 22 cows had oedema of their udders and 10 had ventral oedema.
- Cow 2147 had a sore foot. The hoof care in the herd was very good.
- Results of udder parenchyma on individual cows are presented in the printouts. Do not react to any scores of 1. These fibroses often resolve and cannot be found with a second examination. Any parenchyma scores of 3 are very serious and, except in cases of clinical mastitis, is an indication of severe, permanent damage to the udder.

Laboratory results

Overall 15% (Aug = 23%) of all quarters had mastitis, 6.8% (Aug =11%) showed signs of udder irritation, 24.8% (Aug = 20%) had teat canal infection and 51.6% (Aug =45%) were normal. This means that 22% (Aug =34%) of all quarters had a somatic cell count (SCC) of above 400 000 cells/ml and 30% (Aug =43%) of quarters were infected. There was an overall improvement in the udder health status of the herd, but it is not yet ideal.

Bacterial profile of lactating cows in the herd

- 12.5% (Aug =14.3%) of quarters were infected with *Staphylococcus aureus* (STA) of which 1.3% (Aug = 2.7%) were suspected human strains (STH).
- 0.5% (Aug =1.2%) were infected with *Streptococcus agalactiae* (SAG); 1.8% (Aug =3%) SFA; 0.5% (Aug =1%) SPY; 0.4% (Aug =3.4%) SUB and 13.5% (Aug =18%) STE.
- 8 cows had one inactive quarter each while cow 1284 had two (LF and LH). 50% of all inactive quarters were LF quarters.
- 67 cows had mastitis in one quarter, 25 in two, 7 in three and 7 in all four quarters.
- 56 cows had udder irritation in one or two quarters and 4 in three or four quarters.
- The estimated milk loss at present due to high quarter SCC is 1276 litters or R2362 per day (R70 860 per month).
- Individual Cows: Please see the attached data. I marked the STA cows that is suspect to have chronic STA infections.

Results of Bulk Milk hygiene tests

	Bacterial plate count (cfu/ml)	Coliforms (cfu/ml)	E. coli type 1 (cfu/ml)	SCC x 10^3 (cells/ml)	Brucella milk ring test
Bulk milk	>300 000	>110	Positive	389	Negative
Requirements	<50 000	<20	Negative	<500	Negative

Results of Water tests

	Bacterial plate count (cfu/ml)	Coliforms (cfu/ml)	E. coli type 1 (cfu/ml)
Water	84	0	Negative
Recommendations	<100 cfu/100ml	None / 100 ml	Negative

Comments on somatic cell counts (SCC) from your E-Report (dated 5 Sept 2016):

The SCC of the cows in early lactation is too high. The first and second lactation cows have far too high SCC which means that cows are infected or udders are irritated early during lactation. The graph showing only SCC below 250 000 cells/ml could improve to 75% and more. The SCC history table shows an overall improvement with the low SCC increasing but the high SCC is still too high.

Dr Inge-Marié Petzer

CHAPTER 3

Somatic cell count thresholds in composite and quarter milk samples as indicator of bovine intramammary infection status

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SIGNIFICANCE OF THE WORK

Somatic cell count (SCC) as indicator of udder health status in quarter milk samples has been well researched and reported on for many centuries. Although researchers agree that it remains an useful indicator of the udder health status in cows they do not agree on the SCC threshold indicating intramammary infections (IMI). There is currently no international guidelines for a SCC threshold indicating IMI in composite milk samples. The number of lactating cows per herd in South Africa increased causing effective monitoring of the udder health status of the individual cow to become more challenging. In South Africa routine udder health examinations of quarter milk samples have been replaced by composite milk samples being more practical and less costly to analyse. Due to the dilution effect of combining the four udder quarters in one sample results of these samples are currently difficult to interpret as single test under field conditions. Some South African milk laboratories culture milk samples only with SCC above a chosen SCC threshold. It is of importance to know what percentage of IMI in general and infections with contagious udder pathogens would be missed at those SCC thresholds. This information can be of key importance to the veterinary practitioner and the manager alike for wise pro-active udder health management decisions, both on herd and individual cow level.

In this first of two manuscripts utilizing 345 461 composite and 89 638 quarter samples from dairy herds in 7 of 9 South African provinces the main objective was to establish a SCC threshold for composite milk samples that could be used for initial screening for IMI.

ABSTRACT

The objective of the study was to establish an operational SCC threshold to predict the presence of intramammary infection (IMI) in composite milk samples and compare findings with those in quarter milk samples. South African dairy producers now prefers composite milk

samples for herd udder health analysis due to increasing cow numbers, convenience of sampling and lower cost.

A retrospective study was done on 345 461 composite and 89 638 quarter milk samples from South African herds. Variance estimates for the proportion of quarter samples testing positive were adjusted to account for the lack of their independence within individual cows. The IMI at SCC thresholds of 150 000 and 200 000 cells/ml differed only by 3.26% in composite milk samples. Youden's Index indicated the optimum SCC thresholds for composite and quarter milk samples as 150 000 and 200 000 cells/ml respectively. At 150 000 cells/ml, Sensitivity (95% CI) in composite milk samples was 65.3% (64.0%, 66.6%) and Specificity was 66.8% (65.7%, 67.9%); and in quarter milk samples Sensitivity was at 200 000 cells/ml 70.8% (69.5%, 72.0%) and Specificity was 63.6% (62.4%, 64.8%). The likelihood of infection for udders and quarters respectively was 1.034 and 1.327 at a SCC threshold of 150 000 cells/ml and 0.864 and 1.177 at 200 000 cells/ml. The area under the curve of the receiver operating characteristics graph was 0.7084 and 0.7277 for composite and quarter samples respectively indicating that the SCC test could be considered as good indicator of IMI in both sample types.

Key words: Composite milk samples, somatic cell count threshold, intramammary infections, dairy cows

INTRODUCTION

Bovine mastitis remains a major challenge and the disease responsible for most economic losses in dairy cows in developed countries despite improvements in management of subclinical mastitis over the past decade (Geary et al. 2012). More than 80% of financial losses due to mastitis have been estimated to occur as a result of the subclinical from of the disease indicating that this should be the key focus in pro-active udder health management (Giesecke et al. 1994).

There is a correlation between the bulk milk somatic cell count (BMSCC) and the estimated percentage of cows with IMI in a herd (National Mastitis Council Guidelines, 2001). Bulk milk somatic cell count is useful for indicating milk quality, safety and suitability for manufacturing of dairy products (Sheldrake, Hoare and McGregor, 1983; Miller and Paape 1985; Reneau and Packard 1991) but it does not provide information about udder health of the individual cow. No consensus exists worldwide regarding the legal limits of BMSCC in milk for human consumption. In the European Union, Australia, New Zealand, Canada and Switzerland the legal BMSCC limit is 400 000 cells/ml, in South Africa 500 000 cells/ml, in the USA 750 000 cells/ml and in Brazil 1 million cells/ml (Ruegg and Pantoja 2013).

Somatic cell counts from quarter samples have been generally accepted and used as the operational measure of inflammation of the bovine lactating gland since 1960 (Heeschen 1996; Barkema et al. 1999; Harmon 2001; Schukken et al. 2003; Biggs 2009; Heeschen 2010),

as an indicator of the severity of IMI (Harmon 1994) and as indicator of economic losses (DeGraves and Fetrow 1993). The SCC threshold level used to describe normal milk, IMI and subclinical mastitis however has often been, and still is a controversial subject (Heeschen 2010). In the 1967 International Dairy Federation (IDF) bulletin an udder quarter was considered to be normal when no pathogens were isolated and the SCC was less than 500 000 cells/ml milk. However, more recently, quarter milk with a SCC of equal or less than 100 000 cells/ml from which no microorganisms has been isolated and without a history of recent infection are considered to be normal (Harmon 2001; NMC Guidelines 2001) while those with a SCC of exceeding 200 000 cells/ml have been regarded as an indication of an inflammatory response and that the quarter is likely to be infected (Dohoo and Leslie 1991; Laevens et al. 1997; Schepers et al. 1997; NMC Guidelines 2001). In 2001 the National Mastitis Council defined subclinical mastitis as an infected quarter with a SCC equal to or above 200 000 cells/ml in the absence of clinical changes to milk (National Mastitis Council Guidelines 2001) based on findings by DeGraves and Fetrow, (1993); Harmon (1994) and Hillerton (1999). This decision was endorsed at an IDF World Dairy Summit in New Zealand (2001), but it was agreed that a tolerance range of up to 400 000 cells/ml was necessary for practical reasons. Further controversy still exists in literature regarding mastitis cure, as the term "cure" may be used when describing only clinical cure of mastitis, clinical cure with reduced SCC, or when clinical symptoms disappear, SCC has reduced and mastitis pathogens are absent (Hiitiö et al. 2012; Roy et al. 2012; Swinkels, Schukken and Cox 2012). The current quarter milk SCC threshold of 200 000 cells/ml does not distinguish between principal udder pathogens although pathogens are known to differ in their pathogenic effects (Barkema et al. 1999, Petzer et al. 2009, Pantoja, Hulland and Ruegg 2009, Petzer et al. 2012). Current information on SCC threshold levels for pathogen specific IMI is needed because SCC are known to differ between udder pathogens and over time (Zadoks and Fitzpatrick 2009, Petzer et al. 2009, Petzer et al. 2012).

The average number of lactating cows per herd in South Africa increased from 100 in 1997 to 238 in Jan 2015 and in provinces ranges from 76 in the Northern Cape to 769 in the Eastern Cape (Lactodata 2013, Lactodata 2015). Quarter milk samples for routine udder health herd examination have been replaced by composite milk samples due to practical and financial reasons. In composite samples milk from the four udder quarters is combined, with a consequent dilution effect that has required different interpretation of the results from quarter milk samples. Although an international SCC threshold has been established for the indication of IMI in quarter milk this has not been agreed for composite milk samples. Identification of pathogens by phenotypically classification, biochemical analysis and genotyping is costly and may well become impractical in both small and large South African herds in future. Knowledge of udder pathogens in herds remains of immense importance for a correct focus of pro-active strategies and monitoring of udder health in dairy herds (Schukken et al. 2003; Ruegg 2011). Early detection of IMI will assist management lessen the severity and duration of mastitis, reduce parenchyma damage, improve bacterial cure and

lessen the risk and duration of bacterial shedding and development of new IMI (Giesecke, et al, 1994, Schukken 2012).

A screening test is required to enhance the likelihood of identifying individual cows at risk of having IMI within herds (Reneau and Packard 1991). Such a test can be considered successful when it is simple, inexpensive and reproducible, widely available and with sufficient discriminatory power. Somatic cell count was chosen in this study for the evaluation of composite milk samples, to be used as a screening test that could identify individual cows with possible IMI. In this paper the incidence of IMI in composite milk samples at various SCC thresholds with indications of probable accuracy (Sensitivity and Specificity) and an "optimum" SCC threshold were evaluated. Results of composite and quarter samples were evaluated and compared to results obtained at similar SCC thresholds.

MATERIALS AND METHODS

Study design and study population

A retrospective observational study was done on milk samples of lactating cows from South African (SA) commercial dairy herds over a period of more than 4 years (January 2008 until April 2012). Samples originated from approximately 830 commercial SA dairy herds that submitted samples as part of their routine udder health monitoring programme and also from herds with increased BMSCC who had sought help. The lactating cow numbers in herds tested varied from approximately 30 to 1 700 cows, while their intervals for herd examinations ranged from monthly and three-monthly, to annually and bi-annually, or in some cases only a once-off test.

Data selection and sampling

In the majority of cases all lactating cows within herds were sampled and not only selected individuals or groups. A total of 386 031 composite cow and 95 228 quarter milk samples were initially under consideration for use, of which 10.51% and 5.87% respectively were defined as being unsuitable and were excluded from the dataset. Samples were regarded as unsuitable when any visible abnormalities such as dirt, floccules, blood or watery milk were detected. Sample results were excluded from this study when cultures indicated contaminated, mixed bacterial growth or when there were doubtful SCC results or missing identification information. The final dataset comprised of 345 461 composite and 89 638 quarter milk samples. Results were exported to and captured in the Milk Sample Diagnostic (MSD) computer program and were identified by producer, cow number, quarter position, date of processing and sample type. In most cases information regarding parity, milk yield and days in milk were not provided. The MSD program was developed over several years by Abaci SA for the Milk Laboratory at the University of Pretoria (Faculty of Veterinary Science), to assist

in the analysis of milk samples received of dairy herds. (Petzer et al. 2016). Milk samples were taken by professional samplers or milkers trained according to a standard operating procedure (Giesecke et al. 1994). Prior to sampling, first milk was stripped from all quarters and teat ends were carefully cleaned and disinfected with methylated alcohol. Approximately 10 ml of foremilk was collected aseptically into sterile marked sample tubes and kept refrigerated until shipment. In the case of composite milk samples the same procedure was followed, only approximately equal volumes of milk from each of the four quarters were collected in one sample tube. Samples were transported on ice to reach the Milk Laboratory at the University of Pretoria (Faculty of Veterinary Science) within 48 hours after sampling. Temperatures and conditions such as sample tubes cleanliness and appearance were noted on arrival at the laboratory and samples which were spoiled or of doubtful quality were not processed. Samples were plated out at the laboratory on the day of their arrival.

Laboratory methods

Microbiology and somatic cell counts were done by the Milk Laboratory at the University of Pretoria (Faculty of Veterinary Science), on all milk samples under consideration for this study. Milk was plated on bovine blood tryptose agar plates (Oxoid, supplied by Quantum Biotechnologies (Pty) Ltd, Ferndale, South Africa). Inoculated agar plates were incubated aerobically at 37 $\pm$1 ^{o}C and examined after 18 to 24 hours and 48 hours. Colonies were initially identified based on colony morphology, haemolysis and potassium hydroxide test (KOH) results (IDF Document 132). The catalase reaction was used to differentiate between staphylococci and streptococci. Staphylase, a coagulase test (Oxoid, supplied by Quantum Biotechnologies (Pty) Ltd, Ferndale, South Africa) was used to distinguish between coagulase positive (*Staphylococcus aureus*) and coagulase negative staphylococci (CNS). The Staph API test (Biomerieux South Africa (Pty) Ltd, Randburg, South Africa) was used for identification of staphylococci. *Streptococci species* were differentiated into the various Lancefield groups using a Strepkit (Oxoid, supplied by Quantum Biotechnologies (Pty) Ltd, Ferndale, South Africa). Gram negative organisms were diagnosed using DNase and MacConkey agar (Oxoid supplied by Quantum Biotechnologies (Pty) Ltd, Ferndale, South Africa) and API 20E (Biomerieux South Africa (Pty) Ltd, Randburg, South Africa).

Intramammary infection was defined as a pure culture when only one species grew on an inoculum. Growth of two distinct species was regarded as mixed growth and a sample was considered to be contaminated when more than two species were present. Samples with growth of two and more colonies from the same bacterial species were recorded (National Mastitis Council Guidelines 2001) except in the case of *Staphylococcus aureus* where one and more colonies were recorded.

Somatic cells were counted by fluoro-opto-electronic means using a Fossomatic 5000 (Rhine Ruhr, P.O. Box 76167, Wendywood, 2144, South Africa).

Data analysis

In this study all pure bacterial cultures isolated from milk samples were considered as intramammary infections (IMI). A further study, using the same dataset investigated and analysed results from 16 different udder pathogens. Somatic cell count was compared with the presence and absence of organisms at eleven threshold levels. Results were analysed at SCC thresholds with increments of 50 000 cell/ml up to 500 000 cells/ml, thereafter the increments escalated to 750 000 cells/ml and greater.

Two by two tables were generated. True positive (TP) and false positive (FP) cases were defined as those milk samples that had SCC above the level investigated and where TP cases were culture positive and FP not. True negative (TN) and false negative (FN) sample were described as having SCC levels below the level of investigation but with either IMI (FN) or without IMI (TN). The results were analysed using a GenStat program (Payne *et al*. 2012). The Likelihood Ratios (LR) was determined using the category-oriented likelihood method (Moosapour *et al*. 2011).

In the case of composite milk samples Sensitivity, Specificity and 95% confidence intervals (CI) of binomial proportions around these estimates were determined using the Mid-P exact test from the OpenEpi freeware program. Quarter samples cannot be considered to be independent but rather clustered within cows (potentially multiple sample sets per cow) (Ampe et al. 2012). Sample dependency of SCC values within each cow was modelled to take into account individual cow factors that may affect quarter samples. An estimator of cluster variance was determined as described by Scheaffer et al., (1996). The 95% confidence intervals were adjusted for clustering by using the design effect (cluster variance / sample random sample variance) (Scheaffer et al., 1996):

$\hat{p} = \frac{\sum_{i=1}^{n} a_i}{\sum_{i=1}^{n} m_i}$ where

$\hat{p}$ = the estimated proportion of unweighted quarters with intramammary infection (IMI)

n = the number of cows

a_i = the number of quarters with IMI per cow

m_i = the number of quarters tested per cow

The estimated variance of the portion ($\hat{V}(\hat{p})$) of quarters with IMI was calculated using the following formula (Schaeffer *et al.* 1996):

$$\hat{V}(\hat{p}) = \frac{\sum_{i=1}^{n}(a_i - \hat{p}m_i)^2}{n-1}$$

The area under the curve (AUC) of the receiver operating characteristics (ROC) curves was used to estimate the discriminative power of the SCC measurement over the range of possible thresholds ranges and was calculated with the trapezoid method (Perkins and Schisterman 2006). The best threshold point for balancing the Sensitivity and Specificity Ratios of the SCC, the one closest to the (0 , 1) point was taken as the threshold point that best differentiated between those udders (composite milk) or quarters with IMI and those without. Youden's index which indicates the maximum distance between the ROC curve and the diagonal chance line was calculated by subtracting 1 from the sum of the maximum Sensitivity and Specificity.

RESULTS

3.1 Composite milk samples

The SCC threshold levels of primary interest initially focused on in this study were 100 000, 200 000 and 400 000 cells/ml for they were the levels in quarter milk samples that have been recommended by the International Mastitis Council (IMC). Because SCC threshold levels used on the various farms may well differ from those recommended by the IMC, other SCC ranges were also investigated in an attempt to minimize diagnostic error under different circumstances.

The percentage of IMI in this dataset of 345 461 composite milk samples was 43.37% (Table 3.1). The percentage of samples with IMI at SCC threshold levels of 100 000, 200 000 and 400 000 cell/ml were 10.89%, 18.31% and 26.26%. Of the 149 817 culture positive samples 57.78% had SCC in excess of 200 000 cells/ml milk compared with 26.67% of culture negative samples. The ratio in composite samples of culture negative to culture positive samples were 2.960, 2.268 and 1.821 to 1 at SCC thresholds of 100 000, 200 000 and 400 000 cells/ml, respectively (Table 3.1).

Table 3.1. The presence and absence of intramammary infection (IMI) within each somatic cell count (SCC) range in composite milk samples (n=345 461).

SCC range cells per ml milk	Sample numbers with IMI	Sample numbers without IMI	Total sample numbers	IMI prevalence per SCC range (95% LCI and UCI)	Cum % with IMI per SCC range
1 000-50 000	19462	77244	96706	0.201 (0.199, 0.204)	5.63
51 000-100 000	18157	34114	52271	0.347 (0.343, 0.352)	10.89
101 000-150 000	14381	19419	33800	0.426 (0.420, 0.431)	15.05
151 000-200 000	11257	12701	23958	0.470 (0.464, 0.476)	18.31
201 000-250 000	9081	8055	17136	0.530 (0.522, 0.537)	20.94
251 000-300 000	7504	6242	13746	0.546 (0.538, 0.554)	23.11
301 000-350 000	6081	4445	10526	0.578 (0.568, 0.587)	24.87
351 000-400 000	5146	3573	8719	0.590 (0.580, 0.601)	26.36
401 000-450 000	4280	2986	7266	0.589 (0.578, 0.600)	27.6
451 000-500 000	3801	2520	6321	0.601 (0.589, 0.613)	28.7
501 000-750 000	12431	7597	20028	0.621 (0.614, 0.627)	32.3
750.000 +	38236	16748	54984	0.695 (0.692, 0.699)	43.37
Totals	149817	195644	345461		

Cum % = Cumulative percentage; LCI = lower confidence interval; UCI = Upper confidence interval

Sensitivity of SCC thresholds as an indicator of IMI in composite samples were 87.0% with 95% upper and lower confidence intervals of (85.5%, 88.5%) at the lowest SCC range investigated (50 000 cells/ml) but dropped noticeably as SCC increased to 74.9% (73.5%, 76.3%) at 100 000, 57.8% (56.6%, 59.0%) at 200 000 and 39.2% (38.2%, 40.2%) at 400 000 cells/ml SCC threshold ranges. At the 200 000 threshold the Specificity was 73.3% (72.2%, 74.5%) (Table 3.2). Likelihood Ratios (LR) for culture negative samples ranged from 3.039 at 50 000 cells/ml to 0.468 at 750 000 cells/ml while the LR became larger than 1 at the 150 000 cells/ml SCC range. The Youden's Index, that balances Sensitivity and Specificity to obtain a mathematical optimum SCC threshold, was indicated at 150 000 cells/ml in composite samples with a Sensitivity of 65.3% (64.0%, 66.6%) and Specificity of 66.8% (65.7%, 67.9%) (Table 3.2).

Table 3.2. Analysing intramammary infection (IMI) in composite milk samples at different somatic cell count (SCC) thresholds.

SCC cells per ml milk	Sensitivity (95% LCI and UCI)*	Specificity (95% LCI and UCI)*	Youden's Index	Likelihood Ratio
50 000	0.870 (0.855, 0.885)	0.395 (0.386, 0.404)	0.265	3.039
100 000	0.749 (0.735, 0.763)	0.569 (0.559, 0.580)	0.318	1.439
150 000	0.653 (0.640, 0.666)	0.668 (0.657, 0.679)	0.321	1.034
200 000	0.578 (0.566, 0.590)	0.733 (0.722, 0.745)	0.311	0.864
250 000	0.517 (0.506, 0.529)	0.775 (0.762, 0.789)	0.292	0.679
300 000	0.467 (0.456, 0.478)	0.806 (0.794, 0.891)	0.274	0.637
350 000	0.427 (0.416, 0.437)	0.829 (0.817, 0.842)	0.256	0.56
400 000	0.392 (0.382, 0.402)	0.847 (0.837, 0.860)	0.24	0.532
450 000	0.364 (0.335, 0.373)	0.863 (0.850, 0.876)	0.226	0.534
500 000	0.338 (0.329, 0.348)	0.876 (0.863, 0.889)	0.214	0.508
750 000	0.255 (0.247, 0.263)	0.914 (0.901, 0.928)	0.17	0.468

*LCI= lower confidence interval, UCI= upper confidence interval.

3.2 Quarter milk samples

Of the 89 638 quarter milk samples analysed, 43 746 (48.8%) had a SCC of above 200 000 cells/ml. The percentage of quarter milk samples in this dataset from which microorganisms were isolated was 33.99% representing 30 467milk samples (Table 3.3). The prevalence of IMI at various SCC ranges was determined with standard errors at 95% confidence intervals. At SCC ranges of 100 000, 200 000 and 400 000 cells/ml the prevalence (upper and lower 95% CI) of IMI were 20.4% (19.7%, 21.2%), 29.1% (28.3%, 30.9%) and 41.1% (38.9%, 43.3%).

Table 3.3. Quarter milk samples indicating the presence and absence of intramammary infection (IMI) for each somatic cell count (SCC) range (n=89 638).

SCC ranges cells per ml milk	Samples numbers with IMI	Samples numbers without IMI	Total sample numbers	IMI prevalence per SCC range (95% LCI and UCI)	Cum % with IMI per SCC range
1 000-50 000	3019	19891	22910	0.132 (0.127, 0.136)	13.18
51 000-100 000	2332	9078	11410	0.204 (0.197, 0.212)	15.59
101 000-150 000	1811	4923	6734	0.269 (0.258, 0.280)	17.45
151 000-200 000	1432	3406	4838	0.291 (0.283, 0.309)	18.73
201 000-250 000	1188	2429	3617	0.328 (0.313, 0,344)	19.76
251 000-300 000	1068	1845	2913	0.367 (0.349, 0.384)	20.7
301 000-350 000	899	1395	2294	0.392 (0.372, 0.412)	21.47
351 000-400 000	790	1133	1923	0.411 (0.389, 0.433)	22.14
401 000-450 000	711	966	1677	0.424 (0.400, 0.448)	22.72
451 000-500 000	617	809	1426	0.433 (0.407, 0.459)	23.21
501 000-750 000	2294	2862	5156	0.445 (0.431, 0.459)	24.9
750.000 +	14303	10437	24740	0.578 (0.572, 0.584)	33.99
Totals	30464	59174	89638		

Cum % = Cumulative percentage; LCI = lower confidence interval, UCI = upper confidence interval

The percentage of quarter milk samples with IMI was 18.73% at 200 000 and 22.14% at 400 000 cells/ml. (Table 3.3). The ratio of quarter samples without IMI to those with IMI remained positive up to a SCC level of 750 000 cells/ml. Only at SCC above 750 000 cells/ml there was a clear negative ratio of uninfected to infected samples.

While discriminative measures are mostly used to make policy decisions, predictive measures are most useful in predicting the probability of a disease in an individual. The Likelihood Ratio for identifying culture negative samples at the various SCC thresholds ranged from 3.145 more likely at 50 000 cells/ml to 0.446 at 750 000 cells/ml. The Youden's index at 100 000 cells/ml was 0.311 and increased to the highest values of 0.344 and 0.345 at SCC thresholds of 200 000 and 250 000 cells/ml quarter milk (Table 3.4). The Youden's Index range from 0 for a poor result to 1 for the perfect test indicating the best balance between the Sensitivity and Specificities of tests.

Table 3.4. Sensitivity and predictability of quarter intramammary infections (IMI) for various somatic cell count (SCC) thresholds.

SCC cells per ml milk	Sensitivity (95% LCI and UCI)*	Specificity (95% LCI and UCI)*	Likelihood Ratio	Youden's Index
50 000	0.889 (0.870, 0.907)	0.336 (0.328, 0.344)	3.145	0.225
100 000	0.819 (0.808, 0.830)	0.492 (0.490, 0.503)	1.868	0.311
150 000	0.757 (0.745, 0.769)	0.577 (0.566, 0.589)	1.327	0.334
200 000	0.708 (0.695, 0.720)	0.636 (0.624, 0.648)	1.177	0.344
250 000	0.667 (0.655, 0.679)	0.678 (0.666, 0.690)	1.046	0.345
300 000	0.631 (0.618, 0.643)	0.709 (0.697, 0.722)	0.899	0.34
350 000	0.600 (0.588, 0.612)	0.734 (0.721, 0.746)	0.817	0.334
400 000	0.573 (0.561, 0.581)	0.753 (0.741, 0.765)	0.757	0.326
450 000	0.549 (0.537, 0.560)	0.770 (0.758, 0.781)	0.732	0.319
500 000	0.528 (0.516, 0.539)	0.783 (0.771, 0.795)	0.702	0.311
750 000	0.449 (0.437, 0.461)	0.882 (0.821, 0.895)	0.446	0.331

*The effect of clustering was taken into account, LCI= lower confidence interval, UCI= upper confidence interval.

The result of the AUC of the ROC, that express the discriminative power of the SSC test to identify IMI in both composite and quarter milk samples, was 0.7084 in composite and 0.7277 in quarter milk samples (Table 3.5). The closer the AUC is to 1 the higher the diagnostic accuracy of the test.

Table 3.5. Receiver operating characteristic curves (ROC) and area under the curve (AUC) for the efficacy of SCC test to identify IMI in quarter and composite milk samples.

SCC threshold (cells/ml milk)	Q Sen	Q Sp	Q 1-Sp	Q. Area	Quarter AUC*	SCC threshold (cells/ml milk)	C Sen	C Sp	C 1-Sp	C. Area	Compo-site AUC*
	1	0	1		0.7277		1	0	1		0.7084
	1	0.336	0.664				1	0.395	0.605		
50 000	0.889	0.336	0.664	0.3174		50 000	0.87	0.395	0.605	0.3692	
50 000	0.889	0.492	0.508			50 000	0.87	0.569	0.431		
100 000	0.819	0.492	0.508	0.1332		100 000	0.749	0.569	0.431	0.1412	
100 000	0.819	0.577	0.423			100 000	0.749	0.668	0.332		
150 000	0.757	0.577	0.423	0.067		150 000	0.653	0.668	0.332	0.0696	
150 000	0.757	0.636	0.364			150 000	0.653	0.733	0.267		
200 000	0.708	0.636	0.364	0.0432		200 000	0.578	0.733	0.267	0.0399	
200 000	0.708	0.678	0.322			200 000	0.578	0.775	0.225		
250 000	0.667	0.678	0.322	0.0289		250 000	0.517	0.775	0.225	0.0225	
250 000	0.667	0.709	0.291			250 000	0.517	0.806	0.194		
300 000	0.631	0.709	0.291	0.0201		300 000	0.467	0.806	0.194	0.0157	
300 000	0.631	0.734	0.266			300 000	0.467	0.829	0.171		
350 000	0.6	0.734	0.266	0.0154		350 000	0.426	0.829	0.171	0.0102	
350 000	0.6	0.753	0.247			350 000	0.426	0.847	0.153		
400 000	0.573	0.753	0.247	0.0111		400 000	0.392	0.847	0.153	0.0075	
400 000	0.573	0.77	0.23			400 000	0.392	0.863	0.137		
450 000	0.549	0.77	0.23	0.0095		450 000	0.364	0.863	0.137	0.0058	
450 000	0.549	0.783	0.217			450 000	0.364	0.876	0.124		
500 000	0.528	0.783	0.217	0.007		500 000	0.338	0.876	0.124	0.0045	
500 000	0.528	0.882	0.118			500 000	0.338	0.914	0.086		
750 000	0.449	0.882	0.118	0.0484		750 000	0.255	0.914	0.086	0.0115	
750 000	0.449	1	0			750 000	0.255	1	0		
maximum	0	1	0	0.0265		maximum	0	1	0	0.0109	

Q. Sen = Quarter milk Sensitivity, Q. Sp=Quarter milk Specificity, Q. area= Quarter milk area, Q. AUC = Quarter milk area under the curve, C. Sen = Composite milk Sensitivity, C. Sp= Composite milk Specificity, C. area= Composite milk area, C. AUC =Composite milk area under the curve, * Area calculated using the trapezoid method even though trapezoid method even though the graph is presented descriptively as steps (rectangles).

DISCUSSION

The average number of cows in South African dairy herds has increased especially in the Eastern Cape and Kwazulu Natal Provinces (Lactodata 2015) with some herds exceeding 1 700 cows. In order to be able to make effective decisions at herd and individual cow level, all lactating cows in a dairy herd were tested both for microbiology and somatic cell count in the Milk Laboratory at the University of Pretoria (Faculty of Veterinary Science). This is however

not the system used in other South African laboratories. Due to the practical difficulty of sampling all cows in the large herds and analytical costs, only cows previously identified with high SCC counts or those found the have high SCC in the present test would be cultured. In some laboratories the chosen SCC threshold for culturing may be as high as 500 000 cells/ml milk (personal communication with laboratory managers). Information of all four quarters, all with possible different infection status, pathogen strains and inflammatory responses is amalgamated into a composite milk sample. These results can be expected to be less informative and the values less sensitive than those from quarter milk samples. The current study has indicated that at a 500 000 cells/ml SCC threshold 66.18% and 45.52% of IMI may be missed in composite and quarter milk samples (Tables 3.1 and 3.3).

Few test results in a laboratory are either simply positive or negative and there is usually a gradual change between results from those considered to be normal to what may be abnormal. Dohoo and Leslie (1991) indicated that a SCC threshold of 200 000 cells/ml of milk was not an absolute value but the SCC threshold was associated with probability of IMI. Milk samples with SCC below that threshold could not be considered to all be culture negative, but they were only more likely to be associated with no IMI. It was in this spirit that this large dataset was analysed in order to quantify the likelihood of predicting the culture negative samples as a whole at various SCC thresholds. This dataset provided an overview of the on-farm operational conditions experienced by South African commercial dairy herds at the time. It did not attempt to distinguish between farming systems, management variations and levels, dairy breeds, cow age, parity, days in milk, quarter position, yield or stress factors originating from environmental, milking machine use, maintenance or machine settings, possible nutrition short comings, social stress or systemic diseases that may challenge the immunity of dairy cows. This study was intended to serve as an initial study to identify the relation between IMI in general and SCC levels while the second part of this study will report on SCC finding regarding major and minor pathogen groups as well as for 16 specific udder pathogens.

Failure to isolate bacteria from quarter samples with high SCC does not necessarily always indicate that the udder or quarter has no IMI. Bacteria may be missed due to very low concentrations present in the milk (Ruegg and Pantoja, 2013). Other studies have found that in 10 – 25% of cases of quarters with high SCC where they failed to isolate bacteria, the possibility existed that bacterial numbers were too low to detect with the laboratory technique (volume of milk used) or bacteria were excreted intermittently by the udder, as is known to occur with *Staphylococcus aureus* IMI (Dohoo and Leslie 1991; Schepers et al. 1997; Pantoja et al. 2009). Schepers et al. (1997) found that 50.2% of the variation in SCC could be explained by the presence of IMI. Some other factors also known to increase SCC are days in milk, month of sampling, udder quarter position, parity, interaction between stage of lactation and parity (Schepers et al. 1997). Somatic cell count is also known to increase in the absence of IMI during stressful events such as extreme environmental temperatures. Transportation stress has been shown to change the peripheral blood neutrophil function by

enhancing their migration capacity across the blood udder barrier with a consequent increase in SCC (Yagi et al. 2004). Wegner et al. (1976) reported an increase in both blood and milk leucocytes concentrations following heat stress. Green et al. (2006) in two consecutive summers found increased proportions of cows with SCC above 200 000 cells/ml without evidence of higher IMI, and considered that heat stress was presumably responsible for 70.8% of the overall SCC increase. In such situations it may not be beneficial to treat cows with intramammary antimicrobials.

Quarter milk samples

The most accurate relationship between IMI and SCC exists where quarter milk samples are taken, and reduced milk yields have been observed and when SCC has exceeded 100 000 cell/ml (Schukken et al. 2003). Somatic cell counts of milk from healthy quarters have been found to be consistently low, and at a threshold of below 200 000 cells/ml has been proposed to be the most practical level for defining udder health (Dohoo and Leslie 1991; Schepers et al. 1997; Pantoja et al. 2009).

In this study the percentage of quarter milk samples with IMI at 100 000 (15.59%) and at 200 000 cells/ml (18.73%) differed only slightly indicating a reduced risk of missing IMI when using a threshold of 200 000 cells/ml, compared to a threshold of 100 000 cells/ml. Furthermore over 63.03% of all quarter samples tested were culture negative at the 200 000 cells/ml SCC threshold, indicating the usefulness of SCC as a screening test in quarter milk samples. Compared with similar studies by Eberhart et al. (1979) and Malinowski et al. (2006) the percentage of uninfected quarters in this study at threshold levels of 100 000 and 200 000 cells/ml were noticeably higher than their findings of 50.0% and 59.6% respectively.

Although the Sensitivity ratio predicting accuracy in identifying IMI at 50 000 cells/ml was relatively high at 88.9% with 95% upper and lower confidence intervals of (87.0%, 90.7%) it reduced rapidly to 81.9% (80.8%, 83.0%), 70.8% (69.5%, 72.0%) and 57.3% (56.1%, 58.1%) at SCC levels of 100 000, 200 000 and 400 000 cells/ml respectively. These Sensitivity Ratios, although slightly lower, compared favourably with those found by Schepers et al. (1997) of 83.2%, 74.5% and 60.8% at similar SCC thresholds. The Specificity that predicted accuracy of culture negative results increased from a low of 49.2% (49.0%, 50.3%) at 100 000 cells/ml to 75.3% (74.1%, 76.5%) at 400 000 cells/ml (Table 3.4). Specificity values reported by the current study for SCC ranges of 100 000, 200 000 and 400 000 cells/ml were however markedly lower than the 80.5%, 89.6% and 95.0% reported by Schepers et al. (1997). Hillerton (2000) proposed Sensitivity of 80% and Specificity of 99% as appropriate target values for screening tests, but these were clearly impractical in this study, even for quarter milk samples. The 95% upper and lower Confidence Interval (CI) for Sensitivity and Specificity that was adapted for clustering varied little for all SCC threshold levels, indicating that cow factors had little or no influence on the SCC levels of the quarters in this study, but it should be taken into consideration that this could possibly have been masked by the large dataset.

Clinicians are often more interested in the predictive values of a test indicating a disease than in the Sensitivity or Specificity Ratios of the test. Ruegg and Pantoja (2013) found positive predictive values relatively poor for indicating recovery from IMI when using the 200 000 SCC thresholds. The Likelihood Ratio (Table 3.4) obtained in this study confirmed that finding of Ruegg & Pantoja (2013). The Likelihood Ratio 1.177 at a SCC level of 200 000 cells/ml increased to below 1 only at a SCC threshold of 300 000 cell/ml. A Likelihood Ratio of less than 1 indicates that the SCC level is more commonly associated with lack of IMI than with IMI. Although the highest Youden's index (0.345) was found to be at a SCC threshold of 250 000 cells/ml in quarter milk, the threshold of 200 000 cells/ml with an almost similar value (0.344) was selected. This was done due its higher Sensitivity (70.8% versus 66.7%) at a SCC of 200 000 cells/ml with the aim of identifying cases with IMI rather than those without (Table 3.4). Even the highest values obtained with the Youden's index were closer to 0 than 1 indicating only fair accuracy. The efficacy of the SCC test for indicating IMI in quarter milk was found to be good but not excellent when the AUC in the ROC curve was determined at 0.7277 (Table 3.5).

Different populations, for example younger cows or herds with a low or high BMSCC may justify the use of different SCC thresholds for screening purposes. When the prevalence of IMI in a herd is high, the SCC threshold used may shift towards higher Specificity levels, in order to identify the true negative cows, while Sensitivity might be of greater value in identifying true positive cases in herds where IMI prevalence is low. Thus prior to analysing the data for practical operations, clarity should be obtained as to the requirement for that specific dairy herd. The operational threshold selected should depend on what the practitioner wants to achieve with this information and what is therefore the most important aspect to optimize. One of the first questions to answer is whether it is more important to identify samples with IMI or samples without IMI. Because Sensitivity and Specificity Ratios are inversely related in most cases, one of the two will be favoured when setting a threshold in a test. When the aim is to eradicate IMI with *Streptococcus agalactiae* from a herd through separation and treatment of cows with infected udders, for instance, it could be beneficial to test all cows in the herd, even if the prevalence is low, or to set a low threshold because of the low Sensitivity at higher SCC levels. When culling is the purpose for testing, as may be the case with *Staphylococcus aureus*, the SCC threshold may be increased to high levels such as 800 000 cells/ml (Sol et al. 1991; Swinkels et al. 2012). In the latter case the SCC test should rather be used as a screening and not a diagnostic test to select cows for further bacterial analysis. When a decision needs to be made whether subclinical IMI with *Staphylococcus aureus* should be treated and the SCC level, the number and position of quarters infected, parity, days in milk and treatment regime is known, the probable outcome of treatment success can be calculated (Sol et al. 1997). Knowledge of cows or quarters infected with coagulase negative staphylococci (CNS) may be of little to no benefit and the cost of further testing may not be justified. Cows with CNS are neither treated nor separated when infected.

It may therefore be beneficial to use variable SCC thresholds when evaluating dairy herds depending on the main udder pathogens isolated from that herd.

Composite milk samples

Few studies have been done on composite milk samples compared with the number of studies done investigating SCC and IMI in quarter milk samples. A composite sample is a combination of milk from the four udder quarters, and it is to be expected that the Sensitivity and Specificity of this sample type should differ at similar SCC levels from that in quarter milk samples.

In this study composite milk samples of 52.91% and 59.84% had SCC equal to or less than 150 000 and 200 000 cells/ml respectively, and 15.05% % and 18.31% were culture positive at the same SCC levels (Tables 3.1). This is a disturbingly high percentage if only viewed from a point of possible misdiagnosis of IMI when choosing either of these SCC thresholds. However it needs to be considered that a substantial portion of these IMI may be minor pathogens and coagulase negative staphylococci (CNS). The proportion of major pathogens and contagious species isolated from low SCC were not examined in this paper, but will be reported in a subsequent paper.

Sensitivities of the SCC test for the composite milk samples were 65.3% with 95% CI of (64.0%, 66.6%) at 150 000 and 57.8% (56.6%, 59.0%) at 200 000 cells/ml and decreased to a low of 25.5% (24.7%, 26.3%) at the highest SCC range. These results indicated that SCC for composite milk samples at 200 000 cell/ml was less able to detect IMI than at SCC for quarter milk samples at 200 000 cells/ml where the Sensitivity was 70.8%. Although still less precise, results of the test at SCC of 150 000 cells/ml were more comparable with results obtained in quarter milk samples (Tables 3.2 & 3.4). Specificity and the ability to detect culture negative samples accurately using SCC was higher in composite samples than quarter milk samples. It increased from 66.8% (65.7%, 67.9%) at the SCC threshold of 150 000 cells/ml to 73.3% (72.2%, 74.5%) at 200 000 cells/ml compared to 63.6% (62.4%, 64.8%) for quarter milk sample at a SCC threshold of 200 000 cells/ml (Tables 3.2 & 3.4). Dohoo and Leslie (1991) reported in composite samples overall higher Sensitivity (83.4%) and slightly lower Specificity (58.9%) for major pathogens at 200 000 cells/ml. The SCC threshold point in composite samples where Sensitivity and Specificity were evenly balanced using the Youden's index, was indicated at 150 000 cells/ml (Table 3.2). This was found to be the optimum mathematical SCC threshold to identify IMI when examining composite samples without including any further information such as cow and environmental information.

Likelihood Ratios were lower in composite samples than those at similar SCC thresholds in quarter milk samples. At 150 000 and 200 000 cell/ml the Likelihood Ratios to detect culture negative composite samples were 1.034 and 0.864 compared to 1.177 in quarter samples at 200 000 cells/ml (Tables 3.2 and 3.4).

Comparing quarter and composite cow milk sample results

The percentage samples from which organisms were isolated at a threshold of 200 000 cells/ml were surprisingly similar in composite (18.31%) and quarter (18.73%) milk samples (Tables 3.1 and 3.3). It was however possible that the dilution effect of the composite milk sample caused more IMI to be at a subminimum level of detection for conventional laboratory microbiological detection systems (Dohoo and Leslie 1991; Pantoja et al. 1997; Hulland and Ruegg 2009).

For every one infected composite or quarter milk sample at a SCC threshold of 50 000 cells/ml 3.969 composite and 6.589 quarters were uninfected respectively. At a SCC threshold of 200 000 cells/ml the ratio of infected to uninfected were 1 to 2.268 in composite milk samples compared to 1 to 4.340 in quarter milk samples (Tables 3.1 and 3.3) making it more likely to correctly identify quarters with IMI using SCC.

Although Specificity of the SCC test was higher in composite milk samples at a threshold of 200 000 cells/ml than for quarter milk samples, this difference between the sample types remained fairly constant at all SCC levels tested. Sensitivities of SCC for composite and quarter samples however started at a similar level at the 50 000 cells/ml SCC threshold (87.0% and 88.9%) but differed increasingly as the SCC level increased. Sensitivity decreased more rapidly in composite samples to 25.5% and quarter samples to 44.9% at the high SCC level (Tables 3.2 and 3.4).

An “optimum” threshold was sought for the SCC test that could indicate IMI in milk samples at a level effective for screening of samples to be cultured under South African conditions. In composite milk samples the best threshold level proved to be 150 000 cells/ml while SCC threshold levels from 200 000 cells/ml were indicated for quarter milk (Tables 3.2 and 3.4). This result is in agreement with the 200 000 cell/ml threshold recommended by the National Mastitis Council when using the SCC test to indicate the presence of IMI in quarter milk samples (National Mastitis Council 2001). The area under the curve (AUC) was found to be 0.7084 in composite and 0.7277 in quarter samples indicating that SCC was a good but not an excellent test for indicating IMI in both sample types (Table 3.5).

CONCLUSIONS

The large dataset used in this study was a reflection of actual samples received by the laboratory for analysis and interpretation. Samples were received from herds that differed in management levels, exposure to environmental conditions, microbial IMI and cow factors (age, parity, days in milk and milk yield).

The SCC test was found to be good but not excellent as an indicator of IMI in both quarter and composite sample types. The current threshold for SCC of 200 000 cell/ml used to detect only IMI in quarter milk samples was reconfirmed to be optimal. Based on the findings of this study

a SCC threshold of 150 000 cells/ml milk can be recommended for use in composite milk samples and could be used in practice as a selection criteria to select samples for culturing in large herds. This study indicated that the likelihood of identifying culture negative samples at a SCC threshold of 200 000 cells/ml in quarter milk samples was 1.177 compared with 1.034 in composite milk samples at a SCC threshold of 150 000 cells/ml. Although less true culture negative milk samples can be expected to be identified for composite milk compared to quarter milk samples due to a lower sensitivity of the SCC test in composite samples, it was indicated that SCC could be used at the lower threshold level, to indicate IMI in composite milk samples.

It should be noted however that these optimal statistically determined SCC threshold levels need to be adapted depending on specific operational circumstances. Even at these "optimal" SCC levels, using the SCC as the only test to predict the presence of IMI is helpful as screening test to select samples for microbiological determinations, but is not ideal as a stand-alone test to indicate IMI.

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CHAPTER 4

Pathogen specific bovine intramammary infections: The validity of a somatic cell count threshold as an indicator of intramammary infection in quarter and composite milk samples

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SIGNIFICANCE OF THE WORK

The pro-active approach necessary in the management of udder health in dairy herds require the knowledge of the most prominent udder pathogen. It is no longer enough to merely know whether this is primary a contagious (host adopted) or environmental organism for prevention strategies that differ substantially within these groups. This study was aimed at assisting with the effective (both cost effective and the accuracy) diagnosis of intramammary infections is lactation cows. Although the IDF recommend that cows with somatic cell counts (SCC) higher than 200 000 cells/ml is more likely to come from quarter of cows with intramammary infections (IMI), no mention is made of the probable difference between the major and udder pathogens.

In case of outbreaks of for instance *Streptococcus agalactiae* consultants need to know what percentage of cow could be miss diagnosed when only samples with SCC of above 200 000 cells/ml is cultured. With this knowledge a decision can be made whether or not all cows should be sampled or not. This together with the estimated 15% production lost in a lactation, a more informed decision can be made.

In this manuscripts utilizing 345 461 composite and 89 638 quarter samples from dairy herds in 7 of 9 South African provinces the main objective was to establish a SCC threshold for composite milk samples that could be used for initial screening for IMI.

ABSTRACT

The objective of this study was to determine whether somatic cell count (SCC) was an effective test, with a sensitivity exceeding 85%, to determine species specific bacterial infections. In addition the relation between the SCC and various udder pathogens groups

were investigated. Somatic cell count thresholds of >200 000 cells/mL were used in quarter and > 150 000 cells/mL in composite milk samples.

A retrospective study was conducted on a dataset for 89,635 quarter and 345,467 composite cow milk samples. Eleven SCC threshold values were used to evaluate the diagnostic efficacy for the following bacteria: Gram-positive major pathogens: *Staphylococcus aureus*, *Streptococcus agalactiae, Streptococcus dysgalactiae*, *Streptococcus uberis;* Gram-negative major pathogens: *Escherichia coli*, *Klebsiella pneumonia*, Serratia spp*.;* minor pathogens: coagulase negative staphylococci, Micrococcus spp., *Staphylococcus pseudintermedius*, *Streptococcus pyogenes*, *Enterococcus faecalis*, *Enterococcus canis, Trueperella pyogenes* and other *Enterobacteriaceae.* Sensitivity and specificity were calculated taking the effect of clustering into account with quarter milk samples.

Most samples yielding major Gram-positive pathogens (88.9% in quarter and 79.9% in composite samples) and minor pathogens (61.4% in quarter and 51.7% in composite samples) had SCC >200 000 cells/mL. Sensitivity of the SCC test to detect major pathogens at a SCC threshold of >200 000 cells/mL in quarter samples and at >150 000 cells/mL in composite milk samples were 88.2% and 84.2% respectively but specificity was low (57.7% and 52.8%).

Keywords: Intramammary infections, mastitis diagnosis, pathogen-specific, somatic cell count

INTRODUCTION

Mastitis remains the most costly disease in dairy cattle (Geary et al. 2012). Subclinical mastitis is considered the most important form of mastitis, as it has been estimated to be responsible for more than 80% of economic mastitis losses (Giesecke et al. 1994, Shim et al. 2004) due to long-term reduction in milk quality and production (Halasa et al. 2009, Holland et al. 2015). Somatic cell counts (SCC) have generally been accepted and used as a measure of inflammation (Barkema et al. 1999, Biggs 2009, Heeschen 2010), as a measure of the severity of intramammary infection (IMI) (Harmon 1994) and as an indicator of economic losses (DeGraves and Fetrow 1993). The SCC threshold value used to describe normal milk, IMI and subclinical mastitis has, however, often been and still is controversial (Heeschen 2010). Dohoo et al. (2011) included the following criteria for diagnosing IMI: the number of colonies isolated, whether the organism was recovered in pure culture, and whether or not IMI was present with an increased SCC. Their conclusions were based on a study in which culture results of single milk samples were compared with a set of triplicate quarter milk samples (used as 'gold standard'). The authors concluded that results of a single sample may be used for the diagnosis of IMI in quarter milk when a minimum of one or two colonies were recorded without taking the SCC into consideration. The term 'IMI' used in this study refers to the presence of a bacterial species isolated from a milk sample as a pure culture, regardless of the level of SCC. A pure culture was defined when only one species grew on an inoculum.

Early detection of subclinical mastitis remains important for treatment success (Ruegg 2004), particularly in cases of contagious pathogens (*Staphylococcus aureus* and *Streptococcus agalactiae*) where the infected udder is the primary source of infection for other cows (Neave et al. 1969, Fox and Gay 1993, Keefe 1997). However, performing microbiology on milk samples of all cows in a herd can be expensive, and therefore, other effective diagnostic and screening tests are essential to identify affected cows for further investigation. The principal reason for increased SCCs in milk of dairy cows remains IMI (Giesecke et al. 1994). In 2001, the National Mastitis Council (USA) stated that when a quarter SCC is equal to or exceeds 200 000 cells/mL, the likelihood is high that it is infected or recovering from an infection (National Mastitis Council 2001). A study conducted on a large data set that did not differentiate between udder pathogens and where results were obtained using Youden's index and ROC graphs indicated that 200 000 cells/mL or more in quarter milk samples and 150 000 cells/mL or more in composite milk samples were the optimum SCC to indicate IMI and culturing of positive samples (Petzer et al. 2016).

Although more than 100 different microorganisms can cause mastitis, only a few species of staphylococci, streptococci and Gram-negative organisms are currently considered to be of economic importance (Zadoks and Fitzpatrick 2009). There have been changes in the relative proportion of mastitis pathogens over the past 60 years in First World countries due to changes in herd and parlour management, udder health monitoring, treatments and differences in genetics (Zadoks and Fitzpatrick 2009). Research in countries practising good udder health has recorded a lower incidence of mastitis caused by contagious bacteria but increases in Gram-negative bacteria. In South Africa, most cases of subclinical mastitis have been caused by coagulase-negative staphylococci (CNS), *S. aureus*, *Str. agalactiae* and *Str. uberis* (Petzer et al. 2009). Coagulase negative staphylococci, regarded as minor pathogens, are now causing an increasing number of clinical mastitis cases and increasingly high SCC (Zadoks and Watt 2009; Petzer et al. 2009).

The primary objective of this study was to determine whether or not the SCC test could be used with a reasonable level of accuracy (sensitivity >85%) to identify species-specific IMI. The study investigated how SCC levels differ between the various udder pathogens and pathogen groups in both quarter milk samples and composite milk samples. In addition, the percentage of infection with a species with SCC below 200 000 cells/mL in quarter milk samples and below 150 000 cells/mL in composite milk samples was quantified.

Decisions in the field need to rely on current farm information to predict future events accurately so that measures can be taken to prevent disease in advance. Early identification and dealing with cows positive for contagious udder pathogens can lower the risk of transmission for new infections significantly (Petzer et al. 2016). An effective, inexpensive test is therefore required to aid in early detection of the presence of bacteria.

METHODS

A retrospective study was conducted of milk cultures of lactating cows obtained from South African commercial dairy herds over a period of 4 years. Samples originated from approximately 830 South African dairy herds that submitted samples as part of their routine udder health monitoring programme and also from herds with increased bulk milk somatic cell count (BMSCC) who had sought help. The South African national average daily milk yield was 20.2 kg (Lactodata 2013) and lactating cow numbers varied from approximately 30 to 1700 cows in herds tested in this data set. Intervals for herd examinations ranged from monthly to annually and bi-annually, or in some cases only as a once-off test. The study population included different dairy breeds and cows differed in parity, milk yield and days in milk.

Quarter and composite milk samples were investigated. Milk samples were taken by professional samplers or milkers trained according to a standard operating procedure (Giesecke et al. 1994). Prior to sampling, first milk was stripped from all quarters and teat ends were carefully cleaned and disinfected with methylated alcohol. Approximately 10 ml of foremilk was collected aseptically into sterile marked sample tubes and kept refrigerated until shipment. In the case of composite milk samples, the same procedure was followed, only approximately equal volumes of milk from each of the four quarters were collected in one sample tube. Samples were transported on ice to reach the Milk Laboratory at the University of Pretoria (Faculty of Veterinary Science) within 48 h after sampling. Temperatures and conditions such as sample tube cleanliness and appearance were noted on arrival at the laboratory, and samples that were spoiled or of doubtful quality were not processed. Samples were plated out at the laboratory on the day of their arrival.

A total of 95 228 quarter milk samples and 386 031 composite milk samples obtained from routine herd investigations were initially under consideration for use, of which 5.9% and 10.5%, respectively, were defined as being unsuitable and were excluded from the data set. Samples were regarded as unsuitable for this study when any visible abnormalities such as dirt, flakes or blood were detected, when cultures indicated contamination or mixed bacterial growth (≥2 bacterial species), or when data were incomplete. In cases where two major pathogens were isolated from the same sample, an identifying code was used in order to be able to use this information for herd management decisions, but this information was not included in the data set. The final data set comprised 89 638 quarter milk samples and 345 461 composite milk samples.

Milk from samples was plated out on bovine blood tryptose agar plates [Oxoid, supplied by Quantum Biotechnologies (Pty) Ltd, Ferndale, South Africa]. Inoculated agar plates were incubated aerobically at 37 ±1°C and examined after 18 to 24 h and 48 h. All samples in the current data set were diagnosed by using one or more colonies in cases where *S. aureus* was suspected and two or more in all other cases. Only pure cultures were used. Colonies were

initially identified based on colony morphology, haemolysis and potassium hydroxide (KOH) test results (International Dairy Federation 1981; Sears *et al.* 1993). The catalase reaction was used to differentiate between Gram-positive staphylococci and streptococci. Staphylase, a coagulase test [Oxoid, supplied by Quantum Biotechnologies (Pty) Ltd, Ferndale, South Africa], was used to distinguish between coagulase-positive and coagulase-negative staphylococci. Maltose agar plates (Merck NT Laboratory Supplies, Halfway House, South Africa) and Staph API [Biomerieux South Africa (Pty) Ltd, Randburg, South Africa] were used for further identification of staphylococci. Catalase-negative streptococci were differentiated into the various Lancefield groups using a Strepkit [Oxoid, supplied by Quantum Biotechnologies (Pty) Ltd, Ferndale, South Africa]. Gram-negative organisms were diagnosed using DNase and MacConkey agar [Oxoid supplied by Quantum Biotechnologies (Pty) Ltd, Ferndale, South Africa] and API 20E [Biomerieux South Africa (Pty) Ltd, Randburg, South Africa]. *Staphylococcus aureus* isolates were further identified by phage typing (Blair & Williams 1961). Typing was performed by using an international set of 23 phages. The strains were typed as one of four groups or as non-allocated. All *S. aureus* isolates that were identified as being from phage group 3 were indicated by the abbreviation STH whereas all those that were not from this group were identified as STA. Phage typing was performed to differentiate between the *S. aureus* isolates for another study investigating the possible role of reverse zoonosis in udder health. Isolates that have been identified as STA and STH are further being genotyped but does not form part of this study. The reason for differentiating between them in this study is to compare their SCC threshold levels as this may have an influence on the management decisions on farm.

Somatic cells were counted by fluoro-opto-electronics using a Fossomatic 5000 (Rhine Ruhr). SSC was evaluated by comparing the presence or absence of 8 and 10 specific pathogens isolated at 11 threshold levels in quarter and composite sample types, respectively. SCC thresholds were used with increments of 50 000 cells/mL up to 500 000 cells/mL; thereafter, the two thresholds were 750 000 cells/mL and above.

IMI was diagnosed in single quarter milk samples at any level of SSC and only using pure cultures when at least one or more *S. aureus* colonies and two or more colonies of any other bacteria were isolated (Dohoo et al. 2011). In the case of composite cow milk samples, the same principle was used but referred to as culture-positive samples. Two by two tables were generated using the GenStat programme (Payne et al. 2012) and the positive likelihood ratio was used.

In the case of composite milk samples sensitivity, specificity [95% confidence intervals (CI)] of binomial proportions around these estimates was determined using the Mid-P exact test from the OpenEpi freeware programme. Sample dependency of SCC values within each cow was modelled to take into account individual cow factors that may affect quarter samples. An estimator of cluster variance was determined and the 95% CI were adjusted for clustering by

using the design effect (cluster variance or sample random sample variance; Scheaffer et al. 1996).

RESULTS

4.1 Quarter milk samples

Bacteria were isolated from 33.9% of quarter milk samples examined. Of these culture-positive samples, 12.43% were major Gram-positive, 0.3% major Gram-negative and 21.2% minor pathogens. The most frequently isolated bacteria were CNS (19.2%), *S. aureus* (STA and STH) (6.8%), *Str. agalactiae* (2.1%), *Str. uberis* (2.5%) and *Str. dysgalactiae* (1.1%). The percentage of quarter milk samples with an SCC in excess of 200 000 cells/mL was 48.8% and bacteria were isolated from 49.9% of these samples (Table 4.1).

Table 4.1. Bacteriologically negative and positive quarter milk samples indicating pathogen-specific bacteria and groups isolated from samples with and without somatic cell counts (SCC) above 200 000 cells/mL milk.

Bacteria isolated	Total numbers of samples from which bacteria were isolated	Bacterial species as a % of the total samples	Bacterial species as a % of bacteria isolated	Number of quarters with a SCC >200 000 cells/mL per bacterial species	*As a % of all quarters with a SCC >200 000 cells/mL per bacterial species	As a % of bacteriological diagnoses with SCC >200 000 cells/mL
Staphylococcus aureus (STA)	3657	4.08	12.03	3125	7.15	85.45
Staphylococcus aureus (STH)	2430	2.71	7.99	2286	5.23	94.07
Streptococcus agalactiae	1875	2.09	6.17	1707	3.91	91.04
Streptococcus uberis	2212	2.47	7.28	1856	4.25	83.91
Streptococcus dysgalactiae	964	1.08	3.17	924	2.11	95.85
Major Gram-positive pathogens	11138	12.43	36.64	9898	22.65	88.87
Escherichia coli	141	0.16	0.46	128	0.29	90.78
Klebsiella pneumonia	45	0.05	0.15	42	0.10	93.33
Serratia spp.	102	0.11	0.34	99	0.23	97.06
Major Gram-negative pathogens	288	0.32	0.95	269	0.62	93.40
All major pathogens	11426	12.75	37.59	10167	23.27	88.98
Staphylococcus pseudintermedius	445	0.50	1.46	237	0.54	53.26
Coagulase negative staphylococci	17249	19.24	56.74	10352	23.69	60.02
Streptococcus pyogenes	56	0.06	0.18	53	0.12	94.64
Enterococcus faecalis	651	0.73	2.14	557	1.27	85.56
Enterococcus canis	33	0.04	0.11	26	0.06	78.79
Trueperella pyogenes	19	0.02	0.06	16	0.04	84.21
Micrococcus spp.	141	0.16	0.46	104	0.24	73.76
Other Gram-negative bacteria	379	0.42	1.25	309	0.71	81.53
All minor pathogens	18973	21.17	62.41	11654	26.67	61.42
Culture negative samples	59236	66.09		21876	50.06	36.93
All culture positive samples	30399	33.91		21821	49.94	71.78
All samples tested	89635			43697		48.75

[a]The number of quarter milk samples, per pathogen group or species, that had an SCC of below 200 000 cells/mL expressed as a percentage of the total quarters (43 697) identified with SCC below 200 000 cells/mL. Totals are written in bold.

The cumulative percentages of the eight most prominent bacterial species isolated in quarter milk samples are indicated at 11 different SCC threshold levels. Less than 10% of the following bacterial species were isolated at an SCC of below 200 000 cells/mL: *Str. dysgalactiae* (4.2%),

S. aureus (STH) (5.9%) and *Str. agalactiae* (9.0%). In contrast, 40.0% of CNS and 46.2% *S. pseudintermedius* were isolated at an SCC of below 200 000 cells/mL (Table 4.2).

Table 4.2. Cumulative percentages of eight mastitis pathogens and culture-negative samples isolated from quarter samples distributed on various cell count thresholds (n = 89 635).

SCC x 10^3 cells/mL	% CNS	% STA	% STH	% STI	% SAG	% SUB	% SDY	% SFA	% No growth
50	14.77	7.07	3.00	22.38	2.65	5.17	1.41	3.97	34.55
100	26.17	11.71	4.79	34.03	5.80	9.98	2.82	7.93	40.32
150	34.94	12.69	5.52	42.19	7.13	14.15	3.59	11.41	56.88
200	39.98	14.55	5.93	46.74	8.96	16.09	4.15	14.44	63.07
250	47.34	21.07	10.38	53.85	12.48	21.10	6.67	20.34	69.01
300	52.06	23.11	13.12	59.21	15.51	24.30	8.72	24.31	72.22
350	55.93	24.66	14.45	63.17	18.66	27.77	10.64	28.10	74.64
400	59.24	26.49	15.45	66.43	21.88	30.87	12.69	31.55	76.61
450	62.13	28.53	17.38	69.23	25.16	33.20	13.97	34.48	78.29
500	64.62	30.14	18.84	73.19	26.80	35.83	16.03	36.55	79.69
750	73.31	36.19	25.83	79.25	37.45	46.38	24.62	45.34	84.67
Sample size	17 249	3 657	2 430	445	1 875	2 212	964	651	59 236

CNS, coagulase-negative staphylococci; STA and STH*, Staphylococcus aureus;* STI, *Staphylococcus pseudintermedius;* SAG, *Streptococcus agalactiae;* SUB, *Streptococcus uberis;* SDY, *Streptococcus dysgalactiae;* SFA*, Enterococcus faecalis;* no growth, culture-negative samples.

The cumulative percentages of the eight most prominent bacterial species isolated in from quarter milk samples are indicated at 11 different SCC threshold levels. Less than 10% of the following bacterial species were isolated at a SCC of below 200 000 cells/mL: *Streptococcus dysgalactiae* (4.2%), *S. aureus* (STH) (5.9%) and *Str. agalactiae* (9.0%). In contrast 40.0% of CNS and 46.2% *S. pseudintermedius* were isolated at SCC below 200 000 cells/mL (Table 4.2).

Sensitivity (95% CI) at an SCC threshold of 200 000 cells/mL for all bacteria that were isolated (Table 4.3) and for major and minor pathogen groups identified was 70.8%, 88.2% and 60.7%, and specificity was 66.6%, 57.7% and 55.6%, respectively. The sensitivity for detecting *Str. dysgalactiae* (95.6%), *S. aureus* (STH) (93.3%), *Str. agalactiae* (90.5%) and the major Gram-negative pathogen group (93.1%) using SCC was high. The highest sensitivity obtained with the SCC test indicating a minor pathogen species was 85.4% for detecting *Enterococcus faecalis.* Positive likelihood ratios at 200 000 cells/mL to indicate major and minor pathogens were 2.085 and 1.369, respectively (Table 4.3).

Table 4.3. Sensitivity and specificity with 95% confidence intervals (CI) for pathogen-specific detection using an SCC threshold of 200 000 cells/mL milk in quarter milk samples and positive likelihood ratios at SCC thresholds of 200 000 cells/mL.

Number of samples	Bacteria isolated	Sensitivity (95% LCI and UCI)*	Specificity (95% LCI and UCI)*	Positive Likelihood Ratio
89635	Bacteria pooled	0.708 (0.695, 0.720)	0.666 (0.636, 0.690)	2.120
3657	*S. aureus* (STA)	0.846 (0.828, 0.863)	0.537 (0.519, 0.554)	1.827
2430	*S. aureus* (STH)	0.933 (0.911, 0.955)	0.533 (0.511, 0.555)	1.998
1875	*Streptococcus agalactiae*	0.905 (0.873, 0.937)	0.530 (0.499, 0.562)	1.926
2212	*Streptococcus uberis*	0.833 (0.814, 0.851)	0.530 (0.509, 0.552)	1.772
964	*Streptococcus dysgalactiae*	0.956 (0.928, 0.985)	0.527 (0.494, 0.559)	2.021
288	Major Gram-negative pathogens**	0.931 (0.901, 0.961)	0.523 (0.464, 0.582)	1.952
11426	Major pathogens	0.882 (0.866, 0.898)	0.577 (0.561, 0.594)	2.085
445	*S. pseudintermedius*	0.528 (0.482, 0.575)	0.522 (0.475, 0.569)	1.105
17249	CNS	0.594 (0.585, 0.603)	0.549 (0.541, 0.558)	1.317
651	*Enterococcus faecalis*	0.854 (0.826, 0.881)	0.524 (0.485, 0.563)	1.873
249	Other minor pathogens***	0.795 (0.744, 0.846)	0.522 (0.459, 0.586)	1.663
379	Other Gram-negative bact.****	0.773 (0.726, 0.820)	0.523 (0.466, 0.579)	1.621
18973	Minor pathogens	0.607 (0.598, 0.616)	0.556 (0.547, 0.567)	1.367
59236	Culture negative samples	0.364 (0.352, 0.376)	0.293 (0.281, 0.305)	0.515

Confidence intervals have been adapted for clustering; LCI/UCI lower and upper confidence intervals; ***major Gram-negative pathogens: *E. coli, Serratia spp. and Klebsiella pneumonia;*c*other minor pathogens: *Micrococcus spp., Enterococcus canis, Streptococcus pyogenes and Trueperella pyogenes; *****other Gram-negative bacteria*:* all Gram-negative bacteria isolated excluding *E. coli, Serratia spp.* and *Klebsiella pneumonia.* CNS, coagulase negative staphylococci; Totals are written in bold.

4.2 Composite milk samples

Major Gram-positive, Gram-negative and minor pathogens were, respectively, isolated from 8.9%, 0.3% and 34.2% of composite samples examined. The most frequently isolated bacterial species in this sample type were CNS, *S. aureus* (STA and STH) and *Str. uberis.* Of the samples examined, 40.1% of bacteria-positive samples and 62.3% of all examined had an SCC in excess of 200 000 cells/mL. Approximately, 80% of the major Gram-positive and Gram-negative groups and 51.7% of minor pathogen group had SCC exceeding 200 000 cells/mL. More than 80% of all major pathogens investigated in this study had SCC exceeding 200 000 cells/mL except *S. aureus* (STA) and *Str. uberis*. In 81% of samples positive for *Trueperella pyogenes,* a minor pathogen, the SCC exceeded 200 000 cells/mL (Table 4.4).

Table 4.4. Composite milk samples either negative or positive for bacteria indicating individual pathogen-specific bacteria and groups isolated from samples with and without somatic cell counts (SCC) above 200 000 cells/mL milk.

Bacteria isolated	**Total numbers of samples where bacteria were isolated**	**As a % of the total samples**	**As a % of bacteria isolated**	**Number of samples with a SCC > 200 000 cells/mL**	***As a % of all cows with SCC >200 000 cells/mL per bacterial species**	**As a % of bacteriological diagnoses with SCC >200 000 cells/mL**
S. aureus (STA)	9550	2.77	6.39	7188	5.19	75.27
S. aureus (STH)	3972	1.15	2.66	3417	2.47	86.03
Streptococcus agalactiae	4759	1.38	3.18	4089	2.95	85.92
Streptococcus uberis	9173	2.66	6.14	7035	5.08	76.69
Streptococcus dysgalactiae	3159	0.92	2.11	2744	1.98	86.86
Major Gram-positive pathogens	30613	8.87	20.49	24473	17.67	79.94
Escherichia coli	341	0.1	0.23	325	0.23	95.31
Klebsiella pneumonia	282	0.08	0.19	271	0.2	96.10
Serratia spp.	240	0.07	0.16	233	0.17	97.08
Major Gram-negative pathogens	863	0.25	0.58	829	0.6	96.06
Major pathogens	31476	9.12	21.07	25302	18.27	80.39
S. pseudintermedius	1458	0.42	0.98	685	0.49	46.98
CNS*	111461	32.30	74.59	56706	40.94	50.88
Streptococcus pyogenes	127	0.04	0.08	97	0.07	76.38
Enterococcus faecalis	2603	0.75	1.74	1986	1.43	76.30
Enterococcus canis	159	0.05	0.11	103	0.07	64.78
Trueperella pyogenes	147	0.04	0.10	120	0.09	81.63
Micrococcus spp.	1006	0.29	0.67	508	0.37	50.50
Other Gram-negative bact.	986	0.29	0.66	824	0.59	83.57
Minor pathogens	117947	34.18	78.93	61.29	44.07	51.74
Culture negative samples	195644	56.70		52166	37.67	26.66
All culture positive samples	149423	43.30		86331	62.33	57.78
All samples examined	345067			138497		40.14

The number of composite milk samples, per pathogen group or species, that had an SCC below 200 000 cells/mL expressed as a percentage of the total composite samples (138 497) identified with SCC below 200 000 cells/mL. *CNS, coagulase negative staphylococci; Totals are written in bold.

In composite milk samples, 9.5% *Str. dysgalactiae*, 10.9% *Str. agalactiae,* 10.9% *S. aureus* (STA), 19.9% *S. aureus* (STH) and 40.6% CNS had SCC less than or equal to 150 000 cells/mL milk (Table 4.5).

Table 4.5. Cumulative percentages of 10 bacteria species and culture-negative samples isolated from composite cow milk samples distributed on various cell count thresholds (n = 345 461).

SCC x10^3 cells/mL	% CNS	% STA	% STH	% STI	% SAG	% SUB	% SDY	% SFA	% SCA	% SPY	% No growth
50	15.32	7.32	3.50	17.42	3.11	6.67	2.41	6.84	11.95	6.30	39.48
100	29.56	14.31	7.43	32.30	6.64	12.41	5.89	12.87	16.98	12.60	56.92
150	40.61	19.92	10.90	44.31	10.86	18.27	9.47	18.44	26.42	18.90	66.84
200	49.12	24.73	13.97	53.02	14.08	23.31	13.14	23.70	35.22	23.62	73.34
250	55.87	28.83	16.72	58.85	17.15	28.28	16.37	28.28	37.11	26.77	77.45
300	61.36	32.28	18.78	64.40	20.17	32.48	19.12	33.19	39.62	33.07	80.64
350	65.68	35.25	21.12	68.31	23.53	36.32	22.06	37.88	43.40	40.16	82.92
400	69.28	37.81	23.04	71.81	26.08	39.94	25.20	41.80	48.43	42.52	84.74
450	72.21	40.08	25.08	74.42	28.18	43.27	27.92	45.10	49.69	44.88	86.27
500	74.77	42.23	26.89	76.20	30.32	46.03	31.31	48.44	52.20	48.03	87.56
750	82.65	50.48	33.46	82.30	39.02	57.25	42.92	60.35	61.64	62.20	91.44
Sample size	111461	9550	3972	1458	4759	9173	3159	2603	159	127	195644

CNS, coagulase-negative staphylococci; STA and STH, *Staphylococcus aureus*; STI, *Staphylococcus pseudintermedius*; SAG, *Streptococcus agalactiae*; SUB, *Streptococcus uberis*; SDY, *Streptococcus dysgalactiae*; SFA, *Enterococcus faecalis*; SCA, *Enterococcus canis*; SPY, *Streptococcus pyogenes*; no growth, culture-negative samples.

At a 200 000 cells/mL SCC threshold, the sensitivity for detecting major Gram-positive, Gram-negative and minor pathogens was 79.9%, 95.5% and 51.7% with specificity of 50.3%, 52.8% and 53.5%, respectively. At a 150 000 cells/mL SCC threshold, the sensitivity improved to 84.2%, 96.1% and 60.1% for the same microbial groups (Table 4.6).

Table 4.6. Sensitivity and Specificity with 95% lower (LCI) and upper (UCI) for various bacterial species and groups indicated in composite samples at SCC threshold levels of 150 000 and 200 000 cells/mL.

Bacteria isolated	**Sensitivity at 150 000 cells/mL threshold (95% LCI & UCI)**	**Specificity at 150 000 cells/mL threshold (95% LCI & UCI)**	**Sensitivity at 200 000 cells/mL threshold (95% LCI & UCI)**	**Specificity at 200 000 cells/mL threshold (95% LCI & UCI)**
S. aureus (STA)	0.801 (0.783, 0.819)	0.521 (0.514, 0.529)	0.735 (0.730, 0.770)	0.523 (0.515, 0.531)
S. aureus (STH)	0.891 (0.862, 0.921)	0.525 (0.517, 0.533)	0.860 (0.832, 0.890)	0.525 (0.518, 0.533)
Streptococcus agalactiae	0.891 (0.865, 0.919)	0.524 (0.516, 0.532)	0.859 (0.833, 0.886)	0.525 (0.517, 0.532)
Streptococcus uberis	0.817 (0.799, 0.836)	0.521 (0.514, 0.529)	0.767 (0.749, 0.785)	0.523 (0.515, 0.530)
Streptococcus dysgalactiae	0.905 (0.873, 0.939)	0.526 (0.518, 0.533)	0.869 (0.837, 0.902)	0.526 (0.518, 0.538)
Major Gram-positive Pathogens	0.842 (0.811, 0.875)	0.499 (0.492, 0.507)	0.799 (0.769, 0.831)	0.503 (0.495, 0.511)
Major Gram-negative pathogens*	0.961 (0.897, 1.028)	0.528 (0.520, 0.536)	0.955 (0.891, 1.022)	0.528 (0.520. 0.536)
S. pseudintermedius	0.557 (0.520, 0.596)	0.529 (0.521, 0.537)	0.470 (0.436, 0.506)	0.529 (0.522, 0.537)
CNS	0.594 (0.580, 0.608)	0.498 (0.489, 0.507)	0.509 (0.496, 0.522)	0.539 (0.530, 0.548)
Enterococcus faecalis	0.816 (0.781, 0.851)	0.527 (0.519, 0.535)	0.763 (0.730, 0.797)	0.527 (0.520, 0.535)
Other Gram-negative bacteria**	0.827(0.771, 0.885)	0.528 (0.521, 0.536)	0.836 (0.780, 0.894)	0.528 (0.521, 0.536)
Streptococcus pyogenes	0.811 (0.665, 0.980)	0.529 (0.522, 0.537)	0.764 (0.623, 0,928)	0.529 (0.521, 0.537)
Enterococcus canis	0.736 (0.611, 0.879)	0.529 (0.521, 0.537)	0.648 (0.532, 0.782)	0.529 (0.521, 0.537)
Trueperella pyogenes	0.871 (0.729, 1,032)	0.529 (0.521, 0.537)	0.816 (0.680, 0.973)	0.529 (0.521, 0.537)
Micrococcus spp.	0.588 (0.542, 0.636)	0.529 (0.521, 0.537)	0.505 (0.463, 0.550)	0.529 (0.512, 0.537)
Other minor pathogens combined***	0.653 (0.612, 0.695)	0.529 (0.521, 0.536)	0.575 (0.537, 0.616)	0.529 (0.521, 0.537)
Minor pathogens	0.601 (0.587, 0.615)	0.491 (0.483, 0.501)	0.517 (0.505, 0.530)	0.535 (0.526, 0.545)
Bacteria pooled	0.653 (0.640, 0.666)	0.668 (0.657, 0.679)	0.578 (0.566,0.590)	0.733 (0.722, 0.745)

*Major Gram-negative pathogens: *Escherichia coli, Klebsiella pneumonia* and *Serratia spp*.; **other Gram-negative bacteria: all Gram-negative isolated excluding *E. coli, Klebsiella pneumonia* and *Serratia spp*.; ***Other minor pathogens combined: *Streptococcus pyogenes, Enterococcus canis, Trueperella pyogenes* and *Micrococcus spp*. CNS, coagulase negative staphylococci. Totals are written in bold.

In almost all cases in composite samples, the probability of identifying pathogen groups or specific pathogen species was only slightly higher than the likelihood of identifying culture-negative samples at both 150 000 cells/mL and 200 000 cells/mL SCC thresholds. *S. pseudintermedius* was less likely to be identified than culture-negative samples when using an SCC threshold of 200 000 cells/mL (Table 4.7).

Table 4.7. The positive likelihood ratios at somatic cell count (SCC) thresholds of 150 000 and 200 000 cells/mL for detecting various bacterial species and groups in composite samples.

Bacteria isolated	**Positive Likelihood Ratio at 150 000 cells/mL**	**Positive Likelihood Ratio at 200 000 cells/mL**
Staphylococcus aureus (STA)	1.672	1.541
Staphylococcus aureus (STH)	1.876	1.811
Streptococcus agalactiae	1.872	1.808
Streptococcus uberis	1.706	1.608
Streptococcus dysgalactiae	1.909	1.833
Major Gram-positive pathogens	1.681	1.608
Major Gram-negative pathogens*	2.036	2.023
Staphylococcus pseudintermedius	1.183	0.998
Coagulase negative staphylococci	1.183	1.104
Enterococcus faecalis	1.725	1.613
Other Gram-negative bacteria**	1.752	1.771
Minor pathogens	1.181	1.112
Bacteria pooled	1.967	2.165

*Major Gram-negative pathogens: *Escherichia coli, Klebsiella pneumonia* and *Serratia spp*.; **other Gram-negative bacteria: all Gram-negative isolated excluding *E. coli, Klebsiella pneumonia* and *Serratia spp*. Totals are written in bold.

DISCUSSION

This study provided an overview of the incidence of IMIs and culture-positive samples under operational conditions experienced by South African commercial dairy herds at the time of the investigation. This study was intended as an initial investigation on data already available from routine samples.

Quarter milk samples

Observations of quarter milk are not independent and within-cows dependency was considered in the study design. The within-herd correlations were not taken into account in this initial study to enable evaluation of results as they are obtained under field conditions on a daily basis by diagnostic laboratories. The ratio of culture-negative to culture-positive quarter milk samples with SCC less than or equal to 200 000 cells/mL was approximately four to one, whereas almost equal numbers of culture-negative and culture-positive samples were present at SCC exceeding 200 000 cells/mL (Table 4.1). Although the ratio of positive cultures increased with increasing SCC conforming to general accepted standards (National Mastitis Council 2001, Biggs 2009, Roy et al. 2012), it was surprising to learn that such a large portion of samples with SCC exceeding 200 000 cells/mL were culture negative (Table 4.1). This

finding indicates that the SCC test is only a survey tool to aid in the identification of IMI under field conditions (Table 4.1). Although numerous factors can influence the SCC at individual cow- and udder-quarter level, such as parity, lactation stage, incorrect milking machine settings, stress and other factors including genetics, the most important cause remains the infection status of the mammary gland (Schepers et al. 1997).

A further objective of this study was to determine whether the SCC levels differed in milk samples identified with different pathogen species and between pathogen groups. Although only very small percentages of major Gram-negative bacteria (*E. coli, K. pneumonia* and *Serratia* spp.) were isolated from South African dairy herds, most were associated with high SCC (Table 4.1). In contrast to the finding of Harmon (1994) that quarters with SCC below 200 000 cells/mL were not likely to be infected with major mastitis pathogens, we isolated 11.0% of major pathogens from low SCC samples (Table 4.1). This value could even be slightly higher as samples that yielded a mixed growth of bacteria were not included in this study. Few researchers in other countries sample as a rule all lactating cows in herds for microbiological evaluation, but rather rely on identification of clinical mastitis and cases with high SCC to identify problem animals. Cows infected with major pathogens that have low SCC may therefore pass unnoticed. In herds with poor parlour hygiene and management, the risk of transmission from unidentified cows causing new IMI can be high (Sears et al. 1990, Zadoks et al. 2002).

Malinowski et al. (2006) found very high SCC (some exceeding 10 million cells/mL) for the Gram-negative pathogen group, *Str. agalactiae* and *T. pyogenes*, whereas Schepers et al. (1997) identified *S. aureus* as being responsible for the highest SCC. In this study from the eight pathogens that were quantified according to SCC levels, *Str. dysgalactiae* had overall the highest SCC followed by *S. aureus* (STH and STA) and *Str. agalactiae* (Table 4.2). Although only small numbers of *T. pyogenes* were isolated in this data set, more than 84% of these bacteria had SCC exceeding 200 000 cells/mL (Table 4.1). Another bacterium, *Str. pyogenes*, which is not often isolated from cow's milk but is frequently isolated from human throat swabs and is known to cause reverse zoonosis (Aboul Dahab et al. 1993), was found to be responsible for high SCC (Table 4.1).

A study conducted by Nickerson and Boddie (1994) showed that the mean SCC for samples identified with the CNS strains *S. chromogenes* and *S. hyicus* was 168 000 and 193 000 cells/mL compared with 39 000 cells/mL from uninfected quarters. In this study, CNS were the most frequently isolated bacteria and were equal in numbers to all the major pathogens combined. More than 61% of samples with minor pathogens had SCC greater than or equal to 200 000 cells/mL (Table 4.1). This finding indicates the extent to which minor pathogens may contribute towards high bulk milk cell counts. Malinowski et al. (2006) indicated that most milk samples with CNS, *S. aureus* and streptococcal IMI had SCC ranging from 200 000 to 2 million cells/mL. Of the more than 17 000 milk samples from which CNS was isolated in this study, 60% had SCC above 200 000 cells/mL, and of those, 26.7% had SCC exceeding

750 000 cell/mL (Tables 4.1 and 4.2). Currently, CNS is still generally regarded as a minor udder pathogen, but scientists may need to reconsider this status in future when more information regarding pathogenicity of different CNS stains become available.

The sensitivity for detecting pooled udder pathogens at a 200 000 cells/mL SCC threshold was low (70.08%), but it was moderately high (88.2%) for detecting major udder pathogens (Table 4.3). Sears *et al*. (1990) found a 74.5% sensitivity at 200 000 cells/mL for a single bacterial culture to detect *S. aureus*. This sensitivity improved to 94% and 98% when two or three consecutive samples of the same quarter were evaluated. Under field conditions, cows are usually selected for culling based on the evaluation of single samples but follow-up samples should preferably be examined to improve diagnostic accuracy. The sensitivity of SCC for different minor pathogens varies from as low as 52.8% for *S. pseudintermedius* to 85.4% in the case of *Enterococcus faecalis* (Table 4.3).

The specificity at a 200 000 cells/mL SCC threshold to indicate the absence of major (57.7%) and minor (55.6%) pathogen groups was low. These values were not in agreement with results obtained by Dinsmore et al. (1990) of 95% to 100% for *Str. agalactiae.* Although sample dependency of quarter SCC values within each cow was modelled, cow factors did not affect the outcome of results.

Positive Likelihood Ratios were used to indicate the probability of a sample being found accurately positive for specific bacteria when the SCC threshold would be increased when samples were accurately positive for specific bacteria. It was disappointing that at the 200 000 cells/ml SCC threshold, only the major pathogen group and *Str. dysgalactiae* had a small increase in probability of detection and this applied to none of the minor pathogens. Even when all bacteria were pooled, the probability remained small at just above 2% (Table 4.3).

Composite milk samples

The use of quarter milk samples for routine udder health monitoring has become expensive for large dairy herds, and initial udder health surveys are now conducted using composite cow milk samples in South African dairy herds (Petzer et al. 2016). It is important, therefore, to know how reliable SCC of composite milk samples are as indicators of IMI and culture-positive results and to learn more regarding the interpretation of these results. Although a composite milk sample is a combination of four quarters, a number of studies have found their SCC to be a useful indicator in mastitis control practices (Erskine et al. 1987, Bartlett et al. 1992, Barkema et al. 1998, 1999, Reyher and Dohoo 2011).

In both quarter and composite samples with SCC less than or equal to 200 000 cells/mL, the ratio for culture-negative to culture-positive samples was 7:3. In samples with SCC exceeding 200 000 cells/mL, composite samples had a ratio of one culture-negative sample per two culture-positive samples compared with a 1:1 ratio found in quarter milk samples (Tables 4.1

and 4.4). In contrast to results for quarter milk samples, a minority of both infected (44.4%) and uninfected (26.7%) composite samples had SCC exceeding 200 000 cells/mL. This indicated that this threshold might not be ideal to use in surveying composite milk samples for culture-positive samples (Table 3.4). In order to use SCC in composite milk samples as a survey tool providing an indication of specific pathogens at a similar level than 200 000 cells/ml in quarter milk, a SCC threshold lower than 200 000 cells/mL is needed.

Most samples with *S. aureus* (STA and STH), *Str. agalactiae, Str. uberis* and *Str. dysgalactiae* had SCC exceeding 200 000 cells/mL in both sample types but the levels at which they were isolated were noticeably lower in composite samples (Tables 4.1 and 4.4). *Staphylococcus aureus* has been shown in the past to cause a high incidence of mastitis and to be an important cause of continuing udder infections (Barkema, et al. 2006). The highest SCC was evident in this study in culture-positive samples containing *S. aureus* (STH), *Str. dysgalactiae* and *Str. agalactiae* (Table 4.5). Mastitis and IMI caused by CNS have increased in recent years, and SCC in CNS-positive samples have risen significantly (Reksen et al. 2008, Reyher et al. 2012). *Staphylococcus pseudintermedius* appears to be an emerging udder pathogen in South African dairies but was present in samples with low SCC (≤ 200 000 cells/m) both in quarter (46.7%) and composite samples (53.0%) (Tables 4.2 and 4.5).

Petzer *et al*. (2016) have found that an SCC threshold of 150 000 cells/mL was more appropriate to use in composite milk samples when surveying a herd for the purpose of identifying samples with positive bacterial growth. This study showed that the percentages of different pathogen species that were isolated from composite milk samples at a 150 000 cells/mL SCC threshold compared better than the percentages isolated at a 200 000 cells/mL threshold to that isolated from quarter milk samples at an SCC threshold of 200 000 cells/mL. When lowering the threshold to 150 000 cells/mL, an additional 8% of CNS, *S. pseudintermedius* and *Enterococcus canis* could be identified and more than 4% extra infections of *Str. uberis*, *S. aureus* (STA and STH) and *Str. agalactiae* (Table 4.5). The bacteria isolated from composite milk samples with the highest SCC were *S. aureus* (STH), *Str. agalactiae, Str. dysgalactiae* and *Str. agalactiae* (Table 4.5). When only samples with an SCC exceeding 150 000 cells/mL are cultured, 31% of *S. aureus* (STA and STH), 18% of *Str. uberis*, 11% *Str. agalactiae* and 10% of *Str. dysgalactiae* would remain unidentified (Table 4.5). All of these bacteria are known to be contagious, and culture-negative cows milked after them may be at risk of being infected (Petzer et al. 2016). Decision-makers in the field may set their goal to eradicate *Str. agalactiae* from a herd and, therefore, culture samples from all cows. Missing even one cow may start new infections or outbreaks in herds (Petzer *et al.* 2016). In the case of *Str. uberis,* eradication is not an option as this is an environmental udder pathogen and only those with high SCC that are repeatedly isolated from the same cow would be important to identify to achieve the goal set here.

Although Reksen et al. (2008) found sensitivity in composite samples to be low, this study found moderately high to high sensitivities (84.2% and 96.1%) for identifying major Gram-

positive and Gram-negative pathogens at the 150 000 cells/mL threshold but low sensitivity (65.3%) for detecting the minor pathogen group (Table 4.6). Lam et al. (1996) and Reyher and Dohoo (2010) reported at the 200 000 cells/mL threshold sensitivities of 63.0% to 77.1% to detect *S. aureus.* To detect *S. aureus* (STA) and (STH), we recorded sensitivity levels of 73.5% and 86.0%, respectively, at 200 000 cells/mL SCC, and these improved to 80.1% and 89.1% at 150 000 cells/mL SCC, respectively (Table 4.6). The sensitivity at 150 000 cells/mL for detecting *Str*. *dysgalactiae* and *Str. uberis* was 90.5% and 81.7% compared with 73.4% and 62.1% reported by Reyher and Dohoo (2010) at a 200 000 cells/mL SCC threshold. These findings supported the use of a 150 000 cells/mL SCC threshold in composite milk samples for surveying the udders infected with major pathogens.

There was little improvement in the level of specificity of the SCC test when using the lower threshold (Table 4.6). According to Reyher and Dohoo (2010), factors such as parity and days in milk did not influence sensitivity and specificity of composite sampling, only for CNS.

The positive likelihood ratio for detecting specific pathogens species and groups differed very little when the SCC was lowered from 200 000 to 150 000 cells/ml milk (Table 4.7).

CONCLUSION

Electronic somatic cell counting has traditionally been used since the 1960s as a diagnostic tool in udder health assessment (Heeschen 2010). Although most major and minor udder pathogen groups were isolated at SCC exceeding 200 000 cells/mL, over a third in quarter samples and almost a third in composite samples were culture negative at SCC exceeding 200 000 cells/ml. From these results, it is clear that SCC is not an accurate tool when used on its own to identify bacteria-positive cultures in quarter or composite samples. When opting to use SCC as a test indicating specific udder pathogens or groups, the low specificity of the test that was recorded will have a negative effect on the accuracy of results. However, decreasing the SCC threshold in composite samples to 150 000 cells/mL improved the sensitivity of the test to moderately high and high levels of 84% and 96% for the major Gram-positive and Gram-negative udder pathogen groups, whereas specificity remained low at 50% and 52%, respectively.

The level of SCC differed considerably according to the various udder pathogens identified, and species that caused the highest SCC in both sample types were *Str. dysgalactiae, Str. agalactiae* and *Str. uberis.* When SCC thresholds of 200 000 (in quarter milk samples) and 150 000 cells/mL (in composite milk samples) were used to detect *S. aureus* positive cultures, if it was found that 20.5% of quarters and 30.8% of cows positive for *S. aureus* might remain undetected due to their low SCC. These values could increase to 49% quarters and 69% of cows when milk samples with SCC exceeding 500 000 cells/ml were to be cultured. This article indicates the estimated risk of under-diagnosing of pooled bacterial infections, various pathogens and pathogen groups at specific SCC levels in both quarter and composite milk

samples. The knowledge gained in this study can assist with more efficient goal-orientated decision-making on farm level.

The effects of days in lactation, parity, milk yield, management level and prevalence of pathogen-specific IMI or culture-positive samples on SCC thresholds were not measured in this research and need to be further investigated.

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ADDENDUM 4.1

Youden's index was calculated for every bacterial species and bacterial group examined for every SCC threshold level in both quarter and composite samples. The highest Youden's index for each and the SCC threshold level at which it was found are indicated in Table 8. In cases where two Youden's indexs were found to be almost similar both were indicated and the one with the highest sensitivity (the lower SCC level) would be preferred, for the aim would rather be to identify IMI than culture negative samples. The SCC threshold levels at the higest Youden's index varied in major Gram positive pathogens from 300 000 cells/ml (*Str. agalactiae* and *Str. uberis*) to 500 000 cells/ml (*S. aureus* (STA & STH)) in quarter and from 250 000 (*Str. uberis*) to 350 000 cells/ml (*S. aureus* (STA & STH) and *Str. dysgalactiae*) in composite milk samples. The SCC thresholds at the point where sensitivity and specificity were in balance were 450 000 for the major Gram negative pathogens in quarter and 300 000 cells/ml in composite milk. In minor pathogens the highest Youden's index varied from a SCC threshold level of 100 000 cells/ml (CNS, *S. pseudintermedius* and minor pathogens) to 400 000 cells/ml (*Enterococcus canis*) in quarter samples and from 50 000 (*Micrococcus spp*.) to 300 000 cells/ml (minor Gram negative bacteria) in composite samples (Table 4.8).

In Table 9 the difference in the percentage of eight bacterial species is evident at the 200 000 and 150 000 cells/mL SCC threshold levels for quarter and composite milk samples. Bacterial species that was responsible for high SCC were less likely to be missed at these chosen SCC levels than those prone to low SCC. *Staphylococcus aureus* (STH) and *Str. dysgalactiea* were the 2 bacterial species that different the most in the percentage isolated from quarter and composite milk samples while the percentage CNS isolated from both sample types was almost the same (Table 4.9). It may therefore be nessesary to take quarter milk samples when *S. aureus* (STH) is suspected in a herd and culture those with a SCC >200 000 cells/ml or to sample the whole herd (composite milk samples).

Table 4.8. Evaluation of bacterial species and groups according to Youden's index to determine the SCC threshold level at the equilibrium point between sensitivity and specificity in Quarter and Composite milk samples.

Bacteria isolated	**QUARTER MILK SAMPLES**		**COMPOSITE MILK SAMPLES**	
	Highest Youden's Index	**SCC x10^3 cells/ml threshold level at highest Youden's index**	**Highest Youden's index**	**SCC x10^3 cells/ml threshold level at highest Youden's Index**
Staphylococcus aureus (STA)	0.461	500	0.368 / 0.370	350 / 400
Staphylococcus aureus (STH)	0.580	500	0.515	350
Streptococcus agalactiae	0.463	300	0.483	300
Streptococcus uberis	0.382	300	0.363 / 0.364	250 / 300
Streptococcus dysgalactiae	0.543	400	0.407	350
Major Gram positive pathogens	0.464	450	0.417	400
Escherichia coli	0.547	500	0.634	300
Klebsiella pneumonia	0.635	500	0.509	300
Serratia spp.	0.573	450	0.583	250
Major Gram negative pathogens	0.563	450	0.632	300
Coagulase negative staphylococci	0.164	100	0.583	250
Staphylococcus pseudintermedius	0.055	100	0.109	100
Enterococcus faecalis	0.374	250	0.365	250
Other Gram negative bacteria*	0.329	200	0.460	300
Micrococcus spp.	0.260	150	0.096	50
Streptococcus pyogenes	0.499	250	0.253	150
Enterococcus canis	0.329	400	0.138	200
Trueperella pyogenes	0.353/0.354	150 / 200	0.392	150
Other minor (combined)**	0.329	200	0.187	100
Minor pathogens	0.144	100	0.303	250

*Other Gram nagative bacteria = all Gram negative isolated excluding *E. coli, Klebsiella pneumonia* & *Serratia spp.* , **Other minor pathogens (combined) = *Streptococcus pyogenes, Enterococcus canis, Trueperella pyogenes* and *Micrococcus spp*.

Table 4.9. Comparing the percentage of 8 different bacterial species that would not be isolated in quarter milk at a SCC threshold of 200 000 cells/mL and 150 000 cells/mL in composite milk samples.

	% Bacteria missed in quarter milk at SCC threshold of 200 000 cells/ml	**% Bacteria missed in composite milk at SCC threshold of 150 000 cells/ml**
Staphylococcus aureus (STA)	14.55	19.92
Staphylococcus aureus (STH)	5.93	10.90
Streptococcus agalactiae	8.96	10.86
Streptococcus uberis	16.09	18.27
Streptococcus dysgalactiae	4.15	9.47
Coagulase negative staphylococci	39.98	40.61
Staphylococcus pseudintermedius	46.74	44.31
Enterococcus faecalis	14.44	18.44

ADDENDUM 4.2 (Poster – International Congress 2016)

Poster presented at the International Dairy Federation Mastitis Conference 7-9 September 2016, Nantes, France. 2016.

Pathogen specific bovine intramammary infections: The validity of a somatic cell count threshold as an indicator in quarter and composite milk samples. (Inge-Marié Petzer, Joanne Karzis, Edward F. Donkin, Edward C. Webb & Eric M.C. Etter).

CHAPTER 5

Epidemiological and partial budget analysis for treatment of subclinical *Staphylococcus aureus* intramammary infections considering microbiological and cytological scenarios.

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SIGNIFICANCE OF THE WORK

Antimicrobial resistance is currently an actual global topic. This study is a fresh approach towards this challenge. The aims for developing the models were: firstly to provide a consultant, veterinarian or dairy producer with a tool to base decisions on current farm information to accurately predict future events so that measurements could be taken to prevent disease in advance; secondly we wanted to predict cost effectiveness of management changes in combination with an indication of epidemiological outcome in the *Staphylococcus aureus* population in herds. Current availability of advances on farm technology and data capabilities based on scientific approach predictions is making this approach become a reality.

Predictions were based on the combination of financial cost benefit but included also predictions of probable epidemiological outcomes such as number of clinical mastitis cases, new intramammary infections, persistant cases likely to occur and number of subclinical *Staphylococcus aureus cases* that would require treatment for the period chosen. The study further compare these outcomes for different initial herd prevalence of *Staphylococcus* intramammary infections, different management levels and varying duration of intramammary treatments. When results of economic and epidemiological models were compared, the best financial option differed in some scenarios. Such models may assist producers, consultants and veterinarians in optimising decisions balancing cost/benefit with end point IMI dynamics. Where initial effective treatment was applied, a lower number of treatments were needed for the 255 days.

We believe that the models can make a positive contribution to enhance current herd udder health decisions by providing more accurate predictions of outcome of inventions such as the treatment of subclinical *Staphylococcus aureus*.

ABSTRACT

An innovative method was investigated to aid in the elimination of *Staphylococcus aureus* (*S. aureus*) intramammary infections (IMI) from dairy herds. A stochastic model explore the economic benefit of three-day or eight-day treatment of subclinical IMI in all *S. aureus* infected cows or in only those with a somatic cell count (SCC) exceeding 200,000 cells/ml. An epidemiological model was developed to run parallel to the economic model that would predict the *S. aureus* IMI likely to persist, develop new infections and clinical mastitis.

In the economic model a first algorithm was used to consider the low prevalence (LP) scenario and made use of *S. aureus* prevalence information provided by retrospective analysis of microbiological and cytological results in South Africa (2008-2012). The data used considered *Staphylococcus aureus* prevalence from $[1.495; 1.595]_{95\%}$ to $[6.72; 6.95]_{95\%}$ for SCC≤200,000 and SCC>200,000 cells/ml respectively. A second algorithm considered the high prevalence (HP) scenario to evaluate a simulated situation with a 5 to 25% prevalence. Scenarios of low or high transmission ratio (TR) were included in the model according to the hygiene management on the farm. Probabilities and costs were calculated over 255 days. The economic models predicted average cost indices for low *S. aureus* IMI and low TR to vary from -3,179 ZAR (South African Rands) when subclinical cases with SCC higher than 200,000 cell/ml were treated for eight days, to -3,663 ZAR when all subclinical *S. aureus* IMI were treated for three days. With a HP and high TR of *S. aureus* the average cost indices changed from -18,042 ZAR when none to -5,433 ZAR per 255 days when all *S. aureus* IMI were treated for eight days.

The epidemiological model in this study predicted substantial benefit of treatment mainly in high TR scenarios. New IMI decreased up to77% in the three-day and up to 91% in the eight-day treatment scenarios. In the HP scenarios, persistent IMI were reduced by 94%. The number of clinical cases predicted with no treatment for subclinical infections was higher than the total number of clinical and subclinical cases in scenarios where cows were treated three or eight days.

Initial prudent treatment of subclinical IMI resulted in less overall treatments and less new, persistent and clinical cases. Combined results of economic and epidemiological models indicated that the option that cost the least did not always have the best epidemiological outcome. Models may assist in optimising and balancing decisions relating to financial and IMI.

Key words: mastitis treatment, subclinical *Staphylococcus aureus*, stochastic economic model

INTRODUCTION

Bovine mastitis is the single most important disease of dairy cows and imposes economic burdens worldwide on dairy farms in first world countries (Schepers and Dijkhuizen, 1991, Halasa et al. 2007). Despite decades of intensive research and management strategies, bovine mastitis still remains an immense challenge. While the occurrence of clinical mastitis has been reported to have decreased there has been almost no reduction in the prevalence of subclinical mastitis (Pyörälä 2002, Guimarães et al. 2017). Economic pressure drives modern dairy farmers to exert continuous efforts to optimize profitability. Many however appear to be motivated by losses from clinical mastitis and failure to obtain milk price premiums and are often unaware of the presence of subclinical mastitis and of the vast losses caused by it (Huijps et al. 2007). Decisions are often based on their limited perception of the economic losses and not on the actual loses (Vaarst et al. 2002, Huijps et al. 2007). Only once farmers can be convinced of the actual losses caused by subclinical mastitis can they be motivated to invest in pro-active udder health management (Valeeva et al. 2007).

The epidemiology of *S. aureus* IMI depends on specific bacterial characteristics as well as on the susceptibility of the cow to this organism (Piccinini et al. 1999). Irregular shedding patterns of many *S. aureus* strains complicate the diagnosis (Sears et al. 1990), control and management of these infections, while *S. aureus* strains differ in their ability to resist phagocytosis (Piccinini et al. 1999). This bacteria is known to lead often to chronic udder infection, extensive parenchyma damage and lower lifetime expectancy.

Modern dairy farms in South Africa have been characterized by amalgamation of smaller herds into herds of 800 or more lactating cows (Lactodata 2013). As the risk of new infections increase due to movement of cows between herds, the demand for effective and pro-active udder health management grows. This increased demand for pro-active management requires ways to detect and eliminate mastitis at an early stage when animals in the herd are infected. Accurate information such as prevalence of intra-mammary infection (IMI) and identification of individual cows that are infected with contagious udder pathogens are valuable in the quest to eliminate in particular *Staphylococcus aureus* (*S. aureus*) and *Streptococcus agalactiae* (*Str. agalactiae*) IMI from dairy herds (Petzer et al. 2016). Nevertheless, the identification of subclinical IMI in individual cows or quarters can be costly especially in large herds, and many farmers often have to rely on somatic cell count (SCC) as the only test to indicate possible IMI. Somatic cell count has been used for many decades as an important tool to indicate udder health status (NMC Guidelines 2001, 2015) and is closely correlated with economic losses (Raubertas and Shook 1982, Halasa et al. 2007, Guimarães et al. 2017). Cows with high somatic cell counts have been estimated to produce less milk (8% for primiparous cows) and up to 8% less milk solids per lactation for every increase of 250 000 cells/ml milk in the range between 100 000 and 600 000 cells/ml (Raubertas and Shook 1982, Halasa et al. 2007). Losses from cows with subclinical mastitis and

high SCC have been estimated at 1.8 kg/day for primiparous cows, and at 2.5 kg/day in older cows (Bar et al. 2007). Guimarães et al. (2017) found a reduction of 32.3% due to subclinical and 18.2% due to clinical mastitis.

Reviews of past calculations of the economic losses resulting from mastitis have been shown to differ meaningfully (Schepers and Dijkhuizen, 1991; Halasa et al. 2007) but there is agreement that mastitis is responsible for major economic losses. Different costs, losses and benefits have been taken into account in these calculations and economic losses have been shown to differ between farms, farming systems, regions and countries. Losses are higher under intensive farming conditions compared to extensive farming (Schepers and Dijkhuizen, 1991, Halasa et al. 2007). Mastitis losses and costs can be direct, indirect and invisible. Invisible losses are those not noticed by the farmer. Direct costs may include treatment costs (drugs used and time of the labourer or veterinarian), losses due to discarded milk (during treatment and the milk withholding period), cow fatalities (market value of the cow and the lost milk production due to an incomplete lactation) and recurring clinical mastitis in a lactation (Petrovski et al. 2006, Bar et al. 2007) as well as lower reproductive performance (Lavon et al. 2011). Indirect or invisible mastitis losses may include decreased milk yield (current and subsequent lactations), inferior milk quality (composition and hygienic quality, increasing zoonotic risks, lower end-product yields, poorer quality and shorter shelf-life of the product), increased risk of culling (and loss of future income) and loss of milk quality premiums (Hortet and Seegers 1998). Other costs might include the need for continuing farmer education, pre- and post-milking disinfectants, teat sealants, dry-cow treatment, mastitis vaccines and laboratory costs (Petrovski et al. 2006).

This study used stochastic modelling in an innovative way to evaluate the cost of no treatment, compared to three-day or eight-day treatment of subclinical *S. aureus* IMI over a 255 day period when either no cows, all cows or only those cows with SCC exceeding 200 000cells/ml were treated. A duration of 255 days were chosen in this study for this time scale fitted the management plan of farmers that were willing to participate in future research that involve testing the epidemiologic models in practise. Decisions that optimize financial cost and cow health are never easy to make as the causes of mastitis are multifactorial and the benefit of interventions should outweigh the cost and predicted losses. Benefits should however not only be measured in economic terms but should also consider the epidemiological outcome in the herd namely the *S.aureus* status at the end of a selected period.

Staphylococcus aureus is a contagious udder pathogen (Fox and Gay 1993) that is transmitted from cow to cow mainly during milking and it remains a challenge in South African herds. It is known for its chronic destructive nature and for shortening the productive lives of cows that need to be culled prematurely. The epidemiology of IMI caused by *S. aureus* depends on specific bacterial characteristics (Sutra and Poutrel, 1994), the susceptibility and immune status of the cow to the specific organism (Piccinini et al. 1999), cow management and the environmental conditions surrounding

the cow. Middleton et al. (2002) asserted that there was not much difference in the severity of parenchyma damage caused by the various *S. aureus* strains. Irregular shedding patterns of *S. aureus* can complicate the diagnosis (Sears et al. 1990), control and management of these infections (Piccinini et al. 1999). The epidemiological elements of interest in this study were to compare the number of new IMI, persistent infections, cure rate and the number of treatments required within the scenarios of variable treatment duration, treatment groups and transmission risks. Further challenges confirming effective diagnosis may include sample type (udder quarter or composite samples), the small volume of milk used for bacterial culturing, and sub-detectable levels of bacteria in samples (Nelson 1991). These uncertainties were not taken into consideration when developing the current models for they are experienced on an ongoing basis in routine milk sampling and microbiological evaluation for all scenario. Conventional intramammary antibiotic therapy used in the treatment of clinical mastitis has been found to be not very successful in eliminating *S. aureus* (Barkema et al. 2006). A meta-analysis by Sol et al*.* (1997) found that the bacterial cure rate of subclinical *S. aureus* IMI varied greatly. Sol et al. (1997) developed a formula to calculate the probability of cure based on the following criteria: the age or parity of the infected cow; days in milk (DIM); number of quarters per udder infected with *S. aureus,* the SCC level of infected quarters and quarter position (hind or front). Petzer et al. (2016) included the level of udder parenchyma damage found on udder palpation by an experienced veterinarian into these criteria. Adequate treatment duration was indicated as a prime factor for successful treatment (Sol et al. 1997, Deluyker et al. 2005). Barkema *et al.* (2006) found cure rates to vary from 3 to 74% depending on the product used, duration of treatment and whether treatment was administered during lactation or the dry period. According to Sol et al. (1997) a three-day treatment period for subclinical *S. aureus* IMI cured 35% while Sol et al. (2000) and Deluyker et al., (2005) found a 60% cure rate for *S. aureus* IMI when treatment duration was increased to eight days. The outcome of treatment of subclinical *S.aureus* should be used in combination with improved hygiene and parlour management (Allore et al*.* 1998).

The concept of treatment of subclinical IMI on a herd basis in order to eliminate *S. aureus* from a herd is not an established practice as it has been in the case of a *Str. agalactiae* outbreaks when "blitz therapy" has often been advised. Prudent treatment of subclinical mastitis however may have both direct and indirect benefits. Direct benefits for the cow involve lower SCC, higher potential milk yield and a reduced probability of contracting clinical mastitis, while healthy animals in the herd will benefit indirectly by a lower risk probability for new IMI (Ott 1999; Ruegg 2000). Swinkels et al*.* (2005) used a deterministic partial budget model to evaluate the cost/benefit of antibiotic treatment of subclinical *S. aureus* IMI using epidemiological parameters and the estimated losses in milk production. However no indication of the likely *S. aureus* IMI status namely new infections, chronic infections and infections cured during the period or the end point of these treatments has been provided.

The epidemiological model included the expected number of persistent cases and the number of clinical cases assumed to have occurred. This study included both the economic and epidemiological outcomes that would aid in the decision making by veterinarians and farmers. The goal of a dairy producer for his/her herd to eradicate *S. aureus* will have an influence on the choice of a scenario. To our knowledge, so far no cost/benefit predictions have been developed and used in parallel with IMI dynamic outcomes in an attempt to aid decision making.

The objectives of this study were to provide a science-based modeling system to determine cost/benefit when treating or not treating subclinical *S. aureus* IMI in herds; to compare epidemiological outcomes for herds with different initial *S. aureus* prevalence and to simulate the cost/benefit and epidemiology after improved management and intramammary treatment durations.

MATERIAL AND METHODS

5.1 Stage 1 - Basis for both the economic- and epidemiological models

Estimations used in the models were reliable data that were observed at the time based on various previous research results as indicated in the text to follow. The economic model used a stochastic approach to incorporate mainly individual variability (production, production losses and prevalence) adapted to the South African context while the epidemiological model used a deterministic approach based on data obtained from the literature.

5.1.1 *Staphylococcus aureus* dynamic

Persistent cases were those *S. aureus* IMI that remained infected for longer than 30 days (Swinkels et al. 2005). According to Lam (1996) and Zadoks et al. (2002) an existing subclinical *S. aureus* IMI (taken as an old case) could either persist in 78% (76% to 80%) of IMI, develop into clinical mastitis in 19% (17% to 21%) of cases or according to Deluyker et al. (2005) or cure spontaneously in 3% (3% to 6%) of cases. Zadoks et al. (2002) and Lam et al. (1996) found that less new infections compared to old existing infections of *S. aureus* IMI developed into clinical mastitis 17% (11.5% to 22.8%). They observed that 21% (18.1% to 23.9%) of the new cases were perceived to have cured spontaneously (Zadoks et al. 2002) and an estimated 62% (59.1% to 64.9%) of the new *S. aureus* IMI persisted (Swinkels et al. 2005) (Figure 5.1).

5.1.2 Transmission parameter for new *S. aureus* IMI

Cows with *S. aureus* IMI may expose other cows in their herds to *S. aureus.* The magnitude of the risk of new infections for fellow herd mates could be expressed as a transmission parameter indicating the average number of new infections that were

anticipated to occur from one infected cow (Lam 1996; Zadoks et al. 2002). The transmission parameters used to calculate new *S.aureus* IMI in this study for a herd with a low risk scenario was 0.0028 per day and it was 0.0460 per day in those where management was deficient (Lam et al. 1996, Zadoks et al. 2002, Swinkels et al. 2005).

According to Zadoks et al. (2002) and Swinkels et al. (2005) in herds under field conditions with strict measurements to identify *S. aureus* and where treatment of IMI was undertaken, the duration of persistent *S. aureus* cases was on average 51 days. Herds with low risk (well managed) used post teat dip, backwash of clusters and hand disinfection of milkers as standard operating procedures whereas herds with high transmission risk did not perform these procedures. Models based their calculations on 51 day persistent infection of *S. aureus* in low risk (well managed) herds and 115 days in high risk (poorly managed) (Zadoks et al. 2002, Swinkels et al. 2005) (Figure 1). The transmission ratio was calculated for the treatment scenario by multiplying the daily transmission parameter with the estimated duration of a persistent *S. aureus* IMI. In case of the low and high risk scenarios transmission ratio of 0.1428 (0.0028 x 51 days) and 5.29 (0.046 x 115 days) were used respectively (Figure 5.2). As we considered a duration of 255 days we needed to consider 5 time steps in the low risk scenario and 2.2 in the high risk scenario.

5.1.3 Herd prevalence of SCC

According to South African historical data on quarter milk (2008-2011) (Petzer et al. 2017a) a SCC distribution of mean 45.1986% and standard deviation of 0.0765% was present.

5.1.4 Herd prevalence of *S. aureus* IMI

The retrospective analysis of microbiological and cytological results from South African dairy herds (Petzer et al. 2017b) tested in quarter milk from 2008-2012 were used in the current study models. The prevalence of *S. aureus* IMI in samples with SCC ≤ 200,000 and >200,000 cells/ml were separated and used as normal distribution to integrate the variability of the herds. This somatic cell count level was chosen based on the National Mastitis Council Guidelines (NMC 2001) whom define that subclinical mastitis is an infected quarter with a SCC equal to or above 200,000 cells/ml in the absence of clinical changes. *S. aureus* prevalences of $[1.495\%; 1.595\%]_{90\%}$ and $[6.72\%; 6.95\%]_{90\%}$ respectively were used in the models as low prevalences and the simulated situation of a high prevalence of $[4.50\%; 5.50\%]_{90\%}$ to $[24\%; 26\%]_{90\%}$ respectively (Figure 5.2) were explored.

5.1.5 Different treatment scenarios used in models

The models explored three treatment regimens: a) no subclinical *S. aureus* IMI were treated; b) all subclinical *S. aureus* IMI were treated; c) only subclinical *S. aureus* IMI with a SCC in excess of 200,000 cells/ml in udder quarters were treated.

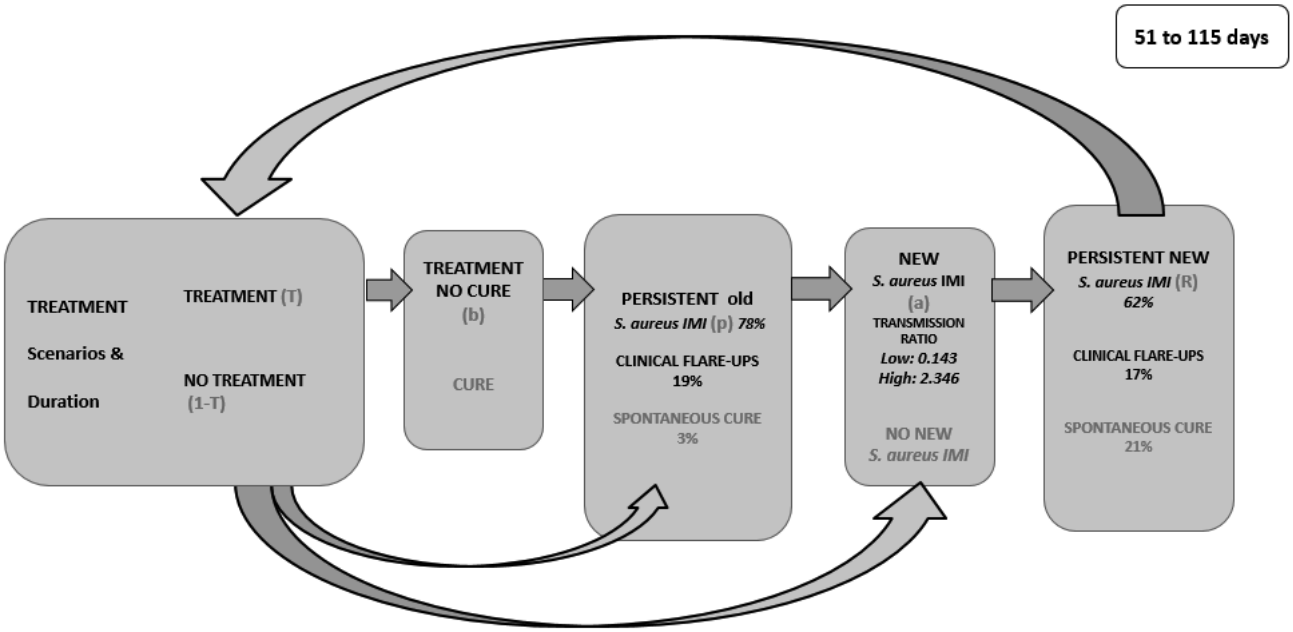

Figure 5.1. Flow diagram used in the modulation of the models.

The final number of clinical flare-ups, new IMI and number of subclinical *S. aureus* IMI that required treatment during the study period were compared between scenarios with different risk and prevalence of *S. aureus* IMI. In order to obtain accurate total number of treatments required per scenario, the number of clinical cases anticipated and the number of subclinical treatments that were projected were added.

5.2 Stage 2: Model development

5.2.1 Epidemiological models (Determining *S. aureus* infection status during the study period and at the end point of measurement)

The model used the *S. aureus* dynamics indicated in Figure 1. Epidemiological models were developed to calculate the persistent *S. aureus* IMI, number of clinical flare-ups, number of subclinical *S. aureus* IMI treated during the three scenarios and number of new *S. aureus* IMI derived from those subclinical cases based on a herd of 100 lactating cows. Developing formulations are advantages for the variables, such as the test

duration, could be change under field conditions and new predictions would be immediately available. Clinical mastitis cases were assumed to be treated or culled including those in scenario A where no subclinical mastitis cases were treated. Models in this study did not consider the remission of clinical *S. aureus* cases to subclinical cases, new infections or persistent infections in order to avoid too much complexity in a first attempt.

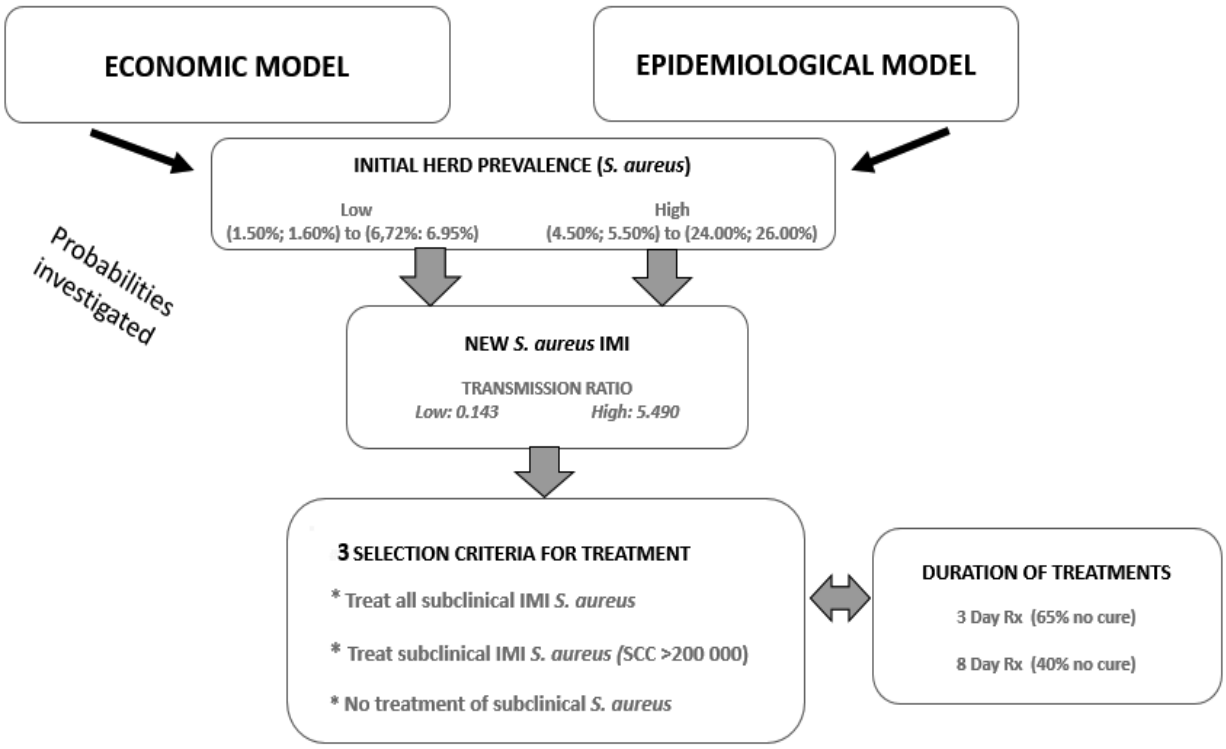

Figure 5.2. Flow chart of treatment scenarios of subclinical IMI S. aureus that were used in both the economic and epidemiological models.

Epidemiological model to predict persistent *S. aureus* IMI

The *S. aureus* IMI cases that still persisted at the end point of the simulation in the model were calculated as arithmetic progression using the following model. The calculation of the number of persistent cases of *S. aureus* IMI after a 255 days period was developed as an arithmetic progression incorporating the initial number of *S. aureus* IMI and recalculating the new number of persistent cases at each step based on the model of the *S. aureus* dynamics indicated in Figure 5.1.

$U_n = U_0 (1-T)\ paR\ (aR\ (1-T+Tbp))^{n-1} + U_0 TbpaR\ [aR\ (1-T+Tbp)]^{n-1}$ formula (a)

Where:

U_n = number of persistent cases of *S. aureus* IMI at end of the period

U_0 = Initial number of *S. aureus* IMI prevalence at the start of the simulation

T = percentage of the *S. aureus* IMI in the herd that was treated

p = percentage of *S. aureus* IMI that was persistent form old cases

R = percentage of persistent *S. aureus* IMI form new IMI

a = percentage of new IMI with *S. aureus* (RR)

b = percentage of *S. aureus* IMI that was not cured

n = number of repetitions of the 51 day or 115 day cycle

(See supplementary material for more detail)

Epidemiological model indicating probable clinical flare-ups

The number of clinical flare-ups anticipated from subclinical cases of *S. aureus* (Sn), compared in the three treatment scenarios, were also calculated using arithmetic progression as follow:

$$Sn = \sum_{i=1}^{n} Cl_i$$

Cl_1 = Clinical Flare-up + New clinical cases after the first cycle

$= U_o (1-T)\ C_0 + U_0\ Tb\ C_0 + U_0 (1-T)\ pa\ C_n + U_0\ TbpaC_n$

$S_n = A + \frac{ßY\left(1-Y^{n-1}\right)}{1-Y}$

with

$A = Cl_1$

$ß = U_0\ paR\ (1-T+Tb)\ (TbC_0 + aC_n - aC_nT)$

$Y = aR\ (1-T+Tbp)$

Where

U_0 = Initial number of *S. aureus* IMI prevalence at the start of the simulation

T = percentage of *S. aureus* IMI in the herd that was treated

p = percentage of *S. aureus* IMI that persisted from old IMI

R = percentage of persistent *S. aureus* IMI from new IMI

a = percentage of new IMI with *S. aureus*

b = percentage of *S. aureus* IMI that was not cured

n = number of repetitions of the 51 day cycle

C_n= % of clinical onset

Cl_1 = number of clinical cases after 1 cycle

Cl_i = number of clinical cases after i cycles

C_o = % of clinical flare-up

(See Supplementary Information 3 for detailed calculations)

Epidemiological model indicating new intramammary infections and numbers of treatment of subclinical cases

In a similar fashion that the number of clinical cases were determined the number of anticipated new *S. aureus* IMI ($\sum_{i=1}^{n} N_i$) and the numbers of subclinical IMI that were treated during the 255 day period ($\sum_{i=1}^{n} X_i$) were calculated.

$$\sum_{i=1}^{n} N_i = \frac{N_1\,(1-Y^n)}{(1-Y)}$$

With

N_1= U_0 pa (1-T+Tbp)

and

$$\sum_{i=1}^{n} X_i = \left(U_0 + \frac{U_0 paR\,(1-T-Tb)(1-Y^{n-1})}{(1-Y)}\right) T$$

(See supplementary material for more detail)

5.2.2 Economic Models

The economic model was based on biological parameters relating to *S. aureus* infection dynamics and transmission ratios derived from previous studies (Lam et al. 1996, Lam et al. 1997, Zadoks et al. 2002, Deluyker et al. 2005,) and from the cost/benefit studies of antibiotic treatment of subclinical S. aureus mastitis (Swinkels et al. 2005).

Two scenario trees incorporating transmission cycles were built to model low risk and high risk scenarios. Each tree presented a certain number of potential outcomes that resulted from a succession of potential events. The succession of a treatment, its potential results, and the evolution of these results, the potential new infection and their potential evolutions represented a step. A cost index was calculated resulting in the addition of all the probable outcomes of the tree. Each outcome has a weighted cost which resulted from the addition of the product of each probability and cost for each transmission cycle (step). In the low risk scenario, the scenario tree had 36 steps with probability and cost associated to each of them resulting in 100 potential outcomes. In the high risk scenario, the scenario tree had 18 steps resulting in 46 potential outcomes. A stochastic approach was used in order to predict partial financial cost in dairy herds with different initial *S. aureus* prevalence using direct and indirect costs (Table 5.1). The model simulated and compared cost of treatments for 3 days, 8 days or no treatment of subclinical IMI with *S. aureus*.

The probable severity of mastitis (change in milk appearance, clinical symptoms present in the udder and / or systemic signs) were incorporated in this model. The cost of the teat treatment was integer in the model for the low risk scenario but was removed for the high risk scenario. The model calculated cost derived from one cow for the 255 days study period. For simplification purposes we took into account the variation in the milk production along the lactation period (milk curve) using a general distribution (not considering different distributions for the different steps).

Table 5.1. Economic parameters used in developing the cost/benefit indices.

Economical parameter	Cost / losses (ZAR)	References
General information		
Milkings /day	2	
Production (Litres/day)	General distribution Minimum = 0; Maximum = 50; 6 different levels of production (15; 20; 25; 30; 35; 50) with their associated probability (32%; 37.5%; 19.5%; 7%; 2%; 2%)	Lactodata, 2013
Milk price (ZAR)/litre	Triangular distribution Minimum = 3.6; Most Likely = 4.1; Maximum = 4.6	National data of South Africa

Production (ZAR/day)	Production * Milk Price	National data of South Africa
Cow price (ZAR)	Uniform distribution Minimum = 5000; Maximum = 10000	National data of South Africa
Duration for lactation (days) chosen for this study	255	
SCC >200,000cells/ml	Normal distribution Mean = 0.451986 SD = $7.65x10^{-4}$	Petzer *et al.* 2017b
High Prevalence scenario: S. aureus positive if SCC >200,000cells/ml	Normal distribution: $[0.24 - 0.26]_{90\%}$	Data created for this scenario
High Prevalence scenario: S. aureus positive if SCC ≤200,000cells/ml	Normal distribution: $[0.045 - 0.055]_{90\%}$	Data created for this scenario
Low Prevalence scenario: S. aureus positive if SCC >200,000cells/ml	Normal distribution: $[0.0672 - 0.0695]_{90\%}$	Milk Laboratory data (2008 – 2012)
Low Prevalence scenario: S. aureus positive if SCC ≤200,000cells/ml	Normal distribution: $[0.01495 - 0.01595]_{90\%}$	Milk Laboratory data (2008 – 2012)
Costs (ZAR)		
3 days treatment (remedies)	-102	Intramammary syringes (Ampicillin + Cloxacilllin)
8 day treatment (remedies)	-272	Intramammary syringes (Ampicillin + Cloxacilllin)
Laboratory analysis	Somatic Cell Count = -5; Microbiology = -20	Milk Laboratory, Faculty of Veterinary Science Onderste-poort, South Africa
Teat dip /cow/milking	t1 = -0.32	Cost as calculated by Ecolab based on their products
Disinfectant used for backflush /cow/milking	t2 = -0.12	Cost as calculated by Ecolab based on their products
Disinfecting hands /cow	t3 = -0.05	Cost as calculated by Ecolab based on their products

Post milking teat disinfection / lactation	(t1+t2+t3)*2*255 = -249.9			
Losses				
Production loss (during and after treatment)	12 days (if 8 days treatment) 5 days (if 3 days treatment)			Results based on Delvotest (DSM) as guidelines to calculate additional days
Production loss SCC >200,000 cells/ml (L/day)	PL1= Triangular distribution Minimum = -25%*Prod; Most Likely value = -13%*Prod; Maximum = -2.5%*Prod			Giesecke *et al.* 1994
Production loss SCC >200,000 cells/ml (ZAR/day)	PL1*Milk price			
Production loss SCC ≤200,000cells/ml (L/day)	PL2 = Triangular distribution Minimum = -7.5%*Prod; Most Likely = -2.5%*Prod; Maximum = 0			Giesecke *et al.* 1994
Production loss SCC ≤200,000cells/ml (ZAR/day)	PL2*Milk price			
Clinical mastitis				
Level of clinical mastitis	Mild	Moderate	Severe	Adapted from Hollard *et al*. 2015,
Loss L/year (due to clinical mastitis)	-300	-400	-1000	
Loss L/day (Yearly loss/Duration lactation)	L1 = -1.176470588	L2 = -1.568627451	L3 = -3.921568627	
Production loss/day	Triangular distribution Minimum = L1; Most Likely = L1; Maximum = 0	Triangular distribution Minimum = L2; Most Likely = L2; Maximum = 0	Triangular distribution Minimum = L3; Most Likely = L3; Maximum = 0	
Probability of clinical mastitis	PC1=Uniform distribution Minimum = 0.6 Maximum = 1	PC2=Uniform distribution Minimum = 0 Maximum = 0.3	PC3=Uniform distribution Minimum = 0 Maximum = 0.1	Adapted from Bar *et al*. 2007

Probability to be culled	PD1=Uniform distribution Minimum = 0 Maximum = 0.1	PD2=Uniform distribution Minimum = 0 Maximum = 0.25	PD3=Uniform distribution Minimum = 0.2; Maximum = 0.95	Adapted from Bar *et al*. 2007
Production loss clinical (ZAR/day)	(L1*PC1+L2*PC2+L3*PC3)*Milk price			
Probability culled if clinical (PD)	IF (PC1*PD1+PC2*PD2+PC3*PD3) ≥1 then PC=1 IF NOT PC= PC1*PD1+PC2*PD2+PC3*PD3			

Statistical analysis – Economic model environment and software

The economic model was run for 10,000 times using a Latin Hypercube simulation using the software package @Risk (@Risk version 5.5.0 Professional edition, 2009,© Palisade Corporation, 31 Decker Road, Newfield, NY) add-in for Microsoft Excel (©Microsoft Office Professional Edition, 2010).

The results of the simulation were used to determine the mean, the 2.5th percentile and the 97.5th percentile of the resulting distribution of the cost index. A sensitivity analysis was run using rank order correlation coefficient, in order to avoid any assumption about the relationship between inputs and outputs (Vose 2000). This analysis provided coefficients that illustrate the impact of the variability or uncertainty of the inputs on the uncertainty of the output (the cost index). They illustrate also the direction of this impact, either increasing or decreasing the output. The inputs with the highest correlation coefficients (> [0.08]) (Figures 5.3a and 5.3b) were used for the sensitivity analysis of the model. This analysis were run for all scenarios.

RESULTS

5.3 Economic models

Estimated parameters from the economic model investigated the scenario of *S. aureus* IMI with a low transmission ratio, also referred to in this study as low risk, regardless of the initial infection prevalence, indicated that treating only *S. aureus* IMI with SCC >200,000 cells/ml for eight days cost the least while treating all IMI for eight days cost the second least (Tables 5.2 and 5.3). In the case of high transmission ratio, eight-day treatment of all IMI was indicated as the least costly option, while not treating at all cost the most (Tables 5.2 and 5.3).

Table 5.2. Results of the economic model comparing cost outcome (cost index) of the three treatment scenarios in herds with a low initial *S. aureus* intramammary infection (IMI) prevalence, (6.83% if SCC > 200 000 1.54% if SCC ≤ 200 000 cells/ml), with high or low transmission ratio.

Risk level for new	**Treatment duration of subclinical *S. aureus* IMI**		**Cost derived from 1 cow per lactation (ZAR)**		
***S. aureus* IMI**	**SCC >200 000 cells/ml**	**SCC ≤200 000 cells/ml**	**Mean**	**5th Percentile**	**95th Percentile**
Low transmission ratio	None	None	-3239	-6910	-1138
Low transmission ratio	3 days	None	-3590	-7443	-1264
Low transmission ratio	3 days	3 days	-3663	-7836	-1309
Low transmission ratio	8 days	None	-3179	-6536	-1186
Low transmission ratio	8 days	8 days	-3236	-6592	-1217
High transmission ratio	None	None	-6122	-12274	-2345
High transmission ratio	3 days	None	-4030	-8171	-1457
High transmission ratio	3 days	3 days	-3681	-7782	-1236
High transmission ratio	8 days	None	-3095	-6381	-1098
High transmission ratio	8 days	8 days	-2616	-5735	-764

Table 5.3. Results of the economic model comparing cost outcome (cost index) of the three treatment scenarios in herds with a high initial *S. aureus* intramammary infection (IMI) prevalence, (25% if SCC > 200 000 and 5% if SCC ≤200 000 cells/ml), with high or low transmission ratio.

Transmission cycle (step) duration)	***Risk level for new* S. aureus *IMI***	***Treatment duration of subclinical S. aureus* IMI**		***Cost derived from 1 cow per 255 days (ZAR)***		
		SCC >200,000 cells/ml	***SCC ≤200,000 cells/ml***	**Mean**	**2.5th Percentile**	**97.5th Percentile**
51 days	Low transmission ratio	None	None	-6387	-14063	-2025
51 days	Low transmission ratio	3 days	None	-7681	-16461	-2518
51 days	Low transmission ratio	3 days	3 days	-7915	-16642	-2600
51 days	Low transmission ratio	8 days	None	-6176	-12911	-2178
51 days	Low transmission ratio	8 days	8 days	-6357	-12963	-2232
115 days	High transmission ratio	None	None	-18042	-35161	-7426
115 days	High transmission ratio	3 days	None	-10402	-20216	-4215
115 days	High transmission ratio	3 days	3 days	-9259	-18650	-3506
115 days	High transmission ratio	8 days	None	-6982	-13539	-2857
115 days	High transmission ratio	8 days	8 days	-5433	-11043	-1932

The number of persistent *S.aureus* IMI were calculated per 51 day cycle in order to observe whether the different treatment options would shorten the duration on *S. aureus* infections in the herd (Table 5.4).

Table 5.4. Results of the three different treatment scenarios summarised to indicate the persistent *S. aureus* IMI per cycles and those still present after a period of 255 days in each case comparing scenarios of low and high initial *S. aureus* prevalence and transmission risks. (Calculation was rounded off to the nearest integer).

Prevalence and Risk Scenario		**Duration (Days)**	**No treatment of subclinical *S. aureus* IMI**	**Treat all subclinical *S. aureus* IMI**		**Treat subclinical *S. aureus* with SCC >200 000**	
	Treatment periods		**0 Days**	**3 Days**	**8 Days**	**3 Days**	**8 Days**
Low Prevalence	**Low Risk**	51	0	0	0	0	0
		102	0	0	0	0	0
		153	0	0	0	0	0
		204	0	0	0	0	0
		255	0	0	0	0	0
		Total cases	0	0	0	0	0
	High Risk	115	3	2	1	2	1
		230	4	1	1	2	1
		255	4	1	0	2	1
		Total cases	11	4	3	5	3
High Prevalence	**Low Risk**	51	1	1	0	1	1
		102	0	0	0	0	0
		153	0	0	0	0	0
		204	0	0	0	0	0
		255	0	0	0	0	0
		Total cases	1	1	0	1	1
	High Risk	115	17	11	7	12	9
		230	25	8	3	11	6
		255	27	8	3	11	5
		Total cases	69	27	13	34	20

The number of clinical cases treated in the scenario where no subclinical cases were treated exceeded the number of treatments in all other scenario (Table 5). This meant that by initially treating the subclinical *S. aureus* IMI, less treatments were needed overall to achieve a better epidemiological outcome over the same period.

Table 5.5. A comparison of expected outcomes of treatment scenarios of clinical flare-ups, new intramammary infections and number of subclinical treatments during the 255 day period in scenario with both low and high initial *S. aureus* prevalence and transmission ratio.

		Assumed scenario	**No treatment of subclinical *S. aureus* IMI**	**Treatment of all subclinical *S. aureus* IMI**		**Treatment of subclinical *S. aureus* IMI with SCC >200 000**	
		Duration of treatment	**0 Days**	**3 Days**	**8 Days**	**3 Days**	**8 Days**
Low Prevalence	**Low Risk**	Clinical flare-ups	1	1	0	1	0
		Total new IMI	1	0	0	1	1
		Subclinical *S. aureus* treated	0	4	4	3	3
	High Risk	Clinical flare-ups	14	2	1	3	2
		Total new IMI	47	11	4	14	5
		Subclinical *S. aureus* treated	0	12	7	9	6

		Assumed scenario	**No treatment of subclinical *S. aureus* IMI**	**Treatment of all subclinical *S. aureus* IMI**		**Treatment of subclinical *S. aureus* IMI with SCC >200 000**	
		Duration of treatment	**0 Days**	**3 Days**	**8 Days**	**3 Days**	**8 Days**
High Prevalence	**Low Risk**	Clinical flare-ups	3	2	1	2	2
		Total new IMI	4	1	1	1	1
		Subclinical *S. aureus* treated	0	16	15	13	12
	High Risk	Clinical flare-ups	53	7	3	11	6
		Total new IMI	180	42	16	53	20
		Subclinical *S. aureus* treated	0	45	27	36	21

Combining results of the economic and epidemiological models allowed for a more informed decision, taking both the end point epidemiological status of the herd at the end of the measure and cost involved to achieve this end point. Only results of the two best economic options (Table 5.2 and Table 5.3) were selected (Table 5.6).

Table 5.6. Combining economic and epidemiological outcomes of treatment scenarios with different *S.aureus* prevalence and transmission risk to determine option providing the best financial option for the optimal epidemiological outcome of *S. aureus* based on the farmers set goal.

Prevalence and Risk scenario		Cost index			*S. aureus* dynamics: during or at the end of 255 days				
			Lowest and 2nd lowest financial outcomes		Persistent (at the end)	Number new (during)	Clinical flare-ups (during)	Number S/C Rx (during)	Total treatments (S/C & clinical) (at the end)
Low Prevalence	Low Risk	1st	Treated 8days (>200 000cells/ml)	-3179	0	1	0	3	3
		2nd	Treated 8days (all subclinical)	-3236	0	0	0	4	4
	High Risk	1st	Treated 8days (all subclinical)	-2616	0	4	1	7	8
		2nd	Treated 8days (>200 000cells/ml)	-3095	0	5	2	6	8
High Prevalence	Low Risk	1st	Treated 8days (>200 000cells/ml)	-6176	0	1	2	12	14
		2nd	Treated 8days (all subclinical)	-6357	0	1	1	15	16
	High Risk	1st	Treated 8days (all subclinical)	-5433	0	16	3	27	30
		2nd	Treated 8days (>200 000)	-6982	2	20	6	21	27

5.5 Sensitivity analysis

The results were consistent when calculating the mean of each correlation coefficient and their standard deviation according to all the different scenarios. The three most important coefficients were the same, regardless of their transmission ratios. They were related in decreasing order of importance (considering their absolute values) to the daily production loss when the SCC was >200,000 cells/ml, the daily production and the daily production loss when the SCC was <200,000 cells/ml. These three prominent coefficients were the most important in terms of their impact on variation of the cost index (range of the cost index confidence interval). This is to be related to their own variations, [-85%; +81%], [-100%; +172%] and [-126%; +100%] for daily production loss when the SCC was >200,000 cells/ml, daily production and daily production loss when the SCC was <200,000 cells/ml respectively (Tables 5.2 and 5.3). The influence of these inputs on the cost index were in inverse direction for production losses whatever the SCC is compared to milk production. The lower the milk production, the higher was the cost index. This meant that the less important the cost of the IMI and its treatment was due to the negative figures for the cost index. In the inverse direction, the lower the value for the production loss (given in negative value) the lower was the cost index (negative value also). This means that the higher the production loss was, the more costly it was in terms of economic impact of the IMI and its treatment. The rest of the coefficients were less prominent and for them important variations were observed between scenarios in high and low transmission ratios. The fourth most important correlation coefficient (milk price in case of low transmission scenario and cow price in case of high transmission scenario) showed more important variations then the three first ones, as its standard deviations varied from 12% to 41% of the means respectively compared to the three most important coefficients which standard deviations varied only between 0.66% and 6.73% from the means. Another input that impacted the variation of the cost index was the milk price, regardless of the transmission ratio, with a correlation coefficient of -0.09 and -0.08 for the low and the high transmission ratio respectively. For the low transmission ratio no other input impacted consistently with correlation coefficient higher than 0.08 in absolute value. In case of the high transmission ratio scenario, other inputs had some impact on the variation of the cost index. These inputs were the cow price, the probability of severe clinical cases and the probability of culling the cow in case of mild clinical cases with correlation coefficients of -0.11, -0.11 and -0.10 respectively.

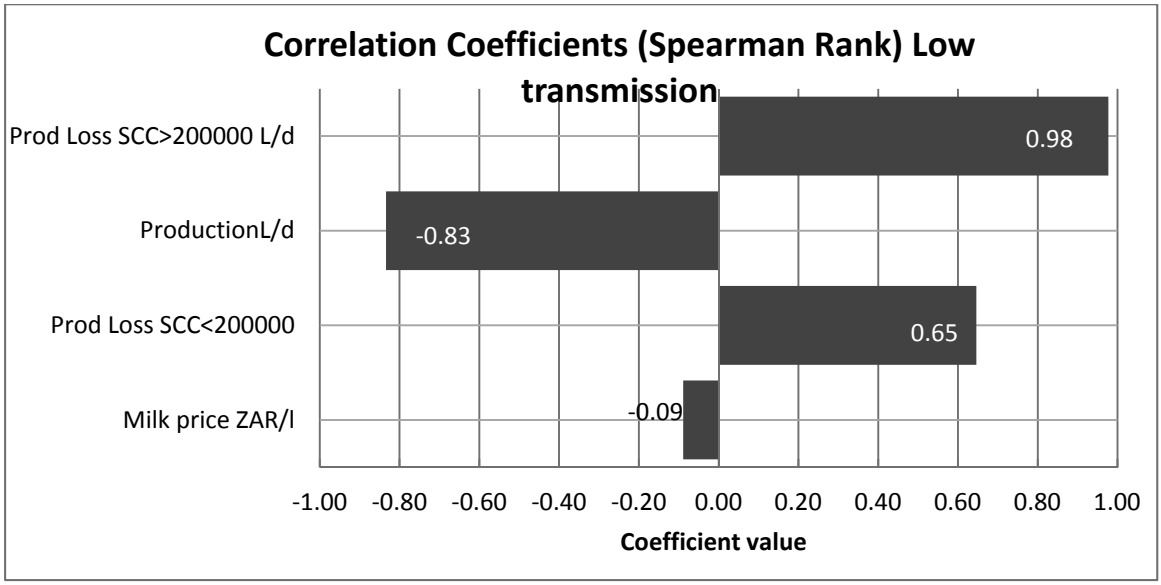

Figure 5.3a. Correlation coefficients (Spearman Rank) of low transmission ratio scenario.

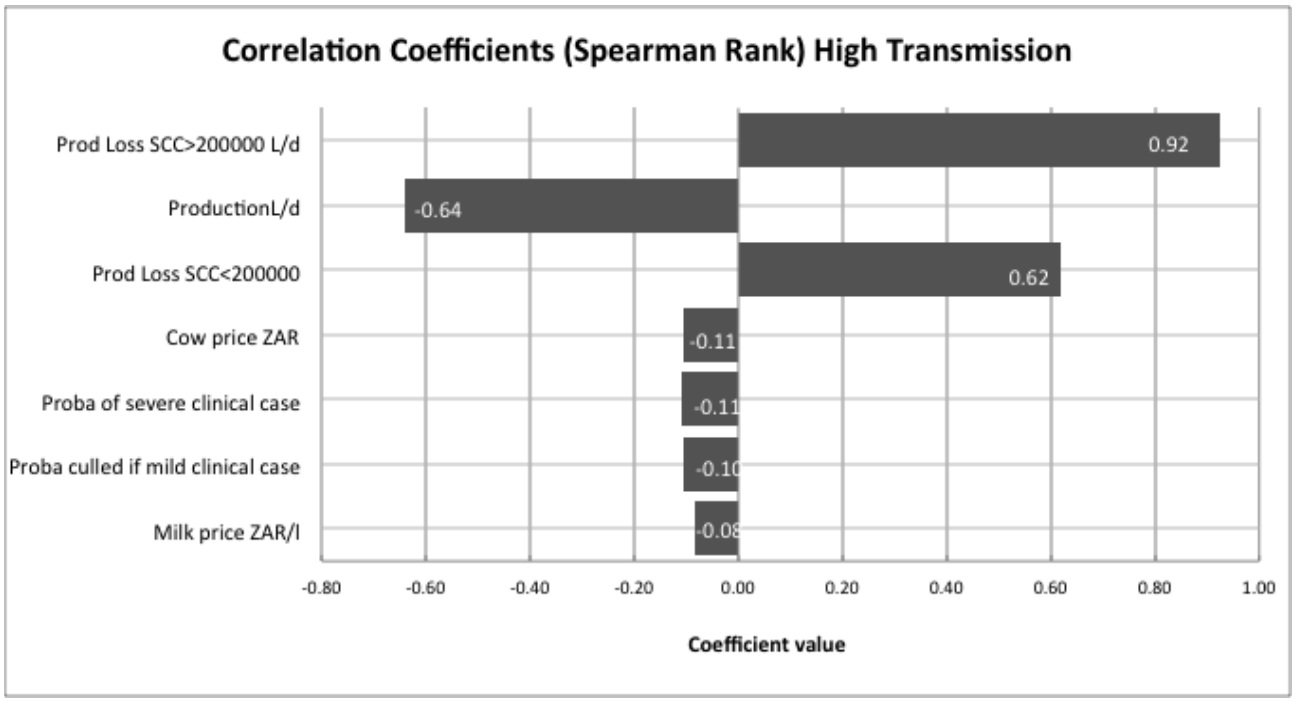

Figure 5.3b. Correlation coefficients (Spearman Rank) of high transmission ratio scenario.

DISCUSSION

The development of our economic and epidemiological models has assisted us to gain understanding concerning the effect of complex interactions between *S. aureus* IMI and management. Our findings corroborate that pro-active treatment of subclinical *S. aureus* IMI resulted in less total treatments over 255 days compared to only treating clinical S.

aureus mastitis cases. Another contribution of the current study was the combined results of financial and epidemiological outcomes of various treatment selections over 255 days. According to Halasa et al. (2007) factors affecting the performance of dairy cows that are internal to a farm are more likely to motivate producers to change them than external factors over which they do not have much control. Producers may be motivated to upgrade parlour hygiene more readily once they have insight into monetary and epidemiological advantage of such efforts.

Economic model

The option with the lowest cost in the low transmission risk scenario, regardless of the initial herd prevalence of *S. aureus,* was to treat IMI with SCC exceeding 200 000 cells/ml for 8 days, while the worst financial option was to treat all of these cases for three days. No treatment of subclinical cases was only marginally more expensive than treating all IMI for eight days.

Considering scenarios with a high transmission risk and low prevalence (LP) of *S. aureus* IMI, the option with the lowest cost was to treat all *S. aureus* IMI for 8 days and the highest cost was not to treat at all. The lowest cost predicted in the high transmission scenario was lower than the lowest cost predicted in the low transmission scenario. This could be explained by the cost involved to achieve a lower transmission in a herd that included that of teatdip, disinfecting of hands and backflushing of clusters used as preventative measures were built in to the models.

The most aggressive treatment scenario (treating all IMI for eight days) was also the best economic option in the HP scenario (Tables 5.2 and 5.3). In the HP scenario, when no subclinical IMI were treated, the gain in the cost index in the low transmission risk scenario was 49.3% (-3239 ZAR and -6387 ZAR) and in the high transmission risk scenario 66.1% (-6122 ZAR and -18042 ZAR) higher than in the LP scenario.

The impact of the production losses on the cost index is easily understandable, as they are involved in the calculation of the cost index at each step of the treatment cycles. For each subclinical case remaining after treatment, the production losses for the remaining time of lactation as for each new infection evolving in subclinical cases, was taken into account. The important individual variability of these losses, [-85%; +81] and [-126%; +100%] when SCC was >200 000 cells/ml and when SCC was <200 000 cells/ml respectively, is therefore responsible for the important variation of the cost index. It would be possible to obtain a more precise cost index in case of a known farm where intra-variability would be lower than the one given for a whole population (Giesecke et al. 1994). Concerning the milk production it is logical that it impacted meaningfully on the cost index as it is used in the calculation of the loss of revenue, in form of remaining production, in case of culling of clinical cases (evolution after inefficient treatment, or status of new infected animal), as well as the loss of production during the treatment and the milk withdrawal period. The incomes from the culling was also incorporated in the

calculation of the cost index but not the replacement. The important variability of the milk production [-100%; +172%] is based on the variation of the production during the lactation that were modelled using 6 different levels of production associated with a certain level of probability. The individual variability is also incorporated in this modelling. This variability is therefore responsible for an important part of the variation of the cost index. At the herd level, when taking the lactation period and production level of his herd into account, it would allow to reduce the variability of this parameter and therefore the variation of the cost index. The impact of the milk price on the cost index is explained by its combination with the milk production and the milk losses when calculating the cost index. Nevertheless its low variability [+/-12%] reflected in its lower impact in the variation of the cost index. In case of the high transmission ratio scenarios other inputs were also involved in the variation of the cost index. They are related to the culling of cows in case of clinical mastitis. Indeed when the transmission ratio is higher, the resulting probability of having cows culled is higher. This explains the more important impact of the variability of the cow price, of the probability of having severe clinical cases and the uncertainty of the probability to cull cows in case of mild clinical cases.

Epidemiological model

An important epidemiological finding of the present study was that treatment of subclinical *S. aureus* IMI effected the transmission risk of infections in all high risk scenarios. In both the low and high prevalence groups persisted cases at the end of the 255 days were more than 50% less compared to the untreated group. Persistent cases were reduced from 11 to 3, 4 and 5 in low prevalence and from 69 to numbers varying from 13 to 34 depending on the treatment in case of the high prevalence. This study highlighted that preventative treatment was only an additional tool in the control of staphylococcal infections in dairy cows. Elimination of infection from a herd would only be possible when no new infection from sources other than shedding herd mates might occur. In reality, infections from other sources are however likely (Barlow et al. 2013). Variation in a TR of *S. aureus* IMI has been observed before and was related to known infection risk factors (Lam et al. 1996, 1997).

Persistent *S. aureus* cases

The number of persistent *S. aureus* IMI can provide insight concerning the potential udder parenchyma damage that can be expected, with a consequent lower lifetime production and reduction of longevity of infected animals. The epidemiological outcome per cycle (the 51 days that *S. aureus* persist) can determine the and whether *S. aureus* IMI could be eliminated prior to 255 days test period when applying some of the treatment options. According to Halloran et al. (1997) a reduction in duration of *S. aureus* infections will also aid to reduce exposure (risk) of other cows.

All persistent IMI were eliminated by day 51 in the low transmission risk scenario (in LP) and by day 102 in the case of HP of IMI (Table 5.4). Treatments aimed at eliminating persistent infections in the low TR scenario therefore did not seem justifiable. *Staphylococcus aureus* persisted in all high transmission risk scenario till the test end point (day 255) although the numbers varied between scenarios. When subclinical IMI were left untreated less IMI persisted cases remained in the initial LP (11 cases) compared with the HP (69 cases) (Table 5.4). Less IMI were present at day 255 in the high transmission risk and HP scenario when treated for 8 days (13 and 20) then those treated for 3 days (27 and 34) (Table 5.4).

New IMI and flare-ups of clinical mastitis

The key advantage of treating subclinical *S. aureus* IMI was apparent in the marked reduction of new IMI, particularly in the high transmission risk scenarios. In LP, new infections were reduced from 47 when no treatment was carried out to only four cases after the most aggressive treatment. Similarly new infections dropped from 180 (untreated) to 16 cases (aggressive treatment) in the HP scenario. These findings resulted in substantial reductions of up to 77% after the three-day treatment and as high as 91% after the eight-day treatment scenario (Table 5.5). According to Zadoks et al. (2002) udder quarters that were previously infected with *S. aureus* and that recovered from these infections were more likely to be re-infected than those that were never infected with *S. aureus*, indicating the lack of acquired immunity (Zadoks et al. 2002).

Only small differences in the number of new IMI were found between groups that were treated and those not treated in the low TR herds. When all subclinical IMI were treated for three or eight days in LP situations, new IMI did not occur until the end of the study. This indicated that *S. aureus* would be eradicated from herds in these scenarios (Table 5.5). Prevalence of *S. aureus* IMI did not seem to be an important initiator of new IMI in this study nor in a study done by Zadoks et al. (2001). New IMI infections were more closely associated with the transmission risk, which represents management in this study. New infections also depended on the duration, severity and shedding patterns of the *S. aureus* IMI (Schukken et al. 2014). In this study severity was addressed by incorporating culling option in the case of clinical mastitis. New cases were less likely to persist than older IMI and shedding patterns was incorporated in the low and high transmission risk and the duration of persistant infection that varied in these risk categories, 51 and 115 days respectively.

Even though treatment and dynamics of clinical mastitis did not form part of the current study, the numbers of clinical mastitis cases anticipated in the treatment scenarios were noted because all cows with clinical mastitis must be treated for ethical and practical reasons. Prudent used of antimicrobial products are of utmost importance to help maintain antimicrobial sensitivity, or the development of antimicrobial resistance, and to prevent antimicrobial residues in milk (Davies and Davies, 2010). In the low

transmission risk scenario, aggressive treatment options failed to prevent clinical mastitis regardless of the initial herd prevalence, while some success was predicted in high transmission risk herds (Table 5.5). When calculating the treatments per scenario, the predicted subclinical IMI treated and the incidence of clinical mastitis were added together to provide a more accurate reflection of a field situation (Table 5.6). Only in high transmission risk herds did the number of clinical flare-ups of *S. aureus* mastitis differ clearly between treatment groups. The model predicted that in the HP high transmission risk scenario, where no subclinical *S. aureus* IMI were treated during the 255 study days, 53 cows would contract clinical mastitis and require treatment. Although almost the same number (52 cases in total) of treatments were predicted for the three-day treat- all scenario in a similar herd, only 7 of these 52 cases were likely to be clinical mastitis. For the eight day treatment scenario markedly less cows were expected to receive treatment: 30 (3 clinical) when all, and 27 (6 clinical) when those with only high SCC were treated. Although at first it may seem to be a contradiction to treat subclinical infections when aiming at lower the overall number of treatments, prudent treatment of subclinical IMI did lead to less treatments overall. Not only were less cows treated, but the potential risk of udder parenchyma damage decreased, because substantially less clinical mastitis cases were expected (Kitchen et al. 1980). Treatment shortened the duration of infections with consequently less shedding of *S. aureus,* which in turn was expected to lower the risk of new IMI (Schukken et al. 2014) as the study period progressed.

Combining cost/benefit and epidemiological outcomes

The two scenarios that were financially the most favourable for each transmission risk and prevalence scenario were compared after 255 days to the epidemiological outcomes. The economic model indicated that the eight-day treatment was effective in the control of this chronic udder disease. Cost index in high prevalence herds were 44.3% to 49.4% higher for infected animals than in low prevalence herd (Table 5.6).

With a low prevalence the number of new IMI, clinic cases and treatments required were almost similar and both options were viable. Nevertheless in the case of the low transmission risk the lower cost option required one less treatment and 57 ZAR less per animal (5 700 ZAR less per 100 animals) but one more new case occurred. If the farmer was aiming to clear the herd of *S. aureus* having less new infection may be worth the low extra cost of the second option. With a high transmission risk and low prevalence the same number of total treatments were required but the higher cost option had one more clinical case. Clinical cases may lead to more udder parenchyma damage and shedding (duration and quantity) (Kitchen et al. 1980) and treatment in this cases would be a reactive action compared to treatment of subclinical cases where the action is pro-active. The cost difference in this case was 47 900 ZAR per 100 cows and the lowest cost option would therefore be preferred.

In the high prevalence low risk scenario for 18 100 ZAR less in a 100 cows herd, there is a tossup between having to treat 2 less cases and having 1 less clinical case. In the worst cases scenario (high prevalence and high risk) although the lowest cost scenario have 3 more treatments it have half of the clinical mastitis cases and a fifth less new cases plus the advantage of saving 154 900 ZAR and therefore will be preferred by the farmers.

Management remains the key factor in order to control and eradicate *S. aureus* IMI from a herd. Prudent treatment of subclinical IMI was however indicated as another tool to aid in this process mainly in HP herds.

CONCLUSION

Models are an innovative approach for simulating epidemiological and economic situations and these outcomes were combined as an aid in better decision making.

Costs in high prevalence scenario were almost double than that seen in the LP scenario while cost in the case of high transmission risk scenario were always higher. The most viable financial and epidemiological options in all cases were the 8 day treatments duration.

In the LP scenario, the total number of treatments of the viable financial situation were similar. In the HP scenario and high TR, although there were three more treatments required in the most viable financial option, less of these cases were expected to result in clinical mastitis.

Treatment had overall effect on lowering the number of persistant cases but even a more important effect in preventing new IMI and clinical flare-ups in all high risk scenario. Treating all *S. aureus* positive cases, regardless of their SCC, is mainly justified in the high risk scenario.

Global concern and new legislation regarding antimicrobial use due to resistance in bacteria is currently evident. Prudent use of antibiotic treatment does not mean withholding treatment. Opting to treat subclinical *S. aureus* may therefore seem at first a contradictory decision but this study indicated that treatment of subclinical IMI with *S. aureus* in high risk herd scenario resulted in less total treatments (clinical and sub-clinical) during the 255 days. Not only were fewer cows treated, but when treating subclinical cases the probability of bacterial cure was likely to be better than in clinical mastitis cases, and less parenchyma damage could be expected. The best case scenario nevertheless remains to create a low TR environment within a *S. aureus* positive herd where the focus will be on excellent management rather than treatment.

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ADDENDUM 5.1 (Persistant *S. aureus* cases)

Calculation of persistant *S. aureus* cases:

$U_1 = U_0 (1-T)\, paR + U_0 TbpaR$

$U_2 = U_1 (1-T)\, aR + U_1 TbpaR$

$U_{n+1} = U_n (1-T)\, aR + U_n TbpaR$

$U_{n+1}/U_n = (1-T)\, aR + TbpaR$

$U_{n+1}/U_n = aR - aRT + TbpaR$

$U_{n+1}/U_n = aR\,(1 - T + Tbp)$

So we can write

$U_n = U_1\,[aR\,(1-T+Tbp)]^{n-1}$

$U_n = U_0\,(1-T)\, paR\,[aR\,(1-T+Tbp)]^{n-1} + U_0\, Tb\, paR\,[aR\,(1-T+Tbp)]^{n-1}$

$U_n = U_0\, paR\,[aR\,(1-T+Tbp)]^{n-1}\,(1-T+Tb)$ formula(a)

Where

U_n = number of persistent cases of *S. aureus* IMI at end of the period

U_0 = initial number of *S. aureus* IMI prevalence at the start of the simulation

T = percentage of the *S. aureus* IMI in the herd that was treated (all)

p = percentage *S. aureus* IMI that persistent form old cases (78%)

R = percentage persistent *S. aureus* IMI form new IMI (62%)

a = percentage of new IMI with *S. aureus* (RR)

b = percentage *S. aureus* IMI that was not cure (65% or 40%)

n = number of cycles

ADDENDUM 5.2 (Clinical cases of *S. aureus*)

Calculation of clinical cases (Cl) of S. aureus IMI.

Cl_1 = Clinical Flare-up + New clinical cases

= $U_o\,(1\text{-}T)\,C_0 + U_0\,Tb\,C_0 + U_0\,(1\text{-}T)\,pa\,C_n + U_0\,TbpaC_n$

Cl_2 = $U_1\,Tb\,C_0 + U_1\,(1\text{-}T)\,aC_n = U_1\,(TbC_0 + aC_n - aC_nT)$

Cl_3 = $U_2\,(Tb\,C_0 + aC_n - aC_nT)$

Cl_{n+1} = $U_n\,(Tb\,C_0 + aC_n - aC_nT)$

Then we replace U_n using formula (a)

Cl_{n+1} = $U_0\,paR\,(1\text{-}T + Tb)\,[aR\,(1\text{-}T+Tbp)]^{n-1}\,(TbC_0 + aC_n - aC_nT)$ formula (b)

We want to calculate the total number of clinical cases and therefor need to add all the clinical cases that developed during the different cycles:

$$Sn = \sum_{i=1}^{n} Cl_i$$

In order to simplify the equation we preferred a change in the variables using A, ß, and Y as new variables as follows:

with A = Cl_1

ß = $U_0\,paR\,(1\text{-}T+Tb)\,(TbC_0 + aC_n - aC_nT)$

Y = $aR\,(1\text{-}T+Tbp)$

So $Cl_{n+1} = ßY^{n-1}$

$S_n = A + ß Y + ß Y^2 + ... + ß Y^{n-2}$

$YS_n = AY + ß Y^2 + ... + ß Y^{n-1}$

$S_n – YS_n = A + ß Y – AY – ß Y^{n-1}$

$$S_n = \frac{A(1-Y)+ßY(1-Y^{n-1})}{1-Y}$$

$$S_n = A+\frac{ßY\,(1-Y^{n-1})}{1-Y}$$ formula (c)

Where

C_n = clinical onset

C_o = % clinical flare-up

Cl_i = number of clinical cases developed during the i^{th} cycle

U_0 = initial number of *S. aureus* IMI prevalence at the start of the simulation

T = percentage of the *S. aureus* IMI in the herd that was treated (all)

p = percentage *S. aureus* IMI that persistent form old cases (78%)

R = percentage persistent *S. aureus* IMI form new IMI (62%)

a = percentage of new IMI with *S. aureus* (RR)

b = percentage *S. aureus* IMI that was not cure (65% or 40%)

n = number cycles

ADDENDUM 5.3 (Numbers treated)

Calculation of the total number of treatments (X) of subclinical S. aureus IMI.

$X_1 = U_0T$

$X_2 = U_1T$

$X_2 = U_0 Tbp\ aRT + U_0 (1-T)\ p\ aRT$

$X_3 = U_2 T$

$X_n = U_{n-1} T$

$\sum_{i=1}^{n} X_i = U_0 T + U_1 T + \ldots\ldots\ldots + U_{n-1} T$

$= (U_0 + \ldots\ldots\ldots\ldots + U_{n-1}) T$

We first need to calculate

$U_0 + \ldots\ldots\ldots\ldots + U_{n-1}$

$U_{n-1} = U_0\ paR\ (1-T + Tb)\ (aR\ (1-T + Tbp))^{n-2}$ formula (a)

$U_1 = U_0\ paR\ (1-T + Tb)\ (aR\ (1-T + Tbp))^{0}$

$U_2 = U_0\ paR\ (1-T + Tb)\ (aR\ (1-T + Tbp))$

$U_3 = U_0\ paR\ (1-T + Tb)\ (aR\ (1-T + Tbp))^{2}$

and we had

$Y = aR\ (1-T+Tb)$

$U_{n-1} = U_1\ Y^{n-2}$

$$\sum_{i=1}^{n} X_i = \left(U_0 + \frac{U_1(1 - Y^{n-1})}{(1 - Y)}\right) T$$

ADDENDUM 5.4

Results at SCC threshold of 150 000 cells/ml in composite milk samples.

Table 5.7. Comparing the number of persistent *S. aureus* cases in herd with initial low and high *S. aureus* herd intramammary infections and risk of infections, for the various treatment scenario over 3 and 8 days, in quarter and composite milk samples at SCC thresholds of 200 000 cells/ml and composite samples at 150 000 cells/ml.

RX Scenario Persistent at end of 255d							
Treatment duration	Risk level	Herd STA prevalence	No treatment	Treat all subclinical STA	Quarter samples: Treat subclinical STA with SCC >200 000	Composite samples: Treat subclinical STA with SCC >150 000	Composite samples: Treat subclinical STA with SCC >200 000
3 days	Low	High	0.0541	0.0000	0.0000	0.0000	0.0000
		Low	0.0142	0.0000	0.0000	0.0000	0.0000
3 days	High	High	26.8534	0.2129	6.897	6.1892	14.2918
		Low	7.0356	0.6321	1.8070	2.7812	3.7444
8 days	Low	High	0.0541	0.0000	0.0000	0.0000	0.0000
		Low	0.0142	0.0000	0.0000	0.0000	0.0000
8 days	High	High	26.8534	0.1310	1.3313	1.2116	5.0523
		Low	7.0356	0.03433	0.3488	0.7813	1.3237

Table 5.8. Comparing the number of new, clinical and total number of subclinical *S. aureus* cases that required treatment in herds with initial low and high *S. aureus* herd intramammary infections and risk of infections, for the various treatment scenario over 3 and 8 days, in quarter and composite milk samples at SCC thresholds of 200 000 cells/ml and composite samples at 150 000 cells/ml.

A) Low Risk / High prevalence	No Rx	Rx all		Rx quarters SCC > 200 000 cell/ml		Rx composite SCC > 150 000 cell/ml		Rx composite SCC > 200 000 cell/ml	
	115d	3 Days	8 Days	3 Days	8 Days	3 Days	8 Day	3 Days	8 Days
Persist End	0.012	0	0	0	0	0	0	0	0
Tot New	4.374	1.137	0.687	1.272	0.914	1.340	1.029	1.382	1.100
Tot Clinical	4.106	2.133	1.289	2.386	1.715	2.513	1.929	2.593	2.064
Tot Rx	-	15.705	15.426	12.484	12.262	10.865	10.672	9.849	9.674
B) High Risk / High prevalence	No Rx	Rx all		Rx quarters SCC > 200 000 cell/ml		Rx composite SCC > 150 000 cell/ml		Rx composite SCC > 200 000 cell/ml	
	115d	3 Days	8 Days	3 Days	8 Days	3 Days	8 Day	3 Days	8 Days
Persist End	54.596	3.271	0.289	8.383	6.012	10.953	8.889	12.565	10.695
Tot New	308.288	53.132	19.715	79.046	52.482	92.071	68.953	100.246	79.290
Tot Clinical	81.202	14.549	5.399	21.645	14.371	25.212	18.882	27.451	21.712
Tot Rx	-	44.671	26.934	35.509	21.410	30.903	18.633	28.013	16.891
C) Low Risk / Low Prevalence	No Rx	Rx all		Rx quarters SCC > 200 000 cell/ml		Rx composite SCC > 150 000 cell/ml		Rx composite SCC > 200 000 cell/ml	
	115d	3 Days	8 Days	3 Days	8 Days	3 Days	8 Day	3 Days	8 Days
Persist End	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Tot New	1.146	0.298	0.180	0.605	0.511	0.351	0.270	0.362	0.288
Tot Clinical	1.076	0.559	0.338	1.135	0.959	0.659	0.506	0.679	0.541
Tot Rx	-	4.115	4.042	3.271	3.213	2.847	2.796	2.580	2.535
D) High Risk / Low Prevalence	No Rx	Rx all		Rx quarters SCC > 200 000 cell/ml		Rx composite SCC > 150 000 cell/ml		Rx composite SCC > 200 000 cell/ml	
	115d	3 Days	8 Days	3 Days	8 Days	3 Days	8 Day	3 Days	8 Days
Persist End	14.304	0.857	0.076	2.053	1.563	2.870	2.329	3.436	2.815
Tot New	80.772	13.921	5.165	18.374	12.884	24.123	18.066	28.600	21.641
Tot Clinical	21.275	3.812	1.414	5.031	3.528	6.606	4.947	7.832	5.926
Tot Rx	-	11.704	7.057	7.339	4.425	8.0966	4.882	9.303	5.610

ADDENDUM 5.5 (Keynote speech IDF congress)

Keynote speech – IDF Mastitis Conference 7-9 September 2016, Nantes, France.

Combining epidemiological and cost benefit analysis to assist in udder health management decisions.

ABSTRACT

Despite decades of intensive research and managemental strategies, bovine mastitis remains an immense challenge. While the occurrence of clinical mastitis has decreased, that of subclinical mastitis has not. Economic pressure drives modern dairy farmers to exert continuous efforts to optimize profitability so benefits associated with controlling and limiting subclinical mastitis will motivate them to invest in pro-active udder health management.

Objective: An innovative method was investigated to aid in the decision making in *Staphylococcus aureus* (*S. aureus*) positive dairy herds. Stochastic cost/benefit models were used to explore 3 or 8day treatments of subclinical *S. aureus* intramammary infections (IMI) in all *S. aureus* infected cows with a somatic cell count (SCC) above 200 000 cells/ml compared to no treatment. Additional models were developed to predict and compare the *S. aureus* subclinical cases that cured, persisted, flared-up clinically and new infections that occurred over 255 days.

Methods: Economic models applied direct costs such as drugs, labour, discarded milk, cow fatalities and laboratory fees and indirect costs of decreased milk yield and penalties. Low and high transmission rates related to management practises were incorporated into the models. Using retrospective analysis of microbiological and cytological results from 2008 – 2012 in South Africa, an algorithm used *S. aureus* prevalence for a *SCC* ≤200 000cells/ml from $[1.495; 1.595]_{90\%}$ and for SCC >200 000cells/ml from $[6.72; 6.95]_{90\%}$. A second model using the same SCC groups explored a 5 to 25% *S. aureus* herd prevalence. Epidemiological models have been developed to calculate persistant, new infections and clinical *S. aureus* cases, incorporating probability of cure of 3 or 8day treatments, spontaneous cure, persistant and new infections for different levels of management.

Results: In the economic model, investigating a low prevalence, the best cost index of -644 ZAR $[-875; -382]_{90\%}$, resulted when no subclinical *S. aureus* IMI were treated. In the model simulating a high *S. aureus* prevalence and good management a less costly scenario was to treat for 8d, only positive animals with the higher SCC resulting in a cost index of -730 ZAR $[-938; -474]_{90\%.}$ In this higher *S. aureus* prevalence situation, all treatment scenarios proved to be more cost effective than when no treatment was used.

Treatment of subclinical *S. aureus* IMI in herds with good management did not prove beneficial in the epidemiological model. A major benefit was seen in high risk herds where new IMI was reduced by 71% in one scenario and 91% in another in the 3 and 8d treatments

scenarios. There was also a reduction of 2 to 49% in treatment numbers compared to the no treatment group (only clinical cases were treated). When results of economic and epidemiological models were combined the best financial option did not always have the most favourable epidemiological outcome.

Conclusions: Stochastic cost/benefit models combined with epidemiological models may have many practical applications that can assist both producers and veterinarians in optimising decisions balancing cost/benefit with end point IMI dynamics. The key beneficial factor however remains the level of management.

ADDENDUM 5.6 (Paper presented at ISVEE Conference)

Paper presented at the ISVEE Conference, Yucatau, Mexico November 3-7, 2015.

Financial cost/benefit when treating subclinical *Staphylococcus aureus* intramammary infections considering micro-cytological scenarios: a stochastic approach.

ABSTRACT

Purpose: An innovative method was investigated to aid in the elimination of *Staphylococcus aureus* (*S. aureus*) intramammary infections (IMI) from dairy herds in South Africa. Stochastic models were used to explore cost/benefit of 3 or 8-d treatment of subclinical *S. aureus* IMI in all infected cows and in only those with a somatic cell count (SCC) above 200 000 cells/ml compared to no treatment.

Methods: The model included direct cost such as additional laboratory fees for performing microbiology and indirect effects of treatment on cure and new infection rate. Herds with low and high transmission rates were incorporated into the simulation. A first model used *S. aureus* prevalence provided by retrospective analysis of micro-cytological results (2008-2012) i.e. 3.93% [3.88; 3.99]$_{90\%}$. A second model explored existing severe situations with a higher prevalence of *S. aureus* in a herd varying from 5 to 25% according to the SCC. To compare the different strategies we used a cost-index resulting for one strategy of the sum of the products of the probability of each possible event by its cost, balanced with the reduction of the IMI. These probabilities and costs were calculated for one animal in a herd over a lactation period of 260 days.

Results: In the case of 3.93% prevalence, the best scenario, with a cost index of -644 ZAR [-875; -382]$_{90\%}$, is to treat 8d only animals with higher SCC. In case of no teat dip the 3d treatment scenario is even cheaper than no treatment at all. In the high prevalence model the best scenario, with a cost index of -730 ZAR [-938; -474]$_{90\%}$, is to treat 8d only animals with higher SCC if teat dip is used and with a cost index of -765 ZAR [-975; -515]$_{90\%}$ if teat dip is not used. In this model all treatment scenarios proved cheaper than no treatment.

Conclusions: Such models have many practical applications that can help with decision making both producers and veterinarians.

Relevance: The level of *S. aureus* IMI that was likely to be missed when using a SCC threshold was based on the retrospective study which indicated that 20.57% and 33.23% of *S. aureus* IMI went undetected at 200 000cells/ml in quarter and composite cow milk samples respectively.

ADDENDUM 5.7 (Poster presented at WBC Conference)

A poster presented at the World Buiatrics Conference, Dublin, Ireland (5-8 July 2016).

A model approach to estimate *Staphylococcus aureus* intramammary infection dynamics for different scenarios when vaccinating for mastitis.

ADDENDUM 5.8

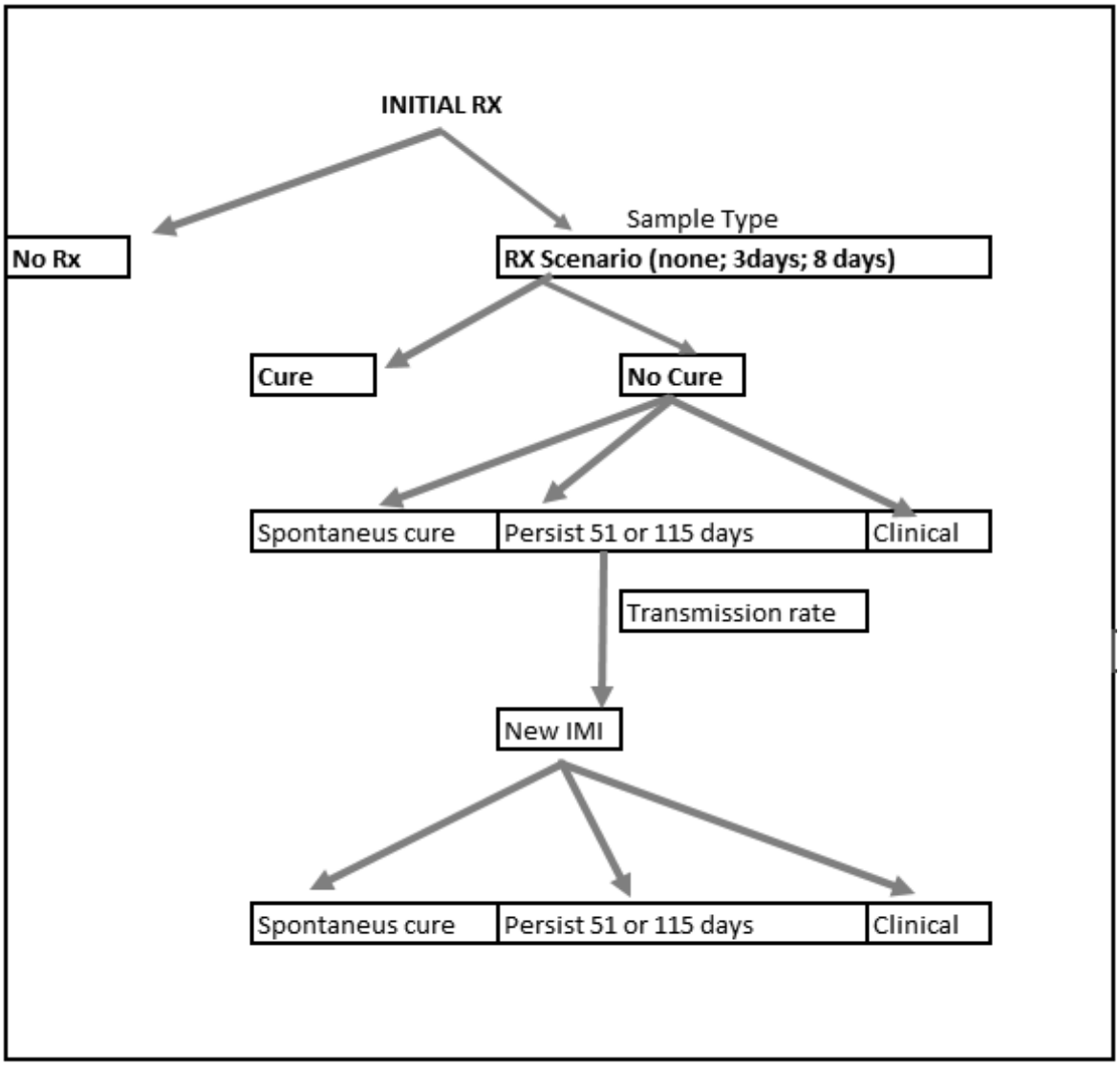

Figure 5.4. Schematic representation of the principle used in a first cycle on which the epidemiological models were based upon.

CHAPTER 6

THESIS SUMMARY

Pro-active dairy herd management decisions based on micro-biology and cytology of milk samples.

A dedicated udder health diagnostic program has been developed and used over a 15 year period in South Africa. This program is used to analyse milk samples of dairy herds based on microbiological and cytological results of individual cows. This is a fresh approach as very few countries worldwide can perform micro-cytology on a complete lactating herd on a relatively regular basis. This program with a current dataset of 1.5 million samples makes a detailed analysis possible and helps to identify and provide insight into possible key sources of udder health problems on farms. The aim of this manuscript is to share the information and experience that was acquired over the years with veterinarians and udder health consultants. This knowledge enables a detailed pro-active udder health approach based on knowledge of pathogens isolated from individual cows or quarters in the complete lactation herd. Results are presented as various group reports indicating trends within herds, cases studies and also providing analysis of the individual cow or quarters.

There are many advantages of having species specific IMI information about udder health in the current Milk Sample Diagnostic (MSD) system. It allows early detection of IMI, rapid follow-up on information from tests; there is a short turnaround time after the receipting of milk samples, and prompt communication of results to herd managers and owners. The program firstly allows evaluation of the herd udder health situation enabling the consultant to identify the main causes of udder health problems in detail. This will assist in identifying and eliminating the sources of the problems timeously. At the same time it provides information on each cow on parity, lactation stage, pregnancy status, production level, and mastitis and SCC history, to enable informed decisions for individual cows and even individual udder quarters. Management decisions can be based on sound information and cows that are cured, or have persistent IMI and new IMI can be identified, based on actual bacterial identification. This improves the accuracy of decisions made.

Somatic cell count thresholds as indicators of bovine intra mammary infection status in quarter and composite milk samples.

Somatic cell count (SCC) as indicator of udder health status in quarter milk samples has been well researched and reported on for many decades, because it is a cost effective technique that is easy to use. Although researchers agree that it remains an useful indicator of the udder health status in cows they do not agree on the SCC threshold indicating intramammary infections (IMI). There are currently no international guidelines for a SCC threshold indicating IMI in composite milk samples. The number of lactating cows per herd in South Africa has increased causing effective monitoring of the udder health status of the individual cow to become more challenging. In South Africa routine udder health examinations of quarter milk samples have been replaced by composite milk samples which are more practical and less costly to analyse. Due to the dilution effect of combining samples from the four udder quarters into one sample results of these samples, the analyses of these samples are currently difficult to interpret as a single test under field conditions. Some South African milk laboratories culture milk samples only with SCC above a chosen SCC thresholds. It is of importance to know what percentage of IMI in general and infections with contagious udder pathogens would be missed at those SCC thresholds. This information can be of key importance to the veterinary practitioner and the manager alike for wise pro-active udder health management decisions, both at herd and individual cow level.

In this first of two manuscripts utilizing 345 461 composite and 89 638 quarter samples from dairy herds in 7 of 9 South African the main objective was to establish a SCC threshold for composite milk samples that could be used for initial screening for IMI.

This large dataset used in this study was a reflection of samples received daily by laboratories that needed analysis and interpretation. Samples are received for herds that differ in management levels, exposure to environmental conditions, microbial IMI and cow factors (age, parity, days in milk and milk yield). The SCC test was found to be sufficient but not excellent as an indicator of IMI in both sample types. This study reconfirmed a threshold for SCC of 200 000 cell/ml use to detect IMI in quarter milk samples to be optimal. Based on the findings of this study a SCC threshold of 150 000 cells/ml milk can be recommended for use in composite milk samples.

It should be noted that these optimal statistically determined SCC threshold levels need to be adapted and interpreted carefully depending on operational circumstances. Even at these “optimal” SCC levels, using the SCC as the only test to predict the presence of IMI is not ideal. Somatic cell count thresholds based on pathogen specific infections in quarters and udders and management levels will still need to be determined to assist in decision making and pro-active management in operational conditions.

Pathogen specific bovine intramammary infections: The validity of somatic cell count threshold as an indicator in quarter and composite milk samples.

The pro-active approach necessary in the management of udder health in dairy herds requires knowledge of the most prominent udder pathogen. It is no longer enough to merely know whether this is primarily a contagious (host adopted) or environmental organism, because prevention strategies can differ substantially between these groups. This study was aimed at assisting with the effective (both cost effective and accurate) diagnosis of intramammary infections in lactating cows. Although the IDF recommend that cows with somatic cell counts (SCC) higher than 200 000 cells/ml are more likely to come from quarter of cows with intramammary infections (IMI), than from uninfected quarters, no mention is made of the probable difference between the major and udder pathogens.

In case of outbreaks for instance of *Streptococcus agalactiae,* consultants need to know what percentage of cows that could be mis-diagnosed when only samples with SCC of above 200 000 cells/ml is cultured. With this knowledge a decision can be made whether or not all cows should be sampled. This together with the estimated 15% production lost in a lactation, will allow a more informed decision to be made.

In this research utilizing 345 461 composite and 89 638 quarter samples from dairy herds in 7 of 9 South African provinces the main objective was to establish a SCC threshold for composite milk samples that could be used for initial screening for IMI.

Although most major udder pathogens and just over half of minor udder pathogen groups were isolated at a SCC of 200 000 cells/mL in both quarter and composite milk samples, the percentages of pathogen species specifically identified within these groups differed considerably. At the selected SCC thresholds of 200 000 cells/mL for quarter and 150 000 cells/mL in composite samples, the bacterial species isolated at the highest percentages were *Str. dysgalactiae, Str. agalactiae* and *Str. uberis.* Less pathogens were isolated and a lower sensitivity of the SCC test was seen for composite milk samples at a SCC threshold of 150 000 cells/mL than for quarter samples. Results for composite samples at 150 000 cells/ml compared favourably with those for quarter milk samples at 200 000 cells/mL.

This research indicated in the case of sixteen contribute udder pathogens, that SCC alone may either to under- or over diagnose pathogen specific IMI in both quarter and composite milk samples. At a SCC threshold of 200 000 cells/ mL 20.48% and 38.70% of *S. aureus* IMI could be expected not to be identified unless cultured from quarter and composite milk samples respectively.

The effects of days in lactation, parity, milk yield, management level and prevalence of pathogen specific IMI on SCC thresholds were not measured in this research and need to be further investigated.

Cost benefit analysis when treating subclinical *Staphylococcus aureus* intramammary infections considering micro-cytological scenarios: a stochastic approach.

Antimicrobial resistance in bacteria is currently perceived to be a critical and urgent topic. This study is a fresh approach towards this challenge. The aims for developing the models were: firstly to provide a consultant, veterinarian or farmer with a tool to base decisions on current farm information in order to predict future events accurately, so that measures could be taken to prevent disease before it occurs; secondly we wanted to predict cost effectiveness of management changes in combination with an indication of the likely epidemiological outcome of the *Staphylococcus aureus* population in herds. The availability of advances in farm technology and data processing capabilities resulting in scientific predictions is making this approach a reality.

Predictions were based on the combination of financial cost benefit but included also predictions of probable epidemiological outcomes such as the number of clinical mastitis cases, new intramammary infections, persistant cases likely to occur and number of subclinical *Staphylococcus aureus* cases that would require treatment for the period chosen. The study further compared these outcomes for different initial herd prevalence of *Staphylococcus* intramammary infections, different management levels and varying duration of intramammary treatments. When results of economic and epidemiological models were compared, the best financial option differed in some scenarios. Such models may assist producers, consultants and veterinarians in optimising decisions balancing cost/benefit with end point IMI dynamics. One of the main findings was to treat more effectively initially in order to treat less.

We believe that the models can make a positive contribution to enhance current herd udder health decisions by providing more accurate predictions of outcome of inventions such as the treatment of subclinical *Staphylococcus aureus*.

Models can be an innovative approach for simulating epidemiological and economical situations and outcomes combine to aid in better decision making. In this study models were used to compare additional costs of culturing all milk samples to culturing only those with SCC >200 000 cells/ml, as well as comparing different treatment durations and management inventions, and the results on the economic and epidemiological outcome. Costs vary between countries and laboratories and this will affect the economic predictions. Even with treatment of only 79% of *S. aureus* cases in high risk herds distinct advantages were evident when subclinical cases were treated.

Combining financial and epidemiological outcomes in the models indicated that in the low prevalence of *S. aureus* in a herd combined with a low transmission risk (LPLT), low prevalence - high transmission risk (LPHT) and high prevalence - low transmission risk (HPLT) scenarios the best economic solution also proved to be the best for the epidemiological outcome *S.*

aureus. With the high prevalence - high transmission risk (HPHT) a the second most economical scenario proved to be the better option for the epidemiological outcome with 11 less new IMI, 4 less clinical and 5 less persistent cases over the period investigated.

Global concern and new legislation in some countries regarding microbial resistance to antibiotics is currently evident. Opting to treat subclinical *S. aureus* may therefore seem at first a contradictory decision to prevent growing antimicrobial resistance. However, when subclinical *S. aureus* IMI were treated fewer cows in total were treated for the 255 day period in all treatment groups compared to those where no subclinical cases were treated. This was due to the larger number of clinical cases that developed and required treatment later on. Treating more infected cows initially did result in treating less overall. Not only were fewer cows treated but by treating subclinical cases the probability of bacterial cure was likely to be better than in clinical mastitis cases and less parenchyma damage could be expected. In the treated groups the number of new IMI was distinctly lower. Persistent cases in the case of 8-day treatments were not only eliminated earlier from herds, but *S. aureus* IMI were substantially lower in all high risk herds compared to the scenario where subclinical *S. aureus* IMI were not treated. In herds with a low risk for new IMI regardless of the initial *S. aureus* prevalence, it was not advantageous to treat subclinical cases.

The best case scenario remains to create a low risk environment within a *S. aureus* positive herd where the focus will be on excellent management rather than only on treatment. For herds that have not yet achieved acceptable levels of management and mastitis prevention, treatment of subclinical *S. aureus* IMI seems an option to consider with the intention of acquiring a *S. aureus* free herd with the least treatments.

Positive advantages of the study

Dataset

The study was based on a very large data set that was obtained from actual udder health investigations in the commercial dairy industry. A further advantage of the data set is that in most cases all lactating cows in a herd were sampled in order to obtain a complete picture of such a herd. All milk samples were cultured and not only selective samples related to the level of somatic cell counts. This is a privilege few researchers have in the field due to the financial burden of extensive milk sample testing.

The data were collected in the different farming systems (Total mixed ration (TMR) to pasture based) as they are experienced in practice under South African conditions and at various levels of management and seasons. Data included outbreak situations and routine monitoring to provide an actual situation the laboratories may be faced with on a daily basis.

Milk Sample Diagnostic (MSD) program

This diagnostic program was developed over several years in practice. It was and is still adapted according to the need for different reports used by consultants and farmers. The collection and use of the information from the MSD program was proven to be effective as numerous outbreaks were dealt with in an effective manner as farmers can testify. Dairy herds were cleared of *Streptococcus agalactiae* from infection rates as high as 35% within 3 to 6 month periods from herds as large as 1200 lactating cows and these herds remained clean from this bacteria for more than 10 years now..

Staphylococcus aureus IMI were completely eliminated from many herds in South Africa even though reverse zoonosis poses a substantial additional challenge in our country. Data that became available indicate additional challenges such as shifts in the mastitis bacteria isolated from pasture based herds where an increase in *Streptococcus uberis* was noted, and in well managed TMR herds Gram negative bacteria increased. Not only were shifts in the bacterial species noted but strain typing is now used for identifying the prominent and dangerous strains under the various farming conditions and for individual dairy farms.

Experience based on case studies

Milk samples were collected from more than 900 commercial herds in South Africa where farmers paid for the samples and the University of Pretoria subsidised the analyses of samples to build up a data base that could be available for research and student training.

It is the opinion of the author that information obtained in this study can be applied in practice to assist future decisions. We have now a better understanding how regarding the use somatic cell counts (SCC) thresholds in composite milk samples as a survey tool. The threshold of SCC levels above which milk samples could be cultured can now be selected per herd, based on the dominant mastitis pathogen in that herd. The goal of the dairy producer (eradication of contagious bacteria from the herd or not) could also be taken into account.

The models provide us now with a different perspective to assist in decisions making. They provided insight into the likely scenario of the epidemiological outcome and over different time periods. These models can also be used to calculate a reasonable time periods after implementations of actions such as mastitis vaccinations to obtain the true benefits. Producers will be able to decide whether the treatment or vaccination will be economically viable in their herds and for what period this treatment needs to be used as a tool, without neglecting good management practices, to obtain optimal results.

Limitations and weakness in the research

The large data set is also a limitation obscuring some findings. Information on days in milk (DIM), milk yield and parity was not available for further detailed investigations at the time of this study.

Inter-herd variation was not considered and although inter-quarter (variation between quarters of the same cow) was calculated, the effect of the large data set may have influenced results.

Although the best information obtained was used and great number of aspects were taken into account in the analyses of the models, some assumptions were also made. For example: when taking milk loss into account it was assumed by the model that all animals calved at day 1 (to be able to follow a lactation curve). More aspects could have been taken into consideration in models but we had to select those that we believed at the time that were the most important, those that had practical implications for an increase complexity of the models might not result in any increased practical benefit.

Future research

Effective the SCC threshold can be sought for different functional groups according to parity, DIM, yield, inter quarter, clustering effect in composite milk samples and quarter milk samples.

Models developed in this study for *S. aureus* could be extended to incorporate more criteria and new similar models could be developed for other mastitis pathogens such as *Streptococcus agalactiae*.

More research is needed to determine more effective transmission factors to bring the outcomes of the models closer to reality. The effect of excellent dairy herd management should be qualified.

As knowledge become available we know that comparisons on a species level is not sufficient for bacteria such as coagulase negative staphylococci (CNS) and *Streptococcus uberis*. We need to know which strains are contagious, opportunistic and environmental and to what level the strains increase somatic cell count in cows.