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EFFICACY OF DIFFERENT DRY-COW INTRAMAMMARY ANTIMICROBIAL
PRODUCTS ON THE PREVALENCE OF MASTITIS IN A HIGH-
PRODUCING DAIRY HERD.

by

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DEDICATIONS

I would like to dedicate this dissertation to my mother, Anni and my late father Hendrik Petzer for the way they always guided and encouraged me with love and by example throughout my life, to my husband Andrew for his unselfish love, his silent strength, for keeping me motivated and for his never ending patience and to dr Werner Giesecke for being my mentor and a valued friend.

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ABBREVIATIONS

AI:	Artificial insemination
ACR:	Automatic cluster removers
BCS:	Body condition score
BMCC:	Bulk milk somatic cell count
Bova:	Bovaclox DC
BSA:	Blood serum albumin
BST:	Bovine somatotropin
BTA:	Blood tryptose agar
Cep:	Cepravin Dry Cow
CMCT:	California milk cell test
CNS:	Coagulase negative staphylococcus
DHIA:	Dairy Herd Improvement Association
Dis:	Dispolac Dry Cow
DNA:	Deoxynucleotidic acid
ECO:	<i>Escherichia coli</i>
EMDA:	The European Agency for the Evaluation of Medicinal Products
Fe:	Iron
FIL:	Feedback inhibitor of lactation
GIT:	Gastro-intestinal tract
IMI:	Intramammary infections
LH:	Luteolizing hormone
MICr:	<i>Micrococcus</i> spp.
MIC:	Minimum inhibitory concentration
M-index:	Mastitis resistance index
mm:	millimetre
ml:	millilitre
Mo:	Molybdenum
MUN:	Milk urea nitrogen
N:	Nitrogen
Naf:	Nafpenzal DC
NAGase:	N-acetyl- β -D-glucosaminidase
Orb:	Orbenin Extra DC
PD:	Pregnancy diagnosis
PGF _{2α} :	Prostaglandin F _{2α}
PMN:	Polymorphonuclear neutrophils
ppm:	Parts per million
PTA:	Predicted transmitting ability
Rx:	Treatment

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Ril:	Rilexine 500 DC
S:	Sulphur
SAG:	<i>Streptococcus agalactiae</i>
SCC:	Somatic cell count
SDY:	<i>Streptococcus dysgalactiae</i>
SFA:	<i>Enterococcus faecalis</i>
spp:	Species
SPC:	Standard bacterial plate count
STA:	<i>Staphylococcus aureus</i>
SUB:	<i>Streptococcus uberis</i>
TCI:	Teat canal infection
TCS:	Teat canal score
TMR:	Total mixed ration
Zn:	Zinc

CHAPTER 1 : INTRODUCTION AND OBJECTIVES

1.1 Introduction

The importance of the dry period with respect to udder health, productivity, overall health and fertility performance in the next lactation has been widely documented ([Leslie, 1994](#); [Enevoldsen and Sorensen, 1992](#); [Sorensen and Enevoldsen, 1991](#); [Eberhart, 1986](#); [Natzke, 1981](#); [Neave, Dodd, Henriques, 1950](#)). The dry period is a period of anatomical and physiological change for many body systems including the mammary gland.

In the absence of effective mastitis prevention and control measures during the dry period, more quarters of the udder will be infected at calving in comparison with the number infected at drying off ([Dingwell, Kelton and Leslie, 2003](#)). Mastitis is considered the most costly disease of dairy cows ([Fetrow, Stewart, Eicker, 2000](#); [DeGraves and Fetrow, 1993](#); [Hoblet, Schnitkey, Arbaugh, 1991](#); [Miller and Bartlett, 1991](#)) affecting both the quality and quantity of milk. Studies have shown that 7% of cows are responsible for 40% of clinical mastitis cases in a herd and that 6% of cows are responsible for 50% of all discarded milk ([Nickerson, 2001](#)).

One of the main factors that influence the manifestation of clinical mastitis in the next lactation is intra-mammary infection (IMI) which develops during or persists through the dry period ([Bradley and Green, 2000](#); [Todhunter, Smith, Hogan, 1995](#); [Oliver, 1988](#); [Smith, Todhunter, Schoenberger, 1985](#); [Oliver and Mitchell, 1983](#)). Most new IMI develop towards the end of lactation, during the initial three weeks after drying off and during the final stages of the dry period ([Schultze, 1983](#); [Natzke, 1981](#); [Bramley, Dodd and Griffen, 1981](#)). The change in the prevalence of IMI at the beginning of lactation is a result of failure to eliminate existing and prevent new IMI during the dry period ([Eberhart, 1986](#)).

The goal of the dry period is to have as few udder quarters infected in the next lactation as possible and to ensure optimum production of milk with a low somatic cell count ([Eberhart, 1986](#)). This goal is achieved through the elimination of existing infections and the prevention of new IMI. Administration of dry-cow antibiotic therapy at the end of lactations is at present the most effective means to eliminate existing IMI and to prevent new ones ([Eberhart, 1986](#)). Treatment during the dry period is said to be almost twice as effective as during lactation. However, by placing emphasis on prevention of new infections, udder health could be achieved more rapidly ([Eberhart, 1986](#)) as new IMI can have a significant impact on milk production in the next lactation ([National Mastitis Council, 1999](#)). It therefore relies on an understanding of both the epidemiology of bovine mastitis and the factors affecting the cow's and the udder's susceptibility to mastitogenic pathogens.

The rate of new IMI is known to be influenced by various factors including challenge by micro-organisms, cow factors and environmental factors. Besides dry cow therapy, proper management of dry-cows forms an extremely important part of an udder health control program. The mammary gland requires a rest period prior to an impending parturition, in order to optimize milk production in the next lactation. The dry period is critically related to the dynamics of IMI within a dairy herd. New IMI picked up during the dry period contribute largely to the increase in the number of infected quarters that occur with each successive lactation of the cow. It was found ([Browning, Mein, Brightling, Nicolls and Barton, 1994](#)) that cows infected in a previous lactation and not effectively treated during the dry period could contribute to more than 76% of infections at calving and nearly 70% at mid-lactation. It can be said that dry-cow therapy, when practised correctly in conjunction with post-milking teat disinfection, constitutes one of the most significant advances yet achieved in mastitis control.

1.2 Objectives

The objectives of this study were to:

- Compare the effectiveness of six different intra-mammary products to cure IMI and prevent new IMI during the dry period.
- Assess the cure-rate and new IMI during the dry period in dairy cattle based on the micro-organisms isolated.
- Assess the influence of cow factors on the efficacy of cure of IMI and prevention of new IMI during the dry period in dairy cattle.
- Assess the effect rainfall on the efficacy of cure-rate and prevention of new IMI during the dry period.
- Assess the influence of dry cow antibiotic treatment on SCC during early lactation.

CHAPTER 2 : LITERATURE REVIEW

Mastitis is a multifactorial disease and generally results from an interaction between a variety of microbial infections, host and management (environment) factors. The risk of mastitis depends on how well the defence mechanisms of the dairy cow can adjust to the challenge from the environment and microbes. From the point of view of mastitis control, most new IMI occur during the dry period ([Natzke,1981](#), [Schultze,1983](#), [Radostits, Gay, Blood and Hinchcliff,2000](#)) and cows with a history of mastitis in the previous lactation are twice as likely to develop mastitis in the following lactation ([Watts and Washburn, 1991](#)). The dry period is eminently suitable for intra-mammary treatments. For the purpose of this dissertation current knowledge regarding the elimination of existing IMI and the prevention of new IMI during the dry period of a cow's lactation cycle will be summarised.

2.1 Micro-organisms most frequently associated with mastitis

Bacteria are the most common cause of mastitis in dairy cows. Reports indicate that more than 137 microbes are incriminated as aetiological agents of mastitis ([Watts,1988](#)).

The microbial causes of mastitis include a wide variety of micro-organisms (aerobic and anaerobic bacteria, mycoplasmas, yeasts and fungi). The most important micro-organisms of bovine mastitis are streptococci, staphylococci, *Escherichia coli* and other coliforms ([Radostits et al.,2000](#); [Giesecke, Du Preez and Petzer,1994](#); [Quinn, Carter, Markey, and Carter,1994](#)). The degree of importance of a specific agent, as a cause of mastitis in dairy cows, is largely dependent on the nature of the organisms, the pathogenicity of the agent, the challenge dose required to cause infection, and is influenced by management practices. Because most pathogens involved in mastitis are ubiquitous, mastitis can be managed but not eradicated.

Common mammary pathogens grow well in milk. This generally requires them to be able to use lactose as a source of carbon and have sufficient proteolytic activity to ensure an adequate supply of nitrogen (N) for the hydrolysis of casein. Once inflammation has developed, leakage of plasma into the milk and increased proteolytic activity in the milk produce compositional changes, which per se are likely to stimulate bacterial growth by providing readily available N. The vitamin requirements of bacteria may be a factor in their growth in milk and therefore in pathogenicity, particularly if vitamins required are unavailable for them because such vitamins are associated with binding of proteins in milk ([Bradley and Dodd,1984](#)).

From an epidemiological viewpoint there are two main sources of mastitogenic pathogens namely contagious or environmental pathogens. This classification is based

on the usual source and means of spread of the organisms involved. The two groups are further sub-divided into major and minor pathogens.

2.1.1 Contagious pathogens

The main source of contagious pathogens is infected udder quarters. Spreading occurs from diseased quarters to healthy quarters in cows. Programmes for the control of contagious mastitis involve the improvement in hygiene and disinfection aimed at disrupting the cow-to-cow mode of transmission. Contagious organisms found during the dry period of a dairy cow are mainly due to persistent infections not cured during lactation.

2.1.1.1 Major pathogens

Major pathogens mainly cause clinical mastitis and include micro-organisms such as *Staphylococcus aureus*, *Streptococcus agalactiae* and *Mycoplasma bovis*.

- *Staphylococcus aureus* (STA)

Staphylococcus aureus are Gram-positive cocci, non-motile, non-sporeforming, facultative anaerobes and catalase positive. STA are commensal bacterins of mammals and humans which most commonly occur on the skin and nasopharynx, but may also be present in the alimentary and genital tracts. It is a potential pathogen and may cause a range of pyogenic conditions, including mastitis.

More than 95% of the sub-clinical and 60% of clinical cases of mastitis in Nordic countries were caused by Gram-positive cocci ([Sandholm, Hokanen-Buzalski, Kaartinen and Pyörälä, 1995](#)). Of these, the most common pathogen was *Staphylococcus aureus* (STA) which was responsible for 30-40% of sub-clinical and 20-30% of clinical cases of mastitis. A survey of Danish herds found that 21-70% of all cows and 5-35% of all quarters were infected with STA ([Aarestrup, Dangler and Sordillo, 1995](#)). STA still remains the most problematic and significant of the bovine mastitogenic pathogens ([Sandholm et al., 1995](#)).

The important source of STA within a herd is chronically infected mammary glands, colonized teat ducts and teat lesions (Roberson, 1999; [Sandholm et al., 1995](#); [Bramley and Dodd, 1984](#); [Giesecke et al., 1994](#)). In recent years, an alarming number of *Staphylococcus aureus* of human origin have been isolated from mastitis cases in South Africa (Petzer, unpublished data). For an actual

infection to be initiated, lowered resistance of some kind is needed. This can be due to changes in environmental temperature, viral infections or epithelial injury ([Sandholm et al.,1995](#)). Faulty milking techniques can thus encourage the transfer of STA into the teat cistern, especially when the teat canal is eroded. STA is transmitted via milkers' hands, communal udder cloths, residual milk in teat cups, and soiled milking equipment during the milking process ([Calvinho, Almeida, and Oliver,1998](#)). STA infections can occur at all stages of lactation, but clinical mastitis is more common during drying off. Once the bacteria adhere to the milk fat inside the udder it can float upwards deeper into the parenchyme tissue of the udder.

STA have the ability to avoid phagocytosis by producing a polysaccharide containing mucus around itself causing the phagocyte not to recognize it. It is further shielded from the body's defences by living intra-cellularly. The extra-cellular defence mechanisms of the host cannot attack intra-cellular organisms and the lower intra-cellular pH reduces the efficacy of many antimicrobial drugs used for treatment of mastitis. Unlike most bacteria STA can resist phagocytosis and can even multiply inside a phagocyte. It also uses the phagocyte as a vehicle to carry it deeper into the udder tissue. When the phagocyte dies the STA is released and it colonizes deep in the udder parenchyme. STA can also survive without a cell membrane in the so-called L-form, which makes it relatively resistant to antibiotics ([Sandholm et al.,1995](#); [Anderson, 1976](#)).

Certain strains of STA may produce enzymes like coagulase, deoxyribonuclease (DNase), hyaluronidase, fibrinolysin, lipase and protease. Enzymes produced by STA destroy oxygen radicals and protect the bacteria against oxidizing agents such as lactoperoxidase, one of the humoral defence mechanisms of the udder. The presence of coagulase and DNase correlates positively with the virulence of the bacteria and is used for identification purposes.

Various toxins are produced by STA such as alpha, beta, gamma and delta-haemolysin, leucocidin and enterotoxin. Of these the most destructive being α -haemolysin which can lead to gangrenous mastitis, which can be fatal to the cow ([Sandholm et al.,1995](#); [Anderson, 1976](#)).

STA is highly contagious compared to common environmental pathogens. It presents itself either as a rare per-acute form, acute mastitis, but most often as chronic mastitis. After STA enters the teat canal the organisms penetrate epithelial cells and move intra-cellularly. By the time clinical mastitis is detected, its logarithmic growth phase is usually complete ([Bramley and Dodd,.1984](#)). Alveoli

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involute and become surrounded by fibrous capsules. Inside the capsule many neutrophils, granulocytes and bacteria can be found. Small to bigger necrotic foci form and in time develop into abscesses. Acute flare-ups of clinical mastitis are seen and mastitis often varies between clinical and subclinical forms when organisms are released intermittently from these sites. Chronic carrier cows with fibrosis of the udder can often be identified by palpation of the freshly milked-out udder.

Cure rates of STA mastitis varying from less than 15% in chronic cases to 35% were reported by researchers (Pyörälä, 2002; [Sandholm et al.,1995](#); [Jarp, Bugge and Larsen, 1989](#); [Owens, Watts, Boddie and Nickerson, 1988](#); [Wilson,1980](#)).

- *Streptococcus agalactiae* (SAG):

Streptococcus agalactiae are Gram-positive cocci, cytochrome-negative, facultative anaerobic organisms and belong to the group of pyogenic, haemolytic streptococci and serologically to Lancefield's group B, although the antibodies towards the G-group may also give positive reactions. Streptococci are homolactic in their metabolism and their growth results in a reduction of pH. SAG is an obligate udder pathogen and is highly contagious and transmission usually occurs during milking.

SAG is a major source of mastitis in dairy herds without an effective control programme and seems to have an increasing prevalence in South Africa (Petzer, unpublished data). Prevalence of infections is 10-50% of cows and 25% of quarters. A prevalence of less than 10% of cows in herds with effective control programmes has been reported ([Radostits et al.,2000](#)).

The main source of infection is from the udders of infected cows in the dairy. Hands of milkers, floors, utensils and cloths are often heavily contaminated. Extra-mammary sources of SAG infection could be lesions on teats, but have been reported as being insignificant ([Bramley and Dodd,1984](#)) in bovines. The bacteria may persist on hair, skin and inanimate material (such as bricks) for up to 3 weeks ([Radostits et al.,2000](#)). SAG outbreaks recently occurred in closed herds in South Africa that were previously SAG negative (Petzer, unpublished data). In a recent study, SAG was isolated from 30% of asymptomatic human carriers ([Narayanan and Ossiani, 2001](#)).

The mechanism by which SAG penetrates the teat canal is influenced more by the diameter of the teat canal lumen than by its length ([Radostits et al.,2000](#)). Once in the udder, SAG has the ability to adhere to parenchyme tissue and the micro-environment of the udder is necessary for the growth of the organism. SAG

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remains a superficial infection that localises on the surfaces of mucous membranes and in the lactiferous ducts and can penetrate the duct wall into lymphatic vessels and the supramammary lymph nodes. There is considerable variation between cows in the development of SAG IMI. It is not clear why, but the integrity of the teat cistern lining is thought to play a major role. The virulence of various strains is related to differences in their ability to adhere to mammary epithelium ([Radostits et al.,2000](#)). SAG infection occurs mainly in older dairy cows and at the beginning of their lactation ([Radostits et al.,2000](#)).

SAG is sensitive to intra-mammary treatment with a wide variety of antimicrobials in lactating cows, resulting in a high cure rate of up to 90-95%. Blitz therapy (treatment of all positive cows) is commonly used to reduce the prevalence of the infection in a herd. ([Radostits et al.,2000](#); [Sandholm et al.,1995](#)).

- Mycoplasma (MYC)

Mycoplasmas are very small procaryotes totally devoid of cell walls, bound by a plasma membrane only. At least 70 species of mycoplasmas are known, of which at least 5 can cause bovine mastitis, the most important being *M. bovis* and *M. bovis genitalium* ([Sandholm et al.,1995](#)). Mycoplasmas require special growth techniques and media in the laboratory and can easily be missed on the routine examination of milk samples in the laboratory ([Jones,1998](#)).

Mycoplasma mastitis is a problem mainly in the USA, particularly in California ([Bramley and Dodd.,1984](#)), United Kingdom, Canada, Israel and Australia, ([Radostits et al.,2000](#)) but has not yet been reported in South Africa.

Attempts to elucidate the epidemiology of the disease have not been successful. It causes a purulent interstitial mastitis. Although infection probably occurs via the teat canal, the rapid spread of the disease to other quarters of the udder and occasionally joints as well as its presence in heifers milked for the first time suggests that systemic invasion may occur. Mycoplasmas are highly contagious organisms and can cause clinical mastitis of an epidemic magnitude. It is most common in large herds.

Depending on the level of milking hygiene in a herd, as much as 50-60% of cows in a herd can be infected if there is an outbreak of MYC mastitis. Cows of all ages and stages of lactation are at risk but those early in lactation are most severely affected. Mycoplasma mastitis causes a sharp drop in milk production and swollen but not painful udders. In the early stage of this mastitis milk rapidly separates into a flaky deposit and a clear supernatant when left to stand in a glass tube ([Jones,1998](#);

[Bramley and Dodd, 1984](#)). Affected cows have a very high somatic cell count and infections tend to become chronic. Therapy is usually ineffective and culling of the infected animals prevents the spread of the disease.

2.1.1.2 Minor pathogens

Several species of bacteria are often found colonising the teat canal and rarely cause clinical mastitis. Minor pathogens have been credited with maintaining a higher than normal SCC and with increasing the resistance of the colonised quarter to invasion by a major pathogen ([Radostiits et al., 2000](#); [Jones, 1998](#); [Smith and Hogan, 1995](#); [Nickerson and Boddie, 1994](#); [Rainard and Poutrel, 1988](#)). Low rates of IMI with major pathogens may be observed in the presence of *Corynebacterium bovis*. However, IMI with coagulase negative *Staphylococcus* spp. (CNS) may be considered an indicator of cows at risk for IMI with major pathogens ([Radostiits et al., 2000](#)). Nickerson et al ([1994](#)) found that quarters infected with CNS and *Corynebacterium bovis* are more susceptible to SAG infection.

- Coagulase negative *Staphylococcus* spp. (CNS)

Coagulase negative *Staphylococcus* are Gram-positive cocci, catalase positive and coagulase negative. They include *Staphylococcus hyicus* and *Staphylococcus chromogenes* which are commonly isolated from milk samples and teat canals, *Staphylococcus xylosus* and *Staphylococcus sciuri* that are free living in the environment and *Staphylococcus simulus*, *Staphylococcus warneri* and *Staphylococcus epidermidis* which are part of the normal skin flora.

CNS is now one of the most common bacteria found in milk, especially in herds in which major pathogens have been adequately controlled. They are increasingly responsible for more than 30% of subclinical and 20% of clinical cases ([Radostiits et al., 2000](#)). They are not nearly as pathogenic as STA and are still regarded as opportunistic mastitogenic pathogens. The prevalence of these bacteria is higher in first lactation heifers than cows and higher immediately after calving than in the remainder of the lactation ([Radostiits et al., 2000](#)). They have also been isolated from teat canals in up to 70% nulliparous heifers in herds ([Nickerson, 1996](#); [Radostiits et al., 2000](#)).

A cure-rate of mastitis caused by CNS of 60-80% is reached during lactation and up to 100% during the dry period ([Sandholm et al., 1995](#)). Longterm intensive programmes of teat dipping will markedly reduce the prevalence of minor pathogens. This could however, increase the risk of the herd for IMI with opportunistic environmental agents.

- *Corynebacterium bovis*

Corynebacterium bovis are pleomorphic, Gram-positive rods occurring in angular arrangement. *C. bovis* is also a minor pathogen and the main reservoir is the infected mammary gland and the teat ducts and can induce a mild inflammatory response with increased SCC ([Radostits et al.,2000](#); [Smith and Hogan,1995](#); [Nickerson and Boddie,1994](#)). It is only mildly pathogenic and spreads from cow to cow in the absence of adequate teat dipping. The presence of *C. bovis* will reduce the likelihood of subsequent infection with STA but may increase the risk of infection with SAG and environmental streptococci ([Radostits et al.,2000](#)).

2.1.2 Environmental pathogens

The most important change in the epidemiology of bovine mastitis over the past decade has been the rise in the importance of environmental pathogens causing clinical mastitis, relative to contagious pathogens. Environmental mastitis is caused by bacteria that are transferred from the environment to the cow, rather than from other infected quarters ([Radostits et al.,2000](#)).

Despite significant progress in the control of contagious pathogens, environmental mastitis continues to be a major cause of financial loss in the United Kingdom ([Bradley and Green, 2000](#)). Dairy herds, in which contagious mastitis has been controlled, and which have low somatic cell counts, often have a higher incidence of clinical mastitis caused by environmental pathogens. The prevalence of environmental mastitis in cows infected is usually very low and thus often has very little effect on the bulk milk somatic cell count (BMCC)([Hogan and Smith,1998](#); [Sandholm et al.,1995](#)).

Environmental mastitis refers to infections caused by two groups of pathogens, the coliform bacteria and non-agalactiae environmental streptococcal species. The usual source of these organisms is the environment of the cow. Examples of conditions and situations that will favour the presence of these micro-organisms are over-crowding with zero-grazing systems, poorly designed housing, wet, unhygienic bedding, dirty lots, milking of wet udders, poor udder preparation prior to milking, housing systems that lead to teat injuries and milking machine problems ([Barkema, Schukken, Lam, Beiboer, Wilmink, Benedictus and Brand,1998](#)). Infection occurs mainly during the late dry period and early lactation when the immune system of the cow is challenged ([Smith, Todhunter and Schoeneberger,1985](#)).

Control strategies for environmental mastitis include improved sanitation and proper udder preparation methods so that the teats are clean and dry at milking time. Special attention is needed at drying off, during the late dry period and during early lactation, when cows are most susceptible. These organisms are usually not well controlled by preventive measures such as dry-cow therapy or teat dipping, because they are ubiquitous, are able to survive outside the udder, and cause infection only when given the opportunity (e.g. milking machine faults, low immunity, unhygienic conditions, etc.) ([Radostits et al.,2000](#)).

2.1.2.1 Major pathogens

- Coliform organisms

Coliforms are Gram-negative rod-like, lactose fermenting bacteria belonging to the family *Enterobacteriaceae*. Most of the coliforms causing mastitis belong to the genera *Klebsiella* and *Enterobacter*. They include *Escherichia coli*, *Klebsiella* spp, *Citrobacter* spp, *Enterobacter* spp and *Aerobacter* spp.

Coliforms are mainly found in dairy cattle which are housed and are the most important udder health problem in well managed dairy herds with a low SCC. The quarter infection rate is 2-4%, mainly during early lactation ([Radostits et al.,2000](#)).

Coliforms are natural inhabitants of the colon flora, which spread via faecal contamination of the environment. They are found in manure, polluted water, soil and poorly managed bedding material (sawdust, shavings and straw) and are opportunistic ([Hogan and Smith,1992](#); [Bramley and Dodd.,1984](#)). Risk factors that predispose dairy cows to coliform mastitis include low SCC, contamination of teat canals (wet conditions), teat injuries, Vit. E and selenium deficiency, a suppressed neutrophil function, low lactoferrin and increased citrate levels in the milk of the peripartum cow and cows in early lactation during cold draughty conditions ([Radostits et al.,2000](#); [Barkema et al.,1998](#); [Hogan and Smith,1998](#); [Sandholm et al.,1995](#); [Giesecke et al.,1994](#)). Approximately 20% of clinical mastitis cases in Nordic countries are caused by coliforms of which about 85% are *Escherichia coli* ([Sandholm et al.,1995](#)).

The clinical course of coliform mastitis depends mainly on the response of the host to the IMI, whereas the virulence of bacterial strains seems to be of less importance ([Sandholm et al.,1995](#)). Coliforms do not colonise in the milk ducts or infect teat lesions ([Bramley and Dodd.,1984](#)). During the puerperal period, when the host immune responses are suppressed, bacteria can replicate to high

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numbers prior to the host recognising the IMI. Coliforms usually do not survive long in the udder and the inflammatory response with severe clinical signs is triggered by endotoxin release by the bacteria. It is often not possible to isolate *E. coli* organisms from milk in cases of *E. coli* mastitis ([Jones, 1999](#)) due to the endotoxin release mainly after the death of the organism. The endotoxin is a lipopolysaccharide of the outer membrane of the cell wall of Gram-negative bacteria. The endotoxins consist of lipid-A, a lipopolysaccharide core and O-antigen and they are able to affect many host cells and organs by integrating in the cell membrane. In response the macrophages as well as endothelial cells release a cytokine-type protein mediator. PMN leucocytes are attracted to the site and release various mediators such as proteases, leukotrienes and prostaglandins causing active cell damage in host tissue and pathogens ([Sandholm et al., 1995](#)) however spectrum is continuously changing ([Myllys, 1995](#)).

Most (80-90%) of coliform udder infections result in clinical mastitis of which 8-10% are per-acute. Subclinical IMI with coliforms are uncommon ([Radostits et al., 2000](#); [Bramley and Dodd., 1984](#)). According to American results, 10% of per-acute cases died, 70% dried off and were culled, and 20% remained in milk ([Sandholm et al., 1995](#)).

The dry udder (steady state) contains high levels of lactoferrin which effectively restrict growth of coliforms ([Sandholm et al., 1995](#)).

- Environmental streptococci.

Environmental streptococci are catalase negative Gram-positive cocci. The most common non-agalactiae environmental streptococcus species are *Streptococcus uberis* (SUB), *Streptococcus dysgalactiae* (SDY) and *Enterococcus faecalis* (EFA). SUB and *Streptococcus agalactiae* (SAG) were isolated in the Nordic countries from 20-25% of subclinical and clinical cases ([Sandholm et al., 1995](#)). Other uncommon environmental streptococci involved with IMI are *S. equi*, *S. viridans*, *S. equinus*, *S. pyogenes* and *S. pneumonia* ([Radostits et al. 2000](#)). Recently *S. canis* has been isolated from a well managed, chronically infected herd in South Africa (Petzer, unpublished data).

In countries where STA and SAG intra-mammary infections have been reduced significantly, environmental streptococci have increased markedly. Todhunter et al. ([1995](#)) found the rate of environmental streptococcal IMI 5,5-fold higher during the dry period than during lactation. Resistance of the cow, mainly a healthy teat canal, is critical to the control of environmental streptococci ([Kirk, 1998](#); [Hogan and Smith, 1992](#)).

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Herds that have implemented an efficient programme for mastitis control have found that environmental streptococci do however, still present problems.

- *Streptococcus uberis* (SUB)

Streptococcus uberis belongs to Lancefield's group E, is opportunistic in nature and is found in high numbers in especially straw bedding used for cattle ([Radostits et al.,2000](#); [Hughes,1999](#); [Hogan and Smith,1992](#)) and in silage. Pastures with shade trees and poorly drained soils can also act as a source of SUB for grazing cattle as is seen in New Zealand. SUB is associated with summer mastitis, which affects dry cows and heifers during the summer months. SUB and *A. pyogenes* have been isolated from the common cattle fly *Hydrotaea irritans* which prefers mastitis milk to normal milk ([Sandholm et al.,1995](#)).

SUB is a common cause of IMI during the dry period, with most clinical cases occurring in older cows and during the first month of lactation. Approximately 50% of new SUB IMI occur during the late dry period and 50% during early lactation ([Radostits et al.,2000](#)). The highest rate of new IMI occurs in summer, both in lactating and dry cows. The increased risk prior to calving could be due to loss of the keratin plugs from teat canals, or immunosuppression.

SUB does not have the same ability to adhere to host cells as the other common streptococci that cause mastitis. SUB seldom colonises the teat canal and is probably propelled directly through the teat canal ([Sandholm et al.,1995](#)). The mechanism by which SUB penetrates the teat canal is influenced more by the length of the teat canal than by the diameter of the teat canal lumen ([Radostits et al.,2000](#)). Some strains of SUB have capsules (non-antigenic, consisting of hyaluronic acid), which increase their resistance to phagocytosis ([Sandholm et al.,1995](#)).

Measures such as teat dips and dry-cow therapy are usually ineffective against SUB mastitis ([Kirk,1998](#)). The rate of elimination of SUB through therapy is poor compared to SAG and SDY, but better in comparison to STA ([Bramley and Dodd,1984](#)).

- *Streptococcus dysgalactiae* (SDY):

SDY belongs to Lancefield's group C. and is now regarded as an environmental pathogen. SDY mostly causes acute mastitis at the beginning of lactation. SDY is usually carried in the tonsils and vagina of carrier animals and infections often occur in dry cows and heifers, demonstrating its independence from the milking process ([Bramley and Dodd.,1984](#)). Outbreaks of SDY often follow an increase in

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the incidence of teat lesions and teat canal injuries resulting from pulsation failure, incorrect vacuum and overmilking. SDY infections often occur in dry cows and heifers and it is believed that SDY is one of the initial organisms which cause summer mastitis, seen mainly in heifers and dry cows in Europe ([Radostits et al.,2000](#); [Sandholm et al.,1995](#); [Bramley and Dodd.,1984](#)).

2.1.2.2 Minor pathogens

Several other micro-organisms are included in the environmental class of infections. These organisms are predominantly opportunistic pathogens. They invade the mammary gland when the defence mechanisms are compromised or when they are inadvertently delivered into the gland during intramammary therapy. This group of opportunistic organisms includes organisms such as *Proteus spp.*, *Prototheca spp.*, *Pseudomonas aeruginosa*, yeast agents, *Serratia spp.* and *Nocardia spp.* ([Watts,1988](#)). Each of these agents has unique microbiologic culture characteristics, mechanisms of pathogenesis and clinical outcomes.

These infections usually occur sporadically. However, outbreaks can occur in herds or in an entire region. When many cases occur, it is usually a result of problems with specific management of hygiene or therapy. For example, mastitis caused by *Pseudomonas aeruginosa* has been reported in several herds as an outbreak associated with contaminated water, contaminated antibiotics, teat dips or equipment ([Jones,1998](#); [Watts,1988](#)). The concentration of iodine containing germicides is often too low in the wash lines to eliminate *Pseudomonas spp.* ([Jones,1998](#)).

2.1.3. IMI in replacement heifers

The mammary glands of heifers have traditionally been regarded as uninfected, but the prevalence of IMI may exceed the level of the adult herd. Heifers are most at risk for contracting IMI during the first few months of their life (suckling each other's teats) and between 18 months of age up to calving, especially in dirty, moist conditions with many flies. IMI of heifers prior to calving varied in various investigations from 86,2% to 93% of animals tested and 70,8% to 75% of quarters tested ([Owens, Nickerson, Boddie, Tomita and Ray,2001](#); [Jones and Bailey,1998](#); [Nickerson,1996](#); [Trinidad, Nickerson and Alley,1990](#)). Pathogens isolated included STA (10-37%), CNS (up to 70,8%) environmental streptococci (7%) and coliforms (4%) ([Nickerson,1996](#)). Heifers are thought to be able to contract IMI from dry cows, especially in confined conditions ([Jones and Bailey,1998](#)).

2.2 Physiology of the mammary gland during the dry period

Dairy cows go through many transitions during their production cycle and the lactation and dry period should be biologically viewed as alternating periods of extreme hard work and essential resting. Milk production after calving is 20-25% less than the peak production at 7 - 10 weeks in multiparous and 15% in primiparous cows. At peak production some 20% and more alveoli may be involuted. After the cow reaches peak production, mammary regression and involution escalates gradually ([Giesecke et al., 1994](#); [Wilde and Knight, 1989](#)) to produce approximately 6-10% less in multiparous and 5-6% in primiparous cows per month.

The predominant galactopoietic hormone in ruminants is growth hormone ([Tucker, 1994](#); [Bauman, 1992](#)). Local mammary factors, such as the feedback inhibitor of lactation (FIL), may play a critical role in the maintenance of lactation ([Wilde and Peaker, 1990](#)). Action of the FIL are minimized by the frequent removal of milk and increased by the accumulation of milk in the alveolar cells ([Wilde and Peaker, 1990](#); [Wilde and Knight, 1989](#)). Concurrent pregnancy also influences persistency of the milk yield during the declining phase of lactation ([Bachman, 1982](#)). The mechanism of this effect is not fully understood. However, the timing of inhibition of milk yield in cattle almost coincides with the period of increased placenta-derived plasma estrogen ([Robertson and King 1979](#)). Estrogen may have an effect on the transition of mammary function from a lactating state to a state of involution or rest ([Athie, Bachman, Head, Hayen and Wilcox, 1996](#); [Bachman, 1982](#)). Mammary involution during the dry period is an enhanced extension of this process leading to complete cessation of the lactation function.

Progesterone has no effect on milk yield in the lactating cow because progesterone has a higher affinity for milk fat than for glucocorticoid receptors and there are no progesterone receptors in the mammary gland during lactation ([Hurley](#)).

2.2.1 Involution of the mammary gland

The dry period can be divided into three phases i.e. cessation of milk production leading to a period of regression or active involution, followed by a steady state involution and a period of regeneration or lactogenesis and colostrogenesis. Active involution starts with the cessation of milk removal and is completed by approximately day 30 of the dry period. The period of steady state involution does not have definite beginning and end points and represents the period during which the mammary gland is maintained at the fully involuted state. The length of the periods of active involution, lactogenesis and colostrogenesis are controlled by hormonal and management

factors ([Smith and Hogan,2000](#); [Hurley,1989](#); [Nickerson,1989](#)). The length of the period of steady state involution depends on the total length of the dry period. Regeneration of secretory epithelial cells, selective transport, accumulation of fluid and the onset of copious secretion characterise the period of lactogenesis and colostrogenesis. This period usually starts approximately 15-20 days pre-partum.

The mammary defence mechanism, which is an integral part of the involution process of the mammary gland, will be discussed as a separate entity to involution in this dissertation (see 2.2.3).

2.2.1.1 Cessation of milk production

Termination of milk removal for at least 36 hours leads to mammary regression and the initiation of the process of mammary involution ([Marti, Feng, Alterman and Jaggi,1997](#); [Giesecke et al.,1994](#)). Mammary regression is a physiological programmed, non-inflammatory process, which destroys the milk secretory epithelium through apoptosis to initiate normal mammary involution. Apoptosis may be identified by characteristic morphological changes: nuclear and cytoplasmic condensation, nuclear fragmentation and formation of apoptotic bodies. It is suggested that apoptosis is a normal physiological event of cell suicide in the ruminant mammary gland ([Bryson and Hurley, 2002](#)). This takes place during normal involution, tissue remodeling and as a response to infections or irreparable cell damage ([Wilde, Addey, Li P and Fernig,1997](#); [Schwartzman and Cidlowski,1993](#)). Apoptotic cell death can be seen in the mammary gland within two days of cessation of milk removal. It does, however, not lead to complete degeneration of the tissue structure ([Hurdley,1989](#)). Due to the destruction of the epithelial cells of the mammary alveoli, the function of the secretory alveoli changes from lacteal secretion to the filtration of certain components from the blood ([Giesecke et al.,1994](#)).

2.2.1.2 Active involution (the early dry period)

The increase in intra-alveolar pressure as a result of milk accumulation after drying off is thought to trigger the events of active involution. The mammary gland continues initially to synthesize and secrete milk after the termination of milk removal, which leads to the accumulation of milk in the mammary gland. Studies conducted in cows producing 9-10 kg of milk per day at the time of drying off, showed that mammary glands accumulated 75-80% of their daily yield at drying off. Maximum fluid volume accumulation occurred between 2 and 3 days post drying off ([Noble and](#)

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[Hurley,1999](#)). This is followed by a substantial decrease in fluid volume in the gland between days 3 and 7 of involution and gradually decreases to at least day 16 of the dry period and even up to day 30.

In bovine mammary cells ultra-structural changes associated with involution start within 36-48 hours after termination of milk removal. The most apparent change is the formation of large stasis vacuoles in the epithelial cells ([Holst, Hurley and Nelson,1987](#)). These vacuoles persist for at least 14 days of involution and usually disappear by day 28. The alveolar luminal area declines during the subsequent 2-3 weeks, while the inter-alveolar stroma increases ([Bryson and Hurley, 2002](#)). By day 28 alveolar structures have collapsed and are considerably smaller than during lactation ([Bryson and Hurley, 2002](#)). Limited apoptosis occurs during the initial two days after termination of milk removal ([Holst, Hurley and Nelson,1987](#)).

Throughout the dry period there are marked changes in the composition of mammary secretions and the concentrations of protective factors such as leucocytes, immunoglobulin and lactoferrin. Changes in the milk composition during the early phases of involution indicate rapid changes in the normal mechanisms involved in milk synthesis and secretion. Milk fat, casein, β -lactoglobulin and α -lactalbumin decrease markedly by day 3 to 4 of involution ([Hurley,1989](#); [Nickerson,1989](#); [Rejman, Hurley and Bahr,1989](#)). The concentrations of lactose and citrate, the major regulators of osmolarity of mammary secretions, only start to decrease markedly from day 3 of involution.

The total protein concentration increases during early involution, partly due to water resorption and partly due to the increase in the concentration of lactoferrin, bovine serum albumin (BSA) and immunoglobulins ([Rejman et al.,1989](#)). The concentration of these proteins changes very little during the first 3 days of involution. However, by day 7 the concentration of serum protein (BSA and IgG₁, IgG₂, IgA and IgM) has increased substantially ([Wilde, Addey, Boddy and Peaker.,1995](#); [Smith, Conrad and Porter,1971](#)). This period of marked increase in BSA and IgG reflects an increase in the permeability of the udder cell barrier to passive diffusion of serum proteins. The concentration of BSA during involution never approaches the level found in mammary secretions with acute infections ([Hurley](#)). The latter suggests that the udder barrier is not totally inactive and that it maintains a degree of control, despite the degenerative processes that occur within the tissue during involution.

The concentration of the iron binding protein lactoferrin increases dramatically during initial involution. The major site of secretion of lactoferrin found in bovine mammary secretions is thought to be the secretory epithelial cell, with the polymorphonuclear neutrophil (PMN) making only a minor contribution. However, the concentration of

lactoferrin in normal bovine milk is 0.1-0.3 mg/ml, as opposed to human milk with much higher concentrations of 3-5 mg/ml. The concentration of lactoferrin increases in bovine secretion to 1,4 mg/ml, 5 mg/ml and 20-30 mg/ml at days 3, 7 and 30 respectively of involution, thus becoming a major protein in the non-lactating mammary gland while casein decreases ([Sordillo, Shafer-Weaver and Derosa,1997](#); [Hurley](#)).

NAGase (N-acetyl-β-D-glucosaminidase) is an intracellular lysosomal enzyme and its activity has been implicated as an indicator of tissue damage during mastitis. Its specific function is not known, but it is thought to have an antibacterial activity. In studies done on NAGase isolated from an infected uterus, it inhibited the growth of *Actinomyces pyogenes*, STA, SAG and *Pseudomonas aeruginosa*, but not *Escherichia coli* and *Enterobacter aerogenes* ([Losnedahl, Wang, Aslam, Sixiang and Hurley,1996](#)). NAGase activity is low in mammary secretions in the early dry period, but increases in the mid dry period.

PMN are the dominant cell types for the first 3-7 days of involution. By day 14 the macrophages are the dominant cell type and by day 30, lymphocytes dominate (see defence mechanism).

Table 2.1 Mean concentrations of different components in normal bovine milk during lactation and after cessation of milking.

Components of mammary secretion	Mean values for periods of normal lactation and suspended milking			
	Normal lactation	Suspension of milking		
		Day 1	Day 7/8	Day 14
Glucose (mM)	1,03	0,68	0,13	0,12
Lactose (mM)	126,8	118,3	36,9	51,4
Na ⁺ (mM)	19,0	27,5	93,2	80,5
K ⁺ (mM)	43,4	40,5	17,8	21,0
Whey-proteins (mg/ml)				
Total	8,92	8,73	29,04	20,75
Serum albumin	0,38	0,48	3,57	4,89
Immunoglobulin	1,43	1,51	13,53	8,91
β-Lactoglobulin	5,41	5,24	7,14	4,95
α-Lactoglobulin	1,7	1,5	4,8	2,0
Somatic cell counts (log ¹⁰ /ml)				
PMN	3,53	4,87	6,03	5,95
Lymphocytes	2,36	4,26	5,93	5,99
Epithelial cells	4,73	4,92	4,90	4,58

Adapted from Giesecke et al., 1994

Comalli, Eberhart, Griet and Rothenbacher ([1984](#)) examined microscopic changes in the bovine teat canal during mammary involution. They found a significant temporary dilation of the teat lumen on day 7 of the dry period. The teat canal epithelium atrophies physiologically during the first 30 days of the dry period. This physiological atrophy is mainly due to a reduction in the stratum granulosum and may have

resulted from continuing keratinization forming a functional teat plug. Changes in the stratum granulosum indicates a decrease in the rate of epithelial cell maturation during involution. The mitotic index in the teat canal epithelium is found to decrease significantly between days 0 to 7 of the dry period.

2.2.1.3 Steady state involution

The optimal length of the dry period in dairy cows should be 55 days, with a range of 42-60 days. This results in a process where involution is being completed, just prior to the hormonal initiation of active regeneration of the mammary gland (colostrogenesis and lactogenesis). If the dry period is less than 40 days it may lead to an overlapping of the active involution and regeneration of the gland ([Hurley,1989](#)). The latter can lead to a suboptimal hormonally mediated lactogenic response and a suboptimal milk yield in the ensuing lactation. With a 60-day dry period, the mammary gland of a normal pregnant dairy cow may be maintained in a steady state of involution for only a very short time, thus in practice the gland usually progresses from active involution to active lactogenesis ([Smith and Hogan, 2000](#)).

2.2.2 Regeneration of the mammary gland (colostrogenesis and lactogenesis)

This phase of the dry period marks the transition from the non-lactating state to the lactating state during the late dry period. In contrast to changes that occurred at drying off, these changes are constructive as opposed to the destructive process of active involution. A major function of the mammary gland during this period is the formation of colostrum with its unique feature of high concentrations of immunoglobulin IgG₁ ([Bramley and Dodd,1984](#); [Smith et al.,1971](#)) and nutrients such as fat and casein. With a 60-day dry period an increase in mammary deoxyribonucleic acid (DNA) synthesis starts about 35 days pre-partum ([Capuco, Akers and Smith,1997](#)).

The concentrations of the major components of milk start to increase from two weeks prior to parturition with marked increases in fat, casein and lactose approximately 5 days preceding parturition. The fluid volume in the mammary gland increases gradually over the last 2 weeks of the dry period and then dramatically during days 1-3 pre-partum. The rapid increase in fluid volume is strongly correlated with the dramatic increase in the concentration of citrate. The concentration of lactoferrin decreases from two weeks pre-partum to levels of only 2-3 mg/ml at calving. The citrate-lactoferrin ratio increases by approximately 100-fold during the last few days pre-partum and suggests a loss of antibacterial properties, particularly with regard to coliform bacteria ([Hurley,1989](#)).

The concentration of immunoglobulins starts to increase 3 weeks pre-partum to reach maximum concentration 5-10 days pre-partum. Approximate concentrations of IgG₁, IgG₂, IgA and IgM in colostrum are 60 mg/ml; 2,5 mg/ml; 4,5 mg/ml and 6,0 mg/ml respectively ([Aslam, Jimenez-Flores, Kim and Hurley,1994](#)).

Macrophages become the predominant cell type while there is about a 100-fold decline in the number of lymphocytes, which coincides with the decrease in the concentration of lactoferrin. It supports the hypothesis that iron-binding proteins may be involved in directing lymphocyte traffic into tissues ([Smith and Hogan,2000](#)).The phagocytic ability of macrophages and PMN appears to be inhibited due to numerous fat globules that may limit their phagocytic potential ([Knight and Wilde,1993](#)).

2.2.3. The defence mechanism of the mammary gland, with particular emphasis on the dry period

The age of the cow, udder and teat conformation, teat canal integrity, antibacterial factors, humoral and cell mediated responses play important roles in the defence mechanism of the udder. These factors can be classified as anatomical-mechanical and chemical-cellular systems.

The most common portal of entry into the mammary gland is through the teat canal (galactogenous route). The primary defence mechanism is therefore the teat canal that prevents pathogens from entering the mammary gland, while the secondary defence mechanism comprises several chemical, immunological and cellular systems in the milk.

2.2.3.1 Cow age and stage of lactation

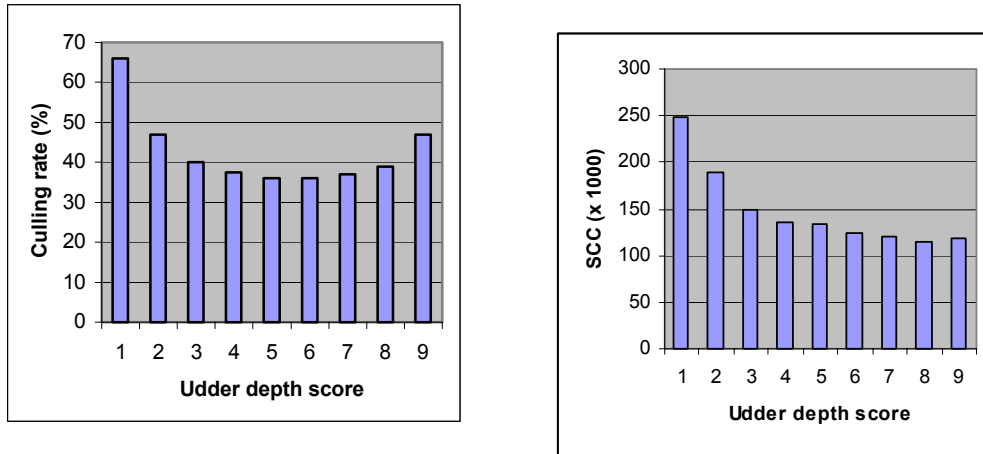
The efficacy of the defence mechanism of the mammary gland decreases progressively with the number of lactations, especially after the fourth lactation ([Quinn et al.,1994](#)). Most new IMI occur during the early part of the dry period, just prior to calving and during the first 2 months of lactation, even in well managed herds ([Smith and Hogan,1995](#)). This is due to the increased stress during the peripartal period causing a depressed immunity, metabolic changes and peak milk production following parturition.

2.2.3.2 Udder and teat conformation

For the modern, high-yielding dairy cow, a sound udder is a prerequisite for a long, productive life. Six linear descriptive traits are recommended by the World Holstein Friesian Federation for Black-and-white breed societies ([Hamoen, 1995](#)). The most important being the suspensory ligaments, udder depth and teat placement. A total udder score is based on the six linear traits and also take the udder texture, veining of the udder, length of the udder floor and udder balance into account. The empty udder in dairy cows can weigh from 20 kg to as much as 100 kg ([Giesecke et al., 1994](#)). Depending on the production level a further burden is added to the mammary suspensory ligaments. Loose mammary attachment and pendulous udders usually occur because the supporting ligaments weaken and stretch due to bad genetics or too much weight, as is sometimes seen with extreme pre-partum oedema. Udders with a distance of more than 40 cm between the tips of the hind teats and the ground have better natural protection, being within the udder shelter, than those closer to the ground ([Giesecke et al., 1994](#)). The teat-end-to-floor distance of the udder has a heritability of 0,2 - 0,7 and is also a risk factor for clinical mastitis ([Radostits et al., 2000](#)).

Emphasis is further placed on udder conformation due to its strong links with the cow's longevity, workability and health. Deficiencies in characteristics of the teat and teat canal, milkability and milk flow rate and the natural defence mechanism of the udder can be predisposing factors to mastitis. Deep udders may result in a higher incidence of teat and udder damage. Too shallow udders might go hand in hand with lower milk production. De Jong ([1997](#)) evaluated data from more than 500 000 cows to compare udder depth and culling rate and 400 000 primiparous cows to compare udder depth with SCC (see Figure 2.1 a & b). Udder depth scores of 5 to 6 were found to be optimal with regard to herd life. Udder depth forms part of the selection criteria for the Mastitis-resistance-index (M-index) which was developed to improve the genetic potential for udder health. The other criteria are teat length, front udder attachment, SCC and milking speed ([De Jong, 1997](#)). Udder depth scores of 1 to 3 (udders hanging on or below the hock joint) and 9 (udders very high in the udder shelter) were found to result in culling rates of higher than 40%. Udder depth scores of less than 3 in primiparous cows, were associated with increased SCC.

Figure 2.1 a & b shows the relationship between udder depth and culling rate, and udder depth and SCC.



Adapted from Harmoen (1995) and de Jong(1997)

Teat size, shape, tips, alignment and placement can play an important role either to increase or decrease the risk of the udder becoming infected. (Sandholm et al.,1995; Giesecke et al.,1994).

2.2.3.3 The teat canal

The teat canal has several anatomical and physiological features that serve as a barrier to penetration by bacteria. The normal teat canal represents a physical barrier to the penetration of bacteria and is the single most important barrier to udder infection (Sandholm et al.,1995; Giesecke et al.,1994; Bramley and Dodd,1984). The teat canal is not so much a single channel from the teat end to the teat cistern but rather a delicate web of tiny channels that open at the base of the teat into a common channel. The teat canal's stratified epithelial lining, spiral longitudinal mucosal folds and sphincter, and the rosette of Fürstenberg, play important roles in the defence mechanism of the mammary gland. The epithelial cells of the teat canal exist in a dynamic state of regeneration and degeneration.

Besides being a physical barrier, the canal contains long chain fatty acids produced by continuous keratinization of epithelial cells that are antibacterial, and a protein called ubiquitin that acts as a general antiseptic against incoming bacteria. In a healthy teat, the keratin plug mechanically traps debris and bacteria and absorb them within the extracellular lipid film (Bramley and Dodd,1984). Resistance to bacterial

penetration of the teat canal can be related, in part, to the presence of keratin ([Murphy,1959](#)) and the diameter of the teat canal ([Radostits et al.,2000](#); [McDonald,1975](#)). Essentially, there are three defence mechanisms offered by the teat canal: 1) adsorptive capacity of keratin for bacteria, 2) desiccation of the canal lumen, and 3) desquamation of the keratin lining during milk flow ([Lacy-Hulbert and Hillerton, 1995](#)).

Teats from which the keratin is reamed lose much of their ability to resist bacterial invasion. The cell layers of keratin of the teat canal are able to absorb bacteria within an extracellular lipid film. Up to a million bacteria can be sequestered by the teat canal at any given time. The most mature keratin cells, lining the milk flow surface may have the greatest capability to entrap or adsorb bacteria. During milking, the pulsatile milk flow, induced by the pulsating action of the pulsator onto the teat liner, removes bacteria-contaminated keratin from the teat canal. The most keratinised epithelial cells of the teat canal are unable to withstand the shearing forces applied to the keratin surface during milking and are very easily removed from the teat canal during milk flow. Up to 40% of the keratinised cells present before milking are removed by the shear-forces associated with milk flow ([Capuco et al.,1997](#)). This means that a proportion of the keratin is removed at every milking by desquamation and has to be replaced by new growth during the inter-milking period. Any process, which reduces the removal rate of the bacteria, will tip the balance in favour of the bacteria and increase the chances of infection ([Lacy-Hulbert and Hillerton, 1995](#)).

The amount of keratin that is collected in relatively long teat canals is more than that collected in relatively short teat canals ([Paulrud and Rasmussen, 2001](#)). This data suggest a positive correlation between the keratin quantity, length and diameter of the teat canal ([Paulrud and Rasmussen, 2001](#)) The probability of infection increased significantly for SUB with a decrease in teat canal length. A significantly higher incidence of infection by SUB was observed in quarters that contained a low wet weight (<1.8 mg) of removable keratin. Cows with wide teat canal diameters and with a thinner keratinous canal lining were found to have an increased susceptibility for common udder pathogens than quarters that did not become infected ([McDonald, 1975](#)).

Epithelial desquamation and milk flow during lactation limit bacterial colonization in the teat canal. However, at the beginning of the dry period when milk production ceases, bacteria are able to colonize the teat canal, multiply and subsequently infect the mammary gland. The cessation of milk flow and poor closure of the teat canal caused by the increase in milk pressure within the udder, as well as oedema of the

teat, are considered to be important predisposing factors, which promote new infections during the period of active involution ([Smith and Hogan, 2000](#)).

A keratin plug closing the teat canal to bacterial invasion during the mid dry period develops as the dry period progresses ([Radostits et al., 2000](#); [Giesecke et al., 1994](#)). A study done by Williamson, Woolford and Day ([1995](#)) showed a distinct difference in the length of time it took for the formation of the teat plug to be completed, ranging from 10 to 50 days. Teat plugs took approximately 10 days to develop in 50% of dairy cows, while 5-10% took more than 50 days. The formation of the teat plugs was twice as fast in cows which received intra-mammary treatment with a long-acting intra-mammary cephalonium product at drying off, compared to cows left untreated during the dry period ([Williamson et al., 1995](#)).

Teat canals of high producing dairy cattle are all too frequently in an appalling condition. The canal is extensively exposed to sporadic and cumulative trauma, which leads to a change in the resistance of the teat canal to bacterial invasion. Resistance of the teat canal decreases when excessive keratin removal from the canal takes place and when eversion and erosion of the teat-end becomes evident. Resistance is also decreased when the pliability of the teat tissue changes due to congestion, oedema and epithelial hyperplasia, as is found immediately pre- and post-partum, or with incorrect use of the milking machine. ([Bulletin of the IDF, 1994](#)). When the teat canal is dilated, the risk of ascending infection is high ([Sandholm et al., 1995](#); [Comalli et al., 1984](#)).

Lymphocytes and plasma cells accumulate beneath and between the epithelial cells of the teat canal wall, particularly around the rosette of Fürstenberg ([Sandholm et al., 1995](#)) to form the second line of defence.

2.2.3.4 Humoral antibacterial factors in mammary gland secretions

The initial inflammatory reaction, which results from the entry of infectious agents into the mammary gland, is followed by the production and release of a complex of cytokines, interferons and interleukins. These are responsible for the fever and the acute phase response, which constitute the primary host mechanism to microbial invasion. Effective humoral and cell-mediated immune responses and the phagocytic activity of macrophages and neutrophils are very important. Four defence mechanisms in particular are important in the mammary gland: phagocytosis, toxin neutralisation, anti-adhesive properties of antibodies and direct bacteriolysis.

- Lactoferrin

Lactoferrin is produced by epithelial cells of the mammary gland, as well as by phagocytes. It is a glycoprotein, which binds two ferric ions to bicarbonate ions. These iron-binding proteins of the host counteract the iron uptake by bacteria by competing for the available iron, thus limiting bacterial growth. Iron is the most limiting nutrient regulating the growth of aerobic bacteria ([Sandholm et al.,1995](#)). Both coliforms and staphylococci have a large demand for iron, while the iron requirement of streptococci is low. Lactoferrin significantly limits the growth of staphylococci and coliform bacteria, but not that of streptococci.

Lactoferrin may control the functional processes of macrophages, lymphocytes and PMN. Lactoferrin may have an autoregulatory role in retaining PMN at inflammatory sites and thus amplifying the inflammatory response ([Smith and Hogan,2000](#)).

The bacteriostatic effect of lactoferrin also depends on the quantity of lactoferrin in the secretion, the iron saturation level of the milk and the concentration of bicarbonate and citrate ions ([Smith and Hogan,2000](#); [Hurley,1989](#)). Bicarbonate boosts the effect of lactoferrin while citrate diminishes it. The molar ratio of citrate to lactoferrin changes significantly at the start of involution from 2,356 mg/ml to 581mg/ml and 49 mg/ml at days 0, 3 and 7 of mammary involution respectively ([Smith and Hogan, 2000](#), [Rejman et al.,1989](#); [Bishop, Schanbacher, Ferguson and Smith,1976](#)). Citrate also binds with ferric ions to form a complex. This citrate-iron complex, however, is a good source of iron for bacteria. Both lactoferrin and bicarbonate levels peak during the dry period when citrate levels are low, except for the start and end of the dry period ([Smith and Hogan,2000](#)). Lactoferrin is mainly bacteriostatic, but can be bactericidal depending on the saturation stage of iron and the degree of proteolytic cleavage. N-terminal peptides of lactoferrin, resulting from pepsin cleavage have strong antibacterial and antifungal properties. Lactoferrin and its N-terminal fragment bind to the external wall of Gram-negative bacteria and damage the surface structures leading to the demise of the bacteria. Lysozyme amplifies this reaction ([Smith and Hogan,2000](#)).

- Lysozyme

Lysozyme hydrolyses the bonds within the structure of the bacterial cell wall, causing osmotic lysis of the bacteria. Compared to blood and other secretions, lysozyme levels are low in bovine milk but high in human milk ([Losned et al.,1996](#)). Although milk levels are low, the interaction of lysozyme is considered to be important ([Sandholm et al.,1995](#)) as other antibacterial factors in milk may enhance its action ([Smith and Hogan,2000](#)). Lysozyme intensifies antibody and complement-mediated

bacteriolysis, stimulates the opsonization of IgM, intensifies the action of lactoferrin and binds the IgM complex to phagocytes ([Smith and Hogan,2000](#)).

- Lactoperoxidase

The lactoperoxidase levels in bovine milk are higher than in other mammals, although levels in bovine colostrum are low ([Losnedahl et al.,1996](#)). Phagocytes in milk produce H₂O₂ as long as there is sufficient oxygen available. Oxygen levels are low in milk and therefore the formation of peroxide is not favoured. In experimentally induced mastitis at drying off, the lactoperoxidase system was active against SUB and suppressed SAG to some degree. The lactoperoxidase system weakens against SAG during the dry period with increases in cysteine and cystine in the udder secretion ([Sandholm et al.,1995](#)).

2.2.3.5 Immunological defence mechanism

- Humoral immune system

The total amount of immunoglobulin, as well as the portions of Ig-sub-classes varies during the stages of lactations. The immunoglobulin content in colostrum is as high as 100 mg/ml to provide passive immunity to the calf. The immunoglobulin however decreases to levels of less than 1 mg/ml within the first week of lactation ([Smith and Hogan,2000](#)). These levels are very low in comparison to human and sow's milk. IgG₁ is the dominant antibody in ruminant milk, while IgA is dominant in the milk of monogastrics. The levels of IgG₁ and IgA are higher in bovine milk than in their plasma, indicating that the epithelium of the mammary gland actively transfers IgG₁ and IgA from the serum to the milk.

Of the immune systems operating in the mammary gland, phagocytosis and killing of bacteria by polymorphonuclear leucocytes (PMN) is the most important. This system does not work in isolation and requires antibodies in milk to opsonize the bacteria prior to phagocytosis. Macrophages and PMN are directed to their target (chemotaxis). Antibodies can neutralise toxins and can in rare occasions be bactericidal. The antibodies function by covering surface structures of bacteria, thus preventing them from adhering to epithelial surfaces. Antibodies of the IgA class do have opsonic activity and can also increase the phagocytic efficiency ([Bramley and Dodd,1984](#)).

- Cellular immunity

- Lymphocytes

Only small numbers of lymphocytes are present in normal milk of which 50% are T-cells, 20% B-cells and the rest null-cells. They are present in the intra-mammary lymph node, at the rosette of Fürstenburg, as well as beneath the mammary epithelium. The role of lymphocytes is not clear. Antigens stimulate the sub-epithelial B-cells to multiply and to differentiate into plasma cells, which produce secretory antibodies ([Sandholm et al.,1995](#)). Lymphocytes are thought to participate in autophagocytosis of secretory cell constituents. A consistent finding has been that lymphocytes of bovine mammary glands are generally less responsive to mitogens than lymphocytes isolated from blood. It is thought that the involuting gland could be more responsive to local immunisation. Diminished lymphocyte activity around calving has been observed ([Radostits et al.,2000](#)).

- Macrophages

Macrophages are phagocytic cells that remove tissue debris and bacteria. They are the first cells to encounter bacteria and function as recogniser and alarm cells initiating immunity in the mammary gland ([Sandholm et al.,1995](#)). They phagocytose bacteria, process antigens for the immune system and regulate the function of the lymphocytes. During initial involution of the mammary gland macrophages accumulate to phagocytose degenerated secretory epithelial cells, fat and casein ([Smith and Hogan,2000](#); [Jensen and Eberhart,1975](#)). The marked decline in fluid volume, fat and casein reduction between days 3 and 7 of involution suggest that all phagocytes would be functionally committed to clear the mammary gland of cellular debris, fat and casein.

- Polymorphonuclear neutrophils (PMN)

The increase in somatic cell count (SCC) seen in mastitis is mainly due to the increase in PMN. The PMN move from the blood through a weak blood udder barrier into the mammary gland. The PMN adhere to the endothelium at the inflammatory site, phagocytose and destroy the bacteria and remove damaged tissue ([Hurley,1989](#)). They kill bacteria by activating oxygen (oxygen burst) through the NADPH-oxidase-myeloperoxidase system ([Sandholm et al.,1995](#)). The action of the PMN is poor in milk due to the low energy reserves (low in glucose) and its low opsonin content (antibodies, complement) and they waste their capacity by phagocytosing casein and fat globules instead of bacteria ([Smith and Hogan,2000](#)). PMN function is impaired during the peripartum period and this may contribute to the increased incidence of mastitis following calving ([Radostits et al.,2000](#)).

2.2.3.6 Spontaneous cure

When a cow rids herself of a clinical udder infection, it is referred to as spontaneous cure, in the narrow sense. Griffen, Dodd and Dodd, ([1982](#)) found in recoveries to be equal to the number of infections by antibiotic therapy given to lactating cows but followed a long period of infection. Nickerson, ([1996](#)) found 20% to 50% of infections to recover spontaneously. The number of leucocytes, as well as their antimicrobial activity becomes elevated. Leucocytes function to overcome the infection aided by antibodies, which are produced in the mammary gland. Spontaneous cure is found to be enhanced in cows vaccinated against STA IMI ([Nickerson,2001](#)). In future we may also be able to boost spontaneous cure with biological agents such as cytokines or interferon ([Nickerson,2001](#)). Most spontaneous cures take place in mild and recently infected cases. Due to secretion high in antibacterial action of the fully involuted mammary gland, spontaneous elimination of IMI can occur more frequently at this time than during lactation. Acute cases of mastitis are not often seen in the fully involuted mammary gland ([Williamson et al.,1995](#)). Ruegg ([2001](#)) and Kirk ([1998](#)) reported the cure-rate for environmental streptococci to be close to 50% in non-lactating cows.

2.3 Dry-cow therapy

With regards to udder health, the main objective during the dry period is to have cows with healthy udders. To achieve this goal, existing IMI should be eliminated and new infections should be prevented from occurring during the dry period.

Dry-cow treatment was adopted as a cornerstone of mastitis control strategies during the 1960's and is still considered to be the most effective way for eliminating existing, mainly contagious IMI, during the early dry period, even in herds with a low cell count ([National Mastitis Council Factsheet](#)). Its efficacy and advantages are well known ([Nickerson 2001](#), [Janosi and Huszenicza,2001](#), [Berry 2000](#), [Erskine 1998](#), [Bolourghi, Hovareshti and Tabatayi,1995](#), [Sandholm et al.,1995](#), [Giesecke et al.,1994](#)).

Most dry-cow antimicrobial products contain mainly narrow spectrum antibiotics in a sustained release base, which will maintain therapeutic levels in the dry mammary gland for 14 to 28 days. These characteristics contribute to cure-rates higher than during lactation, up to 35% fewer new infections post drying off, allow regeneration of tissue, lower the incidence of mastitis at calving and decrease the chances of residues in the milk ([Anderson and Côté, 1996](#)).

2.3.1 Drying off protocol

Critical aspects of drying off are dry-off date, body condition, cow handling, monitoring of udder health, the method of administration and other treatments such as vaccinations, vitamin A, D and E, trace mineral supplements and deworming. Accurate data regarding AI or service dates and pregnancy diagnosis are necessary to determine the expected date of calving correctly. The conception date determines both the length of lactation and the drying off date. The length of the dry period should be approximately 55 days and can vary between 42-60 days ([Giesecke et al., 1994](#)). A short dry period may not allow sufficient time for the mammary gland to regenerate and will lead to a lower milk production in the ensuing lactation. If the dry period is much longer than 70 days, milk production is also lower during the subsequent lactation. Research has shown that the milk production without a dry period between lactations is only 75% as much in her subsequent lactation and 62% in the following lactation ([Smith and Guthrie](#)).

There are various methods of drying dairy cows off. Drying off is a very stressful period due to separation of cows from herd mates, cessation of milking, introduction into a new group, ration changes and even withdrawal of drinking water in some cases. Therefore there is a need to handle cows to be dried off very gently to minimize stress ([Brand, Noordhuizen and Schukken, 1996](#)). Feed intake, especially concentrates, should be limited gradually from approximately 2 weeks prior to drying off. Concentrates can even be taken away completely. In persistent producers it may even be necessary to make use of poor quality roughage for a while and limit water intake. On the correct date the cow should be dried off abruptly. At the last milking care must be taken to milk the udder out completely. Preventive dry-cow therapy is administered and the udder is left undisturbed during the remainder of the dry period.

Administration of intramammary dry-cow antibiotics should be done with the utmost hygiene (adequate teat end preparation) and caution. Unsatisfactory infusion practices can introduce antibiotic resistant environmental organisms present on the teat-end into the mammary gland. This may result in an infection more severe than the one for which the therapy is intended.

Infections with opportunistic micro-organisms such as yeasts or *Nocardia* may cause more extensive udder damage than the original organism for which the therapy was intended. An increase in the incidence of *Nocardia* has been associated with blanket dry cow therapy, especially products containing neomycin ([Radostiits et al., 2000](#)).

The problem of new dry period infections associated with the method of therapy has been the subject of some investigation. Complete insertion of the syringe cannula may lead to the spreading of the micro-organisms already in the teat canal to the teat cistern. It may cause temporary dilation of the teat canal and may force the keratin teat plug aside or partially remove it. The larger than normal canal opened by these practices may allow bacteria easier entrance into the teat cistern. Partial insertion of the infusion cannula into the teat canal (3-4 millimeters) has markedly reduced the risk of new infections at calving ([Radostits et al.,2000](#); [Boddie and Nickerson,1986](#)) due to fewer organisms being delivered beyond the teat canal.

2.3.2 Elimination of existing IMI during the dry period

The dry period offers a valuable opportunity for the elimination of existing IMI while cows are not lactating. Intramammary therapy during the dry period has many advantages over treatment during lactation. During lactation discarding milk that contains antibiotic residues represents serious economic losses. Therapy during the drying off period has no such effect and there is a limited risk of milk contamination (only with premature calving) of antibiotic residues being found. During the dry period elimination of IMI is more likely than during lactation as the drug is not milked out, but remains in the udder, and a higher and more uniform concentration of antibiotic is maintained in the udder. Furthermore, the concentration and effect of several endogenous antibacterial factors are optimal during the dry period (lactoferrin and macrophages in particular), that enhance the effect of the antibiotic therapy (see paragraph 2.2 of this dissertation). The dry period aids in the regeneration of damaged udder parenchyme and the exposure to contagious bacteria is reduced in the absence of the milking process. Effective dry-cow therapy can mean a reduction in the incidence of new mastitis cases in the ensuing lactation and an increase in the number of cows with low somatic cell milk ([Sandholm et al.,1995](#)).

The cure-rate of treatment during the dry period varies significantly between major pathogens. Intramammary infection caused by STA can pose a serious problem to the elimination of IMI of a dairy cow. Better cure-rates are achieved in younger cows and cows with IMI with STA in only one or two udder quarters. For each month that a intramammary STA infection persists, the prognosis worsens by 20% if the initial cure- rate is taken as 100% ([Sandholm et al.,1995](#)). Factors which have been suggested to influence the ability of an antimicrobial agent to eliminate STA include: lack of rapid intracellular penetration or accumulation (β -lactams and aminoglycosides), improper distribution within the intracellular compartments (chloramphenicol), inactivation of antibiotic due to the low pH of phagolysosome (aminoglycoides and macrolides) and a state of low metabolic activity of the

intracellular microbes (β -lactams) ([Yancey, Sanchez, Rzepkowski, Chester, Barnes and Ford, 1991](#)). Dry-cow therapy is effective in controlling SAG (90-95%) and has some efficacy against STA (20-30%) ([Bramley and Dodd, 1984](#); [Dodd, 1983](#); [Ziv, Storper and Saran, 1981](#)). The success of treatment for environmental streptococci is given as 40-50%, CNS as 50-60%, coliforms as 0-10% but no success is achieved in the treatment of *Nocardia*, MYC and fungi ([Yancey et al., 1991](#)).

Extended therapy for chronic STA cases is being introduced. After intramammary infusion 50% of the drug is absorbed from the udder into the bloodstream and 50% of that amount is then re-excreted back into the udder aiding the drug to reach infected areas of the mammary gland parenchyme. With three treatments Nickerson (2001) achieved a 41% success rate in curing chronic STA quarters. A second study included sub-clinical mastitis where 86% of cases were cured and the SCC decreased from 3,4 million to 280 000 cells / ml milk ([Nickerson, 2001](#)).

2.3.3 New intramammary infections during the dry period

The use of intramammary antibiotics to prevent new IMI during the dry period was originally developed as a control measure for summer mastitis ([Pearson, 1950](#)). Mastitogenic pathogens that were highly susceptible to antibiotics were practically eliminated from the cow population, while at the same time resistant bacteria became dominant. The prevalence of infections by streptococci has decreased and has been replaced by staphylococcal infections. In a similar way, Gram-positive infections become less frequent when teat dipping is practised while the prevalence of acute Gram-negative infections such as coliforms and Gram-positive infections such as SUB seem to increase ([Bradley and Green, 2001](#); [Sandholm et al., 1995](#)).

The rate of new IMI is significantly higher during the dry period than during lactation. The highest increase in susceptibility is during the first three weeks of active involution of the dry period. A second period of increased susceptibility occurs prior to parturition during the period of colostrogenesis and lactogenesis. The incidence of new IMI is the lowest during the steady state of involution if compared to any other functional stage of the mammary gland. The degree to which a fully involuted udder can withstand infection also varies with the bacterial species. It is quite resistant to coliform infections, but is susceptible to SUB and SAG. The low rate of new IMI in the fully involuted mammary gland can thus only be partly due to the increased levels of antibacterial factors, and to a reduced rate of bacterial penetration through the teat canal ([Smith and Hogan, 2000](#); [Hurley](#)).

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Factors in the involuting mammary gland that may favour new infections. A large volume of milk accumulates and leaks from the teats, leaving the teat canal open for bacterial invasion:

- milk no longer aids in washing micro-organisms at regular intervals from the teat canal, to prevent their colonization of the canal,
- no teat disinfectant is applied to control micro-organisms on the teat skin and canal and lastly the phagocytic cells are mainly pre-occupied with clearing cell debris,
- the increase in lactoferrin, immunoglobulins, phagocytes and lymphocytes at this stage should render the gland more resistant to new infections.

However, if these changes occur too slowly their antibacterial function is compromised. The efficiency of the PMN is reduced by milk protein, butterfat, and by the absence of specific antibodies. Bacteriostatic antibiotics need actively dividing bacteria to be effective and bactericidal antibiotics are less effective inside leucocytes because of the higher pH ([Smith and Hogan.,2000](#); [Hurley](#)).

Although the focus of mastitis control schemes for the dry period has often been on contagious pathogens, environmental bacteria need also be considered. Williamson et al.([1995](#)) studied the effect of intramammary dry-cow therapy on the rate at which teat canals closed (formation of teat plug). The incidence of open teats (no complete teat plug) was highest for untreated controls. In two of the four herds used for this trial, the incidence of open teats in untreated quarters as opposed to treated quarters was double ($P<0.01$) until 50 days into the dry period. Of all IMI in this trial 83 % occurred within 21 days of drying off. Of these IMI, 97% were in quarters, which at the time of infection, still had open teat canals ([Williamson et al.,1995](#)).

The exposure to contagious bacteria is reduced in the dry period. However, coliforms, SDY, SUB and SFA are ubiquitous in the environment around the cow's mammary gland, which therefore continuously exposes the udder to these pathogens during the dry period. The involuted udder (mid dry period) is naturally resistant to Gram-negative bacteria. However, during the first and last weeks of the dry period new IMI are mainly due to environmental pathogens such as SUB and coliforms ([Williamson et al.,1995](#)). Most currently available dry-cow remedies are formulated for efficacy against Gram-positive cocci and they provide no or little protection against Gram-negative bacteria.

To date, it has been assumed that dry-cow antibiotic therapy could not play a role in the control of clinical coliform mastitis during early lactation ([Smith et al., 1985](#)), primarily due to the inadequate spectrum and the effective action time. However, a wide spectrum intramammary dry-cow product containing framycetin which persists

for up to 14 weeks after drying off has proven the opposite. Bradley and Green (2001) found that cows treated with framycetin were significantly less likely to develop clinical *E.coli* mastitis (odds ratio = 2,11 and P = 0,045) and coliform mastitis (odds ratio = 2,13 and P = 0,036).

2.3.4 Blanket dry-cow therapy

Blanket dry-cow treatments involve the therapy of all quarters of all cows immediately after the last milking, irrespective of the udder health status. The objectives are to eliminate existing and to prevent new IMI during the dry period.

Firstly, it significantly reduces the prevalence of contagious infective quarters in the herd. As a result of enhanced host defence in the mammary gland, success of therapy is more likely for most pathogens during the dry period than during lactation. By reducing the prevalence of infected quarters, uninfected quarters are less exposed to the contagious bacteria (Nickerson,2001). Secondly, fewer new infections (predominantly environmental pathogens) occur during the first two weeks of the dry period. In the absence of any dry-cow treatment, the rate of new infections with contagious pathogens could increase 5 to 7 fold during the first 2 weeks of the dry period compared to the rates during lactation (Hillerton,1996). It is widely used and in the United States 77% of all dairy herds use blanket dry-cow therapy (Smith et al.,1985).

Berry (2000) found that blanket dry-cow treatment reduced the rate of new IMI by 80% and clinical mastitis was prevented during the dry period, especially IMI due to SUB, compared to a untreated control group (see Table 2.2).

Table 2.2 Infection rates based on dry-cow therapy

	No dry-cow therapy	Dry-cow therapy with cephalosporin
Clinical mastitis during the dry period	12 / 134 cows (8,96%)	0 / 117 (0,0%; p=, 001)
New infections at calving	42 / 122 cows (34,4%)	12/117 (10,3%; p<, 001)

Adapted from Berry and Hillerton, 2002

The annual culling rate on conventional dairy farms that use blanket dry-cow therapy as part of their animal health program is 30%, compared to 50-60% on organic dairy

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farms that did not use antibiotics ([Nickerson,2001](#)). The National Mastitis Council at present recommends blanket dry-cow therapy ([National Mastitis Council Fact Sheet](#)).

2.3.4.1 Disadvantages of blanket dry-cow therapy

Greater awareness of and sensitivity to the widespread use of antibiotics in food producing herds and low infection rates at drying off resulted in the re-evaluation of the use of blanket dry-cow therapy. In blanket therapy 15,5 tubes were used for each infection eliminated versus 6,4 tubes in selective dry-cow therapy ([Browning et al.,1994](#)). This more than doubles its cost above selective dry-cow therapy. There is further a possibility of emergence of antibiotic-resistant organisms. More minor pathogens are also eliminated from the mammary gland with dry cow therapy.

An important limiting factor of intramammary dry-cow therapy is its inability to prevent new IMI during the last trimester of the dry period, as most dry-cow antibiotics persist for only 14 to 28 days after instillation and often contain narrow spectrum antibiotics ([Smith et al.,1985](#); [Eberhart and Buckalew,1977](#)).

2.3.4.2 Cost-effectiveness of blanket dry-cow therapy

If a dairy herd does not apply any dry-cow therapy, 8-12% of quarters may develop new IMI during the dry period. It has been shown that prevention of 1% of new infections pays for the entire dry-cow program, including blanket therapy ([Nickerson,2001](#)). A mammary gland cured of infection during the dry period is estimated to produce 90% of its capacity in the subsequent lactation. New IMI contracted during the dry period are estimated to cause a milk loss of between 30-40% in the ensuing lactation ([Nickerson,2001](#)).

2.3.4.3 Variations in the duration of therapeutic levels of blanket dry-cow therapy

Berry ([2000](#)) compared the infection rates at calving between cows treated with an extra long-acting intramammary antibiotic (active for more than 7 weeks) at drying off and cows not treated at all. The therapeutic action of the extra long-acting intramammary product lasted for at least 51 to 70 days. This drug's major advantage is that it protects the mammary gland during the second high-risk period prior to calving. The percentage of untreated cows infected at calving varied between 30-50% and the treated cows between 0 -15%.

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A double dry-cow therapy, one given at drying off and one three weeks later showed little if any value in udder health ([National Mastitis Council Fact sheet](#)). Cummins and McCaskey (1987) also found no difference using a single dry-cow therapy or three therapies with the same product 7 days apart.

2.3.5 Selective dry-cow therapy / Selective quarter therapy

Selective dry-cow therapy is when only infected quarters or mammary glands in a herd are treated with a long-acting dry-cow remedy at drying off. Although dry-cow therapy is the cornerstone of any mastitis control programme, there is continuing controversy regarding the need to treat all quarters of all cows at drying off. This debate has gained momentum as the implementation of basic udder health management practices has caused a reduced prevalence of IMI. Selective dry-cow therapy should be considered in herds with a low prevalence of sub-clinical mastitis and where the bulk somatic cell count is low ([National Mastitis Fact Sheet](#)).

The major advantages in pursuing selective dry-cow therapy are to reduce cost of treatment, to avoid the possible emergence of antibiotic-resistant organisms and to prevent the elimination of minor pathogens ([Eberhart,1986](#)). It may also limit damage to the teat canal by the insertion of a cannula and reduce the likelihood of introducing pathogens during non-aseptic infusion.

2.3.5.1 Disadvantages of selective dry-cow therapy

Selective treatment requires screening of cows to identify infected quarters. The selection criteria for therapy is the first question that comes to mind. Poutrel and Rainard (1981) suggested the selection of cows for therapy that show at least one California milk cell test (CMCT) positive quarter when tested 8 weeks prior to drying off. Østerås, Sandvik, Aursjo, Gjøl and Jorstad (1991) found that one sample taken 1-8 weeks prior to drying off is inadequate to make a decision on selective therapy. The history, individual cow SCC and bacteriological culture are valid decision-making tools, but they may all result in leaving some infected cows without therapy. Østerås, Edge and Martin (1999) found that the geometric mean of the composite cow milk sample SCC of at least 5 to 6 months of lactation was the best predictor. A threshold value of 200 000 cells per ml milk had to be used in this case. Sandholm et al.(1995) found that the decision to treat or not should be made on the basis of the cow and not the quarter. However, the positive and negative predictive values of any of the available tests were found to be unacceptable as a basis for decision making concerning dry-cow treatment ([Radostits et al.,2000](#)).

Selective dry-cow therapy only aims at curing existing IMI and ignores infections that could occur during and after drying off and the preventive benefit of blanket treatment is lost. The history of clinical mastitis, positive CMCT, individual cow SCC, and even bacterial culturing may leave some infected cows untreated. According to the National Mastitis Council Fact Sheet, selective dry-cow therapy may fail to reach 20% to 40% of infected quarters in a herd.

Other studies have shown that the infection rates were higher at calving than at drying off in herds with a low mastitis prevalence, in which selective dry-cow therapy was used ([Smith and Hogan,1995](#); [Eberhart and Buckalew,1977](#)). Schukken, VanVliet, VandeGeer and Grommers ([1993](#)) did a blind trial in a herd with low somatic cell counts, treating two of the four quarters in an udder with a dry-cow remedy, while two were left untreated. The treated quarters showed a significantly lower incidence of clinical mastitis during the dry period.

2.3.5.2 Cost-effectiveness of selective dry-cow therapy

It was reported that selective dry-cow therapy was only beneficial compared to no therapy at all ([Østerås and Sandvik,1996](#)). Natzke, Everett and Bray ([1975](#)) calculated that the production gain from the prevention of infection of only nine quarters (2,2%) in a 100-cow herd would cover the cost of antibiotics for therapy of all the cows. Currently it is clear that the replacement of a blanket dry-cow therapy with a selective programme cannot be economically justified. Effective methods should first be found to protect the cow from new infections during the dry period to make selective dry-cow therapy more effective as a strategy.

2.3.6. Short-acting intramammary teat sinus and cistern antibiotic therapy and teat canal therapy.

A study conducted by the Dairy Research Corporation in New Zealand investigated the efficacy of short-acting antibiotics as dry-cow therapy. The hypothesis was that new IMI during the dry period may originate from bacteria that remain in the teat canal or teat or lower gland cisterns. Four different antibiotic therapy were compared in a parallel study.

Group 1 was left untreated, group two was treated intramammary with a conventional long-acting dry-cow product, and group 3 was treated with a short-acting intramammary product and group 4 with 0.22g of a long-acting dry-cow antibiotic in

the teat canal. The two unconventional dry-cow therapy targeted bacteria in the teat canal (group 4) and bacteria in the teat sinus and lower gland (group 3). The active ingredient of all three therapies was cloxacillin ([Unknown, Dairy Research Corporation limited New Zealand](#)).

Table 2.3 summarises the results. Some advantage of dry-cow therapy is clear from this data. Of the untreated controls 3,5% developed clinical mastitis during the dry period compared to 2,9% that were treated in the teat canal and 2,7% that were treated in the teat/gland cistern. No cases of clinical mastitis developed during the dry period in cows that were treated with long-acting antibiotics.

Table 2.3 Results of therapy of clinical cases with short-acting antibiotics in the teat sinus and teat cistern

Therapy	Clinical cases- Dry period	Clinical cases - Early lactation
Teat canal Rx	2,9%	1,1%
Teat sinus / gland cisterns Rx	2,7%	1,3%
Long-acting dry-cow Rx	0%	0,6%
Negative control	3,5%	1,7%

Adapted from [Dairy Research Corporation limited New Zealand](#) (Author unknown)

The total percentage of major and minor bacteria isolated from samples at calving (table 2.4) of untreated cows was 22,1% compared to 12,8% treated in the teat canal, 10,1% treated in the teat / gland cistern and 4,2% treated with the long-acting dry-cow preparation.

Table 2.4 Results of sub-clinical cases of mastitis at calving

Therapy	SUB intramammary infections at calving	Major pathogens (Total)	Minor pathogens (Total)
Teat canal Rx	4,4%	5,4%	7,4%
Teat sinus / gland cisterns Rx	3,7%	4,8%	5,3%
Long-acting dry-cow Rx	1,1%	1,6%	2,6%
Negative control	6,3%	7,2%	14,9%

Adapted from [Dairy Research Corporation limited New Zealand](#) (Author unknown)

Østerås, Aursjo, Gronningsaeter and Jorstad ([1994](#)) compared the efficacy of a short-acting lactating cow antibiotic given every second day prior to calving, to a long-acting antibiotic. A significantly better effect was only found in preventing new IMI with STA and SDY in treated quarters compared to untreated quarters.

2.3.7 Prophylactic therapy in replacement heifers

Heifers treated with a long-acting intramammary antibiotic close to 60 days prior to calving showed a reduction in IMI at calving ranging from 40% to 97,1% compared to the 2,6% spontaneous recovery in the untreated control group at calving ([Nickerson,1996](#)). Jones et. al., ([1998](#)) found the IMI of pregnant heifers reduced milk production in their subsequent lactation by as much as 18%. Treated heifers produced on average 3kg more milk per day over the first two months of their lactations ([Nickerson, 2001](#)). This additional milk will pay for the mastitis remedy eight-fold if used in the above-described fashion.

2.3.8 Systemic antibiotic therapy.

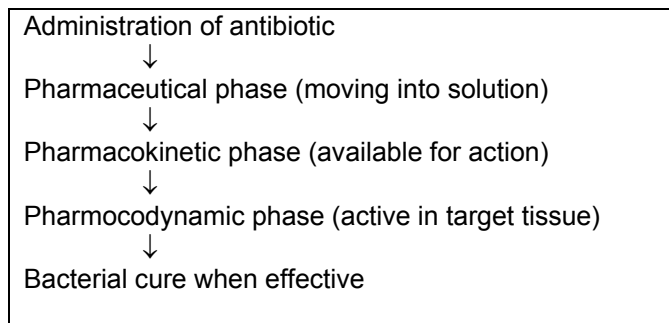
There is clearly no agreement yet on the efficacy of systemic dry-cow therapy. Bolourthi et al. ([1995](#)) found that treating dairy cows systemically with a macrolide, tylosin, at drying off, was as effective as treatment with intramammary products containing nafcillin, penicillin or dihydrostreptomycin. Erskine et al. ([1998](#)) had similar results in a trial comparing systemic treatment with 4 treatments of an oxytetracycline with an intra-mammary therapy of cephalosporin (21,2% and 22,5%). In a study done by Nickerson, Owens and Fox ([1999](#)) the cure-rates of intramammary therapy with cephalosporin were considerably higher (78%) compared to the cure-rates for systemic treatment with tilmicosin (9%). Saran, Soback, Faingold, Ziv, Winkler and Glickman ([1995](#)) used norfloxacin nicotinate as a systemic dry-cow therapy as well as preparations containing either neomycin and cloxacillin or nafcillin, penicillin G and dihydrostreptomycin. The cure-rates following systemic therapy and intramammary dry-cow therapy were respectively 69% and 44% and the new infection rates were 9,4% and 7,1%. Sorback, Paape, Filep and Varma ([1995](#)) found parenteral therapy with a fluoroquinolone, norfloxacin an effective regimen for dry-cow therapy. Wilson et al. ([1996](#)) and Sorback, et al. ([1995](#)) found systemic treatment with florfenicol to have cure-rates of 67% for SAG, 18% for STA and 50% for *E. coli*. Treatment with florfenicol against all agents resulted in a 39% cure-rate compared to 33% with cloxacillin-treated cases.

Dairy herds with intramammary STA infections, should consider including systemic therapy with tetracyclines, macrolides and fluoroquinolones ([Sorback et al.,1995](#); [Wilson, Sears, Gonzalez, Smith, Schulte, Bennett, Das and Johnson, 1996](#)). The use of systemic dry-cow remedies however, possesses an additional risk of antibiotic residues with antibiotics being excreted in the urine and faeces.

2.3.9. Pharmacokinetic considerations for mastitis therapy

The route of administration, dosage form and the dose all derive from pharmacokinetic studies. Pharmacokinetic describes the disposition of a drug in the body in a graphical or mathematical way. Table 2.5 summarises the flow of events following antibiotic administration. The time-course concentration profile of a drug in blood and tissues is dependent on the dose, absorption from the administration site, distribution within the body and elimination of the drug by metabolic and excretory processes. Pharmacokinetic information is needed as various drugs and dosage forms take different routes and time-course profiles within the body.

Table 2.5 A flow diagram of events following antibiotic administration.



Adapted from Van der Wal ([1995](#))

Most preparations used for the therapy of mastitis have been designed with little or no consideration being given to the natural defence mechanism of the mammary gland. Therapy of mastitis has shown to have only limited success during lactation.

The goal in therapy of mastitis is to administer an antimicrobial at a dose and site that will allow accumulation in the mammary gland (pharmacokinetics) above the minimum inhibitory concentration (MIC) for the pathogen for a sufficient period (pharmacodynamics) of time.

2.3.9.1 Parenteral mastitis therapy.

The pharmacokinetics in ruminants after parenteral therapy is complicated by the fact that the volume of the gastro-intestinal tract (GIT) exceeds the extra-cellular volume in the dairy cow. This leads to antibiotic disappearing in the GIT or being inactivated by the liver and not reaching the infectious foci when administered via the parenteral route ([Sandholm et al.,1995](#)). Symbiotic microbes in the GIT have a high capacity to metabolize and inactivate drugs and so has the liver. The liver needs a high

metabolize capacity because of the massive load of plant-derived xenobiotics and microbial products of the GIT ([Sandholm et al.,1995](#)).

For the drugs to reach the udder after systemic administration they must cross the blood-udder barrier. Passage of drugs across the blood-udder barrier takes place by passive diffusion. The extent to which a drug gains access to the mammary gland after systemic administration, depends on its degree of lipid solubility, its ionisation and binding with serum proteins. The ideal antibiotic intended for parenteral mastitis therapy should have a low minimum inhibitory concentration (MIC) against the major udder pathogens, high bio-availability from the intramuscular injection site, should be a weak base, have a long half-life in the body and have little or no drug accumulation in specific organs. Currently no single antibiotic meets all these requirements, but antibiotics such as pirlimycin (a lincosaminide) rapidly crosses the blood udder barrier ([Yancey et al.,1991](#))

2.3.9.2 Intramammary antibiotic therapy

Long-acting antibiotic preparations have been formulated specifically to treat sub-clinical mastitis during the dry period and to prevent new IMI and should have the following characteristics. The antibiotic effect must be long-lasting, as its purpose is to form a deposit in the lactiferous ducts from where the antibiotic is slowly released, without causing tissue irritation ([Janosi and Huszenicza,2001](#); [Sandholm et al.,1995](#)). The duration of the effect of the antibiotic is regulated by pharmaceutical manipulation of the intramammary drugs, precipitating the antibiotics, dissolving them in a slowly absorbing oil or micro-encapsulation. Most intramammary preparations persist only for 14 to 28 days ([Smith et al.,1985](#); [Eberhart and Buckalew,1976](#)).

Intramammary dry-cow remedies contain mostly narrow spectrum penicillins (penicillin, cloxacillin, oxacillin and nafcillin), cephalosporins and spiramycin. These preparations are designed to eliminate contagious mammary gland pathogens such as STA and SAG and to prevent their infection during the early dry period. In intensive systems, where dairy cows are confined to small areas, environmental infections increase during the dry period. Most dry-cow remedies are reasonably effective against environmental streptococci, but are ineffective against coliform bacteria. A study done by Bradley & Green ([2000](#)) demonstrated that infections with environmental pathogens are often acquired during the dry period. In 52% of cases of clinical mastitis caused by environmental pathogens, the same pathogen (as identified by DNA fingerprinting) has been previously isolated from that cow during her dry period.

Withholding periods for milk from animals treated with dry-cow formulations range from 30-70 days post treatment. A general recommendation is that dry-cow intramammary treatment should never be administered within a month of the expected calving date. This may however be too short when cows were treated with some of these antibiotic remedies.

All the intramammary dry-cow antibiotics used in this trial were from the β -lactam and aminoglycoside groups or combinations. Table 2.6 summarizes the spectrum of β -lactam and aminoglycosides.

Table 2.6 List of antibiotics and their activity against different bacterial species

Antibiotic	Gram-neg.	Gram-pos.	Pseudomonas spp.
β -LACTAM			
Penicillin	-	+	-
Ampicillin	+	+	-
Cloxacillin	-	+	-
Cephalosporins 1 st generation	-	+	-
AMINOGLYCOCIDES			
Streptomycin	+	+	+
Neomycin	+	+	+

Adapted from EMEA 1999

2.3.9.2.1 The β -lactam group (Procaine benzylpenicillin, Ampicillin, Cloxacillin, Nafcillin and cephalosporins).

The mechanism of action of the β -lactam group is through inhibition of the bacterial cell wall synthesis. Penicillins inhibit transpeptidase and carboxypeptidase, which result in the weakening of the bacterial cell wall and causes its eventual rupture ([Van der Wal, 1995](#)).

The β -lactams are able to diffuse through membranes. They do however not concentrate within phagocytes. Their intracellular concentration remains below that of the external environment, meaning that penicillins only act intracellularly if given in high concentrations. If benzylpenicillin (Penicillin G) is converted into a basic compound by substituting the carboxyl moiety with a bovic moiety, its intracellular accumulation improves significantly. Unfortunately this chemical modification has no antibacterial activity ([Tulkens, 1991](#)).

Ampicillin, cloxacillin and nafcillin are semi-synthetic penicillins derived from 6-amino penicillanic acid. The active compound of cloxacillin is chemically the benzathine salt of 6-(3-(2-chlorophenyl)-5-methyl-isoxazole-4-carboxamido) penicillanic acid. It contains a semi-synthetic penicillin nucleus, 6-amino-penicillanic acid ([Brander, Push and Bywater](#)).

Both cephalonium and cephalexin are semi-synthetic first generation cephalosporins ([Brander, Push and Bywater](#)).

2.3.9.2.2 Aminoglycosides (Dihydrostreptomycin and neomycin sulphate)

The mechanism of action of the aminoglycoside group is through the inhibition of bacterial protein synthesis. Aminoglycosides do penetrate cells, but at a very slow rate. Intracellular accumulation proceeds for days if the drug is regularly administered till the intracellular concentration is 2-3 times higher than that of the extracellular concentration. Aminoglycosides are not effective against bacteria with rapid growth. The environment within the lysosomes is acidic (pH=5) which reduces the action of aminoglycosides greatly ([Tulkens, 1991](#)).

2.3.9.3 Resistance of bacteria to antibiotics

Bacteria can modify their permeability by becoming impermeable to antibiotics, or by actively excreting the antibiotics that are accumulated within the cell or by producing enzymes capable of modifying and directly inactivating the antibiotics, or they can modify the structure of the antibiotic's target molecule. Antibiotic resistance can develop against both the β -lactam and aminoglycoside group by the increase in bacterial cell permeability and modification of the antibiotic and its target (Table 2.7).

Table 2.7: Main bacterial resistance mechanisms against two classes of antibiotics

Class of antibiotic	Modification of			
	Bacterial cell permeability		Antibiotic inactivation	Target
	Decreased influx	Increased influx		
β -lactam	+	-	+	+
Aminoglycosides	+	-	+	+

Adapted from EMEA 1999

2.4 Management during the dry period

Sound general management during the dry period as well as during calving is of critical importance for udder health. The provision of a stress-free environment and proper housing for cows, particularly during the peripartal period, is essential for the control of mastitis. Biosecurity on a dairy farm should also be borne in mind and infectious diseases such as bovine viral diarrhoea, bovine respiratory disease complex and salmonellosis can cause severe immunosuppression that may influence the prevalence of mastitis.

2.4.1. Groupings

Based on expected calving date and nutrient requirements pregnant heifers and dry cows should be grouped into two groups, an early/mid dry group (phase 1) and a close-up group (phase 2). Heifers and cows in the close-up group should be within 3-4 weeks from their expected calving date. Cows at the different phases of the dry period need different levels of nutrition and care to meet their different metabolic challenges ([Brand et al., 1996](#)). Contrary to normal practice of grouping heavily gravid heifers and cows together, it has been reported that housing pregnant heifers and dry cows together increasing the risk of *E. coli* mastitis ([Barkema, Schukken and Lam, 1999](#)).

2.4.2 Housing

Poor housing facilities and management practices on farms contribute to the contamination of the environment and exposure of teats to environmental pathogens. Dry-cow housing is frequently sub-standard compared to that of lactating cows, increasing the risk for environmental mastitis when cows are not clean or kept in a relatively clean environment. If possible, an exercise yard should be provided.

2.4.2.1. Bedding

The main purpose of bedding is to improve the comfort of the cows and to absorb moisture, help keep the cows clean and restrict bacterial growth. Reducing the number of bacteria results in a decrease in the incidence of environmental mastitis. Coliforms cannot live for long periods of time on the teat skin. One factor that has the greatest impact on bacteria in the cow's surroundings is the choice of bedding

material in stables or free stall barn systems. Bedding material can vary from organic (straw) to inorganic (sand).

Maximum populations of bacteria are often reached within 24 hours after fresh bedding is added, after which the populations remain in a stationary growth phase for up to 7 to 10 days. The general appearance of bedding has little correlation to bacterial load ([Hughes,1989](#); [Hogan and Smith,1992](#)). Finely chopped organic bedding frequently contains very high numbers of coliforms and streptococci compared to clean long straw ([Hogan and Smith,1999](#)). When sawdust was treated with lime an increase of bedding pH and a decrease in Gram-negative bacteria, coliforms, *Klebsiella* and streptococci was seen on day one. After 2 to 6 days both the pH and bacterial counts were similar to the untreated bedding ([Godkin, 1999](#)). Docking and trimming of tail ends and the clipping or cold burning of hair on the mammary gland may assist in keeping udders clean in free-stall systems ([Wallace, 2000](#)).

Pastured cows are generally thought to be at a reduced risk from environmental mastitis compared to cows in confined housing. However, areas under trees where animals congregate, overgrazed pastures or grazing during rainy weather can lead to conditions similar to what housed cows experience ([Hogan and Smith,1998](#)).

2.4.2.2. Calving hygiene

The immune status of a cow is highly challenged during the peripartal period due to many factors and changes occurring in her environment, social interactions and body (hormonal status, nutritional status, milk production and parturition). Cleanliness and low-density of micro-organisms at the time of calving is a prerequisite for optimal peripartal health of both the dam and the calf. Calving facilities and managemental issues such as sanitation, calving observation and comfort is of critical importance. Too frequent use of a maternity area without appropriate sanitation, bedding and disinfection results in the build up of pathogenic organisms and a high prevalence of environmental mastitis ([Bradley and Green, 2000](#)).

2.4.2.3. Fly control

The control of flies presents a very difficult problem. The risk of fly-borne diseases and increased stress levels can translate into significant economic losses. In a study done by Nickerson ([1996](#)), mammary gland infections of heifers at calving were compared (see table 2.8). One group was kept in an environment with proper fly control, while there was no fly control in the second group. A significant difference

was found in the CNS infections (a 10-fold decrease), streptococcal infections were 17% lower and STA 8,5% lower in the group where fly control was applied.

Table 2.8 The effect of fly control during the nine months pre-partum in dairy heifers

Micro-organisms	Percentage of infected heifers	
	With fly control	Without fly control
STA	32,9	41,4
CNS	5,6	55,2
<i>Streptococcus spp.</i>	3,7	20,7
Coliforms	2,2	0
<i>A. pyogenes</i>	0	3,4

Adapted from Nickerson ([2001](#))

Under South African conditions other ectoparasites should be controlled, especially ticks (*Amblyomma hebraeum* and *Hyalomma truncatum*) and biting midges.

2.4.3 Nutritional management of dry cows

Dry-cow nutrition is often one of the most neglected areas of dairy cow management because some producers consider dry cows as being in a resting state and not productive. Cows should be in optimal condition when they dry off (BCS of 3,5). A nutritionally balanced dry-cow feeding program is important to ensure udder health. Proper management during the dry period is a cost-effective investment in the next lactation. The primary objective of dry-cow nutrition is to optimize udder and general peripartal health, to normalize rumen and liver function and to start controlling the negative energy balance and the mineral metabolism in the dry period, with subsequent optimal peripartal health, milk production and reproductive performance ([Brand et al,1996](#)).

2.4.3.1 Macro-nutrition

The nutritional requirements of the dry cow are relatively low compared to the lactating cow. One should therefore also guard against animals becoming too fat with an increase of dystocia and related problems, udder oedema, metabolic disturbances and reduced milk production and fertility performance.

Dry period nutrition aims to achieve the following: Cows should dry off with a condition score of 3,5 and their BCS should remain fairly constant throughout the dry period. Rumen function should be normalized from a high concentrate ration and

should again be stimulated in the transitional stage prior to calving to stimulate rumen bacteria and the development of rumen papillae. Liver function should be normalized to minimize metabolic disease. Mineral reserves of the cow should be replenished and programmes preventing hypocalcaemia, retained placenta, metritis and sub-clinical mastitis should be in place. The foetus completes almost two thirds of its growth during the dry period and its growth will take priority.

During the early and mid dry period the cow needs little more than good quality, coarsely chopped hay. The high forage diet (>85%) is beneficial to maintain maximum rumen volume and motility. This will increase rumination, salivation and increase rumen pH, allowing rumen wall lesions to recover from the high grain diet during lactation ([Brand et al,1996](#)).

The close-up transitional period which starts about 3-4 weeks before calving is characterised by endocrine and physiological changes. It is critical to adapt the rumen, stimulate calcium metabolism and maintain a strong immune system through the peripartal period and to maintain a positive energy balance up to calving. Immunocompetence is compromised in most cows during the peripartal period. Cows should be in a rising plane of nutrition to prevent a negative energy balance and the mobilisation of adipose tissue and to compensate for the decreased feed intake, especially 3-5 days prior to calving ([Curtis, Sniffen, Smith, Powers, Smith, White, Hillerman and Pearson,1983](#)). The transition ration should be higher in energy, protein and other nutrients than recommended by the NRC to compensate for the decrease in dry matter intake (DMI). Strategies for preventing hypocalcaemia include lowering the Ca level in the diet, changing the dietary anion-cation balance and administering vitamin D₃ prior to calving ([Curtis et al.,1983](#)). When balanced correctly, anionic salts cause a mild acidosis, which results in more calcium and phosphorus being released from the bone and an increase in the absorption of these minerals from the intestine ([Byers,1993](#)).

Signs of an inadequate transition nutritional programme during the dry period include cows that are slow to come onto feeds post calving, a high incidence of metabolic disorders, variable milk production, cyclic feed intakes and excessive loss of body condition post calving. Other important economic implications associated with the quantity and composition of the dry-cow ration are dystocia, udder oedema, fatty liver syndrome, ketosis, milk fever, displaced abomasum, mastitis, reproductive problems, sub-acute rumen acidosis and poor immunity. Hypocalcaemia has been associated with an increased risk of dystocia, uterine prolapse, retained placenta, inferior contraction of the ring muscle of teat canals, mastitis, metritis and displaced abomasum ([Brand et al.,1996](#)).

2.4.3.2 Trace minerals and vitamins

Specific nutritional factors have important roles in the resistance to mastitis, especially during the dry period ([Erskine,1998](#); [Eberhart,1986](#)). Dietary vitamin E and selenium are shown to reduce the severity and frequency of coliform mastitis ([Wallace,2000](#)). It serves to reinforce the immune system response by increasing the release of leucocytes and increasing the efficiency of phagocytes, thus protecting mammary parenchyme from free oxygen radicals. Adequate levels of vitamin E and selenium in dry-cow rations appear to be important for udder health at calving and during early lactation ([Weiss, Hogan, Todhunter and Smith,1997](#)). Maddox, Reddy, Eberhart and Scholz ([1991](#)) found that cows given a selenium supplement of 0,35 mg/kg dry matter are better able to resist mastitis caused by *E. coli*. The duration of this mastitis was even shorter when given 2 mg of selenium per day per kg of ration. Recommended blood levels for selenium were found to be 0,2-1,0 g/ml and more than 4 g/ml for vitamin E ([Smith, Hogan and Weiss,1989](#)).

Comparative studies on the intramammary infection rate of heifers at calving receiving and not receiving vitamin E and selenium, showed that the rate of new IMI did not differ. However, the duration of most infections was reduced by 40-50% and clinical mastitis in early lactation was reduced by 57% in heifers which received selenium (50-100 ppm/day) supplementation ([Nickerson, 2001](#); [Oliver, Lewis, Gillespie and Dowlen, 1992](#)). Similar results were obtained by Duval ([2000](#)) who found a reduction in mastitis at calving of 42% and a reduction of clinical mastitis by 32%.

Zinc deficiency reduces potency of both the cellular and antibody response of the immune system to infections. The function of PMN and macrophages entering the udder, especially during early lactation, is impaired. Zinc assists in the maintenance of epithelial cells (cellular repair and replacement) and is also required for the production of keratin ([Costello,1998](#)). A study done at the University of Tennessee found that heifers fed with complexed zinc supplement have less udder oedema than those supplemented with inorganic trace minerals ([Socha, 2001](#)).

Ceruloplasmin is the main copper binding protein in the blood. Copper is involved in enzyme production and activation involving the effective functioning of both PMN and macrophages. A copper deficiency is associated with a decreased ability of these cells to multiply. Bio-availability of copper in ruminants may occur when dietary S, Mo, Zn or Fe is high. Harmon ([1998](#)) studied the effect of copper on mastitis. Heifers were fed two dietary treatments from 84 days prepartum with 6-7 ppm copper and the second group with 20 ppm copper. Liver biopsies at calving showed copper levels of 14 and 209 ppm for the two groups respectively although plasma copper levels were normal in

both groups. The group with high hepatic copper levels had significantly more uninfected quarters (60%) compared to the group with low hepatic copper levels (36%). The low copper group also tended to have higher SCC ([Harmon, 1998](#)).

There is speculation about the role of manganese in helping to prevent mastitis. Manganese is involved in detoxifying free oxygen radicals produced by the immune cells in response to killing bacteria. No evidence of its role in mastitis has yet been published ([Costello, 1998](#)).

Parantainen, Tenhunen, Kangasniemi, Sankari and Atroshi ([1987](#)) noted that the level of silica in mastitis infected milk was only 0,39 mg/ml compared to 0,81 mg/ml in normal milk. The blood serum levels of silica also differed significantly between the non-infected and normal and mastitic cows ie. 1,63 mg/l and 1,02 mg/l. Silica, with a similar role as selenium, has a marked effect on the formation of free radicals, lipid peroxidation and macrophage activity.

As previously indicated, iron may also play a role in the prevention of mastitis due to its association with lactoferrin ([Sandholm et al., 1995](#)).

Supplementation of vitamin A, D, E and trace-elements is important for the transfer of adequate levels of immunoglobulins to the calf via colostrum ([Brand et al., 1996](#)). Vitamin A may enhance keratinization in the teat canal ([Bramley and Dodd, 1984](#)).

2.4.4 Teat dipping during the dry period

Post milking teat dipping with effective teat disinfectant, correctly administered, is considered the single most effective practice for prevention of intramammary infections in lactating cows ([Pankey, Eberhart, Cumming, Daggett, Fransworth and McDuff, 1984](#)). A teat dip should however be formulated in such a way that it does not damage the skin by removal of moisture, lipids and other chemicals, but that it protects its structural and functional integrity. Teat dipping is especially effective against STA, SAG, *Mycoplasma bovis* and *Corynebacterium bovis*.

Researchers agree that the number and type of bacteria on the teat skin have a direct relationship to the incidence and type of mastitis that develops. Teat dipping reduces the bacterial population on the teat skin and a vast amount of published evidence shows that this practice reduces the infection rate amongst dairy cows ([Nickerson, 2001](#); [VanderWal, 1995](#)). A variety of germicides in various teat dips destroy bacteria through chemical or biological action, such as oxidation-reduction

mechanisms, denaturation/precipitation of cytoplasmic proteins, and inhibition of enzyme activity and disruption of cell membranes ([Nickerson,2001](#)).

The efficacy of presently available teat dipping during the dry period however, has been discouraging. Daily teat dipping during the first week post drying off does not prevent IMI with SUB. The latter could partly be explained by the fact that SUB does not colonize in teat canals. A study done by Timms ([1996](#)) on the persistence of various teat dips on the skin of peripartal cows and cows in their early dry period has shown large variations. The average retention time for the persistency of commercially available barrier teat dips ranged from 0 to 18 hours during the peripartal period and 11 to 35,5 hours in the early dry period. Variability was seen within a dip across animals (teat size, shape and condition).

In general, the effectiveness of teat dipping is also dependent upon the prophylactic, curative hygiene management and care and the organic load on the teat skin. Other negative effects of teat dipping include irritation of the teat skin by causing chapping, drying, or a caustic reaction. This is usually due to the chemical composition of the germicide itself, too low or too high pH, breakdown products resulting from improper storage (temperature) and dilutions with non-potable water ([Nickerson,2001](#)).

2.4.5. Teat sealant

A teat sealant was developed for the most vulnerable periods of the dry period of a cow. The idea is to provide an additional barrier for bacteria such as SUB, preventing them from entering the teat canal ([Bradley and Green, 2000](#)). A teat sealant should be used together with proper teat skin care and management.

2.4.5.1 Internal teat sealant

Internal teat seals are gel like and are administered via the teat canal into the teat and udder cistern. They are removed (milked out) with the first milking post calving. Internal teat sealants may be helpful in preventing environmental IMI during the post drying off and immediate prepartal periods. A teat sealant infused into each teat has been developed in Ireland ([Meaney,1977](#)). The sealant is infused into the teats following the last milking prior to drying off. The material remains in the teat cistern and prevents pathogens from gaining entrance to the parenchyme of the mammary gland. A 90% reduction in new infections was reported ([Woolford, Williamson, Day and Copeman, 1998](#); [Meaney,1977](#)). Recent studies have attempted to improve upon

the teat sealant by adding a non-antibiotic bactericidal substance, lacticin 3147 ([Ryan, Flynn, Hill, Ross and Meaney, 1999](#); [Ryan, Hill, Ross and Meaney, 1998](#));).

2.4.5.2 External teat sealant

External teat sealants are applied on the outside of the teat and form a second skin on the teat that peels off in time. A primary goal of mastitis control during the dry period is to minimize bacterial exposure on the teat end at a time when the immunological factors in the udder are pre-occupied or suppressed during the first and last weeks of the dry period. A polyether-polymethane product has been developed to act as a external teat seal (barrier type teat dip) ([Corbellini, Benzaquen, Weinmaier, Introzzi C and Janowics, 2001](#); [Mellenberger, 1997](#); [Timms, 1996](#)), which can persist on the teat ends of dry cows for several days to prevent new IMI without harm to the teat tissue. Pending its effectiveness to adhere to the teat opening until the keratin teat plug has formed in the teat canal, this product has potential for protection against environmental streptococci. Corbellini et.al. ([2001](#)), Mellenberger ([1997](#)) and Timms ([1996](#)) conducted field trials using an experimental persistent teat sealant that was shown to persist for more than 3 days on 98% of dipped teats of dry cows. Cows were teat dipped once at drying off following dry-cow treatment of 47, 52, 68 and 43%. Teat dipped cows showed a reduction in total, major pathogens, environmental *Streptococcus spp.* and CNS intramammary infections. An improved product formulation, tested by Hemling, Henderson, Leslie, Lim and Timms ([2000](#)), was found to provide protection to 70% of the teats for just over 7 days. It is thought that the external teat sealant may become a future alternative to dry-cow treatment in herds with low SCC.

2.4.6. Immunoprophylaxis

A great deal of research aimed at improving resistance of dairy cows to mastitis has been done. Research focuses on the production of vaccines against STA and *E. coli*.

A polyvalent STA vaccine developed in Australia showed an overall decrease in clinical STA infections and levels of sub-clinical mastitis. More studies are however, needed to validate these findings. Heifers were vaccinated at 6 months of age, and at 2-month intervals. The treated heifers showed significantly lower infection rates during pregnancy than the untreated controls (14,4 versus 25,9%). At calving, 8,9% of the vaccinated heifers were infected, compared to 16,1% of the unvaccinated heifers ([Nickerson, Owens, Tomita and Widel, 1999](#)).

The clustering of *E. coli* infections around the time of calving lends itself to control by Gram-negative vaccines. At least two vaccines are available for the control of *E. coli* mastitis. Protection with J5 (a rough mutant strain of *E. coli*) has been explained as being a straightforward mechanism of enhanced antibody production resulting in increased opsonization of coliform bacteria and lipo-polysaccharides. The possibility that the J5 vaccine could decrease the risk factor for coliform mastitis, such as impaired blood PMN diapedesis, has not yet been investigated. Hogan, Todhunter, Tomita, Smith and Schoenberger (1992) found that the J5 vaccine is not associated with a reduction in the number of new dry-cow IMI, but has reduced the incidence and severity of clinical coliform mastitis during the dry period.

The Enviracor E-coli Mastitis vaccine (Penmellyn Veterinary Group, 2000) is given in a 3-dose course: at drying off, 1 month into the dry period and 2 weeks post calving. Claims are being made that this vaccine renders some protection for the first 100 days of lactation against clinical *E. coli* mastitis (Penmellyn Veterinary Group, 2000). The severity of clinical symptoms of coliform mastitis has been reduced by immunisation with the *E.coli* vaccine. Hogan, Smith, Todhunter and Schoenberger (1992) have reported enhanced opsonizing by serum from vaccinated cows and the opsonization with high serum IgM titres to *E. coli* J5.

Researchers at the Veterinary Infectious Diseases Organisation in Saskatoon, have developed a vaccine for the streptococcal form of mastitis but it is not yet commercially available (Radostits et al.,2000).

2.4.7 Summary

In summary optimal management during the dry period is critical in the successful control of mastitis in a herd. New and old uncured infections have a marked influence on the herd SCC and incidence of clinical mastitis. Blanket dry-cow treatment is highly recommended for the control of mainly contagious and, to a lesser degree, of environmental streptococci. The impact of blanket dry-cow treatment on CNS and coliforms is marginal, if any. Supplemental vitamin E and selenium and balanced trace minerals during the dry period can be beneficial. Vaccinating against coliforms can be helpful in problem herds, but vaccines for environmental streptococci are not yet commercially available. Teat sealants containing non-antibiotic antibacterial compounds appear to have potential. Housing and calving areas for cows during the dry period play a role and cows should be kept clean, cool, dry and comfortable.

CHAPTER 3 : MATERIALS AND METHODS

3.1 Model system

3.1.1 Herd

The trial was conducted over a period of 14 months, from January 2001 until March 2002. The trial veterinarian visited the herd on a weekly basis. The animals used in this study were from a high producing, closed Holstein herd which consisted of approximately 305 lactating cows on a farm in the Cullinan district of South Africa.

3.1.2 General herd management programme

The herd was well managed by a competent, dedicated manager. All animals were clearly identified and health, reproduction and milk production data were recorded using an on-farm computer program (Agrimilk, Software Farm). Rainfall during the trial period on the farm was recorded and entered on a calendar.

The herd was Brucella and Tuberculosis free and vaccinations were kept up to date. Cows were vaccinated at drying off with Scourguard (Pfizer Animal Health, PO Box 783720, Sandton, 2146) and Supavac (Intervet SA, PO Box 4278, Edenvale, 1610). Scourguard contains rota- and coronavirus, *C. perfringens* type C toxoid, K99 and *E. coli* bacterin, while Supavac contains toxoids of *Clostridium botulinum* types C1+2 and D, *C. chauvoei* and *Bacillus anthracis*. Bovishield-4 (Pfizer Animal Health) which contains modified live virus strains of IBR, BVD, PI₃ and bovine RSV viral was injected post-partum prior to breeding. All cows and heifers older than 12 months were vaccinated annually during August/September with Lumpy Skin Disease (Onderstepoort Biological Products (OBP), Private Bag X 7, Onderstepoort, 0110), and Three-day-stiffsickness (OBP). Bovine somatotropin (BST) was used selectively after cows were confirmed pregnant. The hooves were professionally trimmed on a regular basis.

The herd participated in the National Milk Recording Scheme of South Africa (option 2) where individual somatic cell counts, butterfat, protein, lactose and milk production are monitored on a 5 weekly basis. Only somatic cell count results from the above list were used in this trial. The herd had an average daily milk production of 35kg per cow during the trial period. Primiparous cows were milked first, followed by the multiparous cows early in lactation. Colostrum was harvested into a bucket and kept from entering the milkline.

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The calving pattern was all year round and breeding was done by artificial insemination (AI), using local and imported semen. The breeding policy was sound and provided for cows to be bred at the first observed oestrus at approximately 60 days post-partum. The local private veterinarian visited the herd on a 4 weekly basis for routine veterinary examinations.

All heifers were scored for linear conformation by a National Inspector of the Holstein Breeding Society of South Africa during their first lactation and strict selection based on conformation and productive performance was maintained in the herd.

The feeding system for lactating cows in the herd was a well managed total mixed ration (TMR) group feeding system. The rations for lactating cows contained a commercial dairy concentrate, lucerne hay, Smutsfinger grass (*Digitaria erianthus*) hay, maize silage, whole cottonseed and molasses meal. Rations were formulated by a qualified dairy nutritionist to meet NRC nutrient requirements. Dietary components were mixed and offered ad libitum as a complete ration twice daily.

3.1.3 The milking system

Cows were milked three times a day with 8 hour intervals (8h00; 16h00 and 24h0). The milking system was a 25point carousel (DeLaval, P.O. Box 513, Heilbron, 9305) and the initial settings were done according to ISO standards. The system had a low milk line, a high vacuum line, automatic cluster removers (ACR) and a duovac system with sufficient reserve vacuum capacity. Maintenance of the milking system was done on a regular basis and teat liners were changed after 2500 milkings. The milking machine was tested prior to the start and on a monthly basis during the trial period with a Westfalia Separator Pulsotest II. A dry test was performed measuring the pulsation at the short milk tubes of the two by two system. A wet test was performed during milking. The vacuum level and fluctuation was measured at the short milk tube during maximum milk flow (second minute) of high, mid and low producing cows. The system vacuum was lowered by 1,5 kPa after the initial herd test due to too high teat end vacuums. The milking machine was monitored on a regular basis during the trial and second minute teat end vacuum was determined of high, mid and low producers, to minimize the risk of machinemediated mammary gland infections. The ACR were evaluated for efficacy to prevent over or under milking. The flow meters were installed effectively to optimize take-off time and low milk flow at the teat end. Flow meters were installed below the level of the udder and long milk lines were less than one meter in length. The system vacuum setting was based on the second minute teat-end vacuum of the high, mid and low producing cows in the herd. The system settings, layout and maintenance were all set to provide

a low risk milking system to promote udder health. Milkers were evaluated and corrected when necessary on the weekly visits. There was however, no constant supervision in the parlour for other milkings.

3.1.4 Dry-cow management

Dry cows were kept in two groups, an early dry (phase 1) and late dry (phase 2) transition or close-up phase. Cows were shifted to the second phase approximately 21 days prior to their expected calving dates. Drying off dates were calculated and cows were dried off approximately 55 days prior to their expected calving date, or when cows produced less than 10 kg milk per day. Cows due for drying off were kept separately and only fed grass hay for 24-48 hours prior to drying off. Cows were dried off abruptly and shifted to the phase 1 dry cows.

During phase 1 dry off cows and pregnant heifers were kept in small kikuyu grass camps with good quality long hay ad lib. The camps always had a good grass mat and provided a clean environment for the cows, even during rainy weather.

When the cows were moved to the second phase dry cows they were injected with: Multimin+Se (Virbac, Private Bag X115, Halfway House, 1685) which contains manganese 20mg, zinc 20mg and selenium 5mg/ml, Vit-A-Plus (Milborrow, distributed by Bayer Animal Health, PO Box 143, Isando, 1600) containing Vitamin A 5 000 000iu and Vitamin E 50iu/ml and Ivomec (Merial South Africa, P.O. Box 5924, Halfway House, 1685) containing Ivermectin 1%*m/v*.

Dry cows and heifers in phase 2 were kept in well "matted" grass camps close to the house to enhance partus observation. These animals were fed a close-up TMR in preparation of the next lactation. The ration consisted of a commercial dry-cow dairy concentrate, Smutsfinger grass hay, maize silage, molasses meal and a trace mineral / vitamin premix. The close-up ration was formulated to meet NRC nutrient requirements for the transitional cow. Animals received this ration until calving, after which they were switched to the normal feeding programme of the lactating cows.

Cows calved on grass in kikuyu paddocks and calves were taken away from cows within 3-12 hours after calving and given colostrum. Cows immediately joined the fresh in milk group where TMR was given ad lib. First time calvers were put in a primiparous cow group post partum and remained separate from the multiparous cows for their first lactation.

3.2 Experimental design and procedure

3.2.1 Initial herd survey

A preliminary herd survey was performed to determine the udder health status of the herd at the commencement of the trial. Foremilk quarter samples were aseptically collected from all lactating and non-lactating cows in the herd (excluding pregnant heifers) according to the International Dairy Federation (IDF) guidelines and standards. Udders and teats were examined clinically during sampling and milk samples were placed on ice and transported to the milk laboratory within 6 hours of sampling. Bulk tank milk samples were taken on a weekly basis to monitor the udder health and milk hygiene status of the herd.

3.2.2 Experimental animals

All cows due for drying off, regardless of parity and milk production, were eligible for inclusion in the trial. Cows with clinical mastitis at drying off or cows that had been treated with antimicrobials within the preceding 14 days and cows with distinctly damaged parenchyme were excluded from the trial.

The number of animals used in the trial was calculated statistically prior to the start of the trial, based on the prevalence of clinical and sub-clinical mastitis in the herd during the initial herd survey. Based on this, 25-35 cows were needed per product tested. A second analysis during the trial was done to re-determine sample size, once data from 20 cows per product was available at that stage.

In order to determine whether further data collection by enrolling more cows in the study was likely to significantly influence the results obtained, a preliminary data analysis was done. New infection rates and cure-rates were compared between groups using Fisher's Exact Test on the complete results obtained thus far from 462 (3x154) quarters. Few statistically significant differences were found between groups. Simulation of further data collection was then done by multiplying each cell in the contingency tables by 1,25 and 1,5 (to simulate the collection of 25% and 50% more data respectively). This was found to result in very little change in the outcome, with marginal changes in significance in three out of 15 between-group comparisons for new infection rates and in one out of 15 between-group comparisons for cure-rates. It was concluded that the enrolment of further cows in the study would probably not materially alter the conclusions of the study and would not justify the further expense and time it would require.

3.2.3 Sampling

Three types of milk samples were collected, i.e. quarter milk samples, composite cow samples and bulk tank milk samples. Quarter and bulk tank milk samples were collected on a weekly basis by the researcher while the herd manager collected cow milk samples on a five weekly basis. Quarter and bulk milk samples and clinical examinations for this trial were done during the 8h00 milking.

3.2.3.1 Sample schedules

3.2.3.1.1 Quarter foremilk samples at drying off.

All cows due for drying off and eligible for inclusion in the trial, were sampled twice prior to drying off. The first of the two samplings was done 24 hours prior to drying off by the manager and kept refrigerated at 4°C for 24 hours. A second sample was collected at drying off just prior to the last milking by the trial veterinarian during the scheduled weekly visits. Only results from the sampling at drying off was used in this dissertation.

3.2.3.1.2 Quarter foremilk samples post calving.

All cows participating in the trial were sampled twice after calving. The first samples were collected as soon as possible after calving but no longer than 7 days after and the second set of samples was taken 7 days later. Data of the first sampling was used in this dissertation.

Results from the other two quarter milk sample sets were only used in the calculation effect of the lowering of the to the SCC limit from 500 000 cells/ml milk to 400 000 cells/ml milk on the diagnosis of mastitis.

3.2.3.1.3 Presentation quarter milk results

A total of 648 quarters were treated with the six intra-mammary at drying off. Three products i.e. Nafpenzal DC, Rilixine 500 DC and Bovaclox DC were used to treat 108 quarters each, 112 quarters each were treated with Orbenin Extra DC and Cpraviv Dry Cow, while 100 quarters were treated with Dispolac Dry Cow.

The number of quarters treated per product varied slightly mainly due to eliminated of cows from the trial due to the following reasons:

- Two cows that contracted clinical mastitis during their dry period were removed from the trial.

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- Five cows were withdrawn as a result of death or other reasons (including contaminated samples).
- Eight cows completed their dry period, calved and were sampled post-calving but did not complete the first three months of their lactation due to culling for various reasons.

3.2.3.1.4 Bulk tank milk samples.

Bulk tank milk samples from both tanks were collected aseptically throughout the trial period every 7 days when the farm was visited.

3.2.3.1.5 National Milk Recording Scheme

The herd manager collected composite cow samples for the National Milk Recording Scheme (Lacto Lab, P.O. Box 326, Irene) from all cows in the herd on a fixed 5 weekly routine. No colostrum milk was used and cows had to be in milk for at least 10 days prior to their participation in the scheme. Milk production and quality data (protein, butterfat, lactose, milk urea nitrogen (MUN) and SCC) for the first three milk recording test dates after calving were obtained from all experimental cows participating in the trial. Protein, butterfat, lactose, MUN and milk production information was used only for herd management purposes. The SCC of the six treatment groups was compared during early lactation.

3.2.3.1.6 Presentation of cow milk results

Individual cow milk samples were collected from 154 cows post calving on three first consecutive samplings of the National Milk Recording Scheme. This brings the total number of cow milk samples post calving to 462.

3.2.3.2 Sampling procedures

3.2.3.2.1 Collecting quarter foremilk samples

For cows included in the study, the udder was prepared for milking as usual. Dirty udders were washed and then dried with individual disposable paper towels, while clean udders were only dry wiped with individual disposable paper towels. At least three jets of milk were milked into a strip cup to evaluate the clinical appearance of the milk and to flush out contaminants. Following foremilk procedures and disinfection

of the teat ends, approximately 5ml of milk was collected aseptically from each quarter of the cow. Teat ends were thoroughly cleaned and disinfected with a cotton wool swab moistened in methylated spirits, starting at the teats on the far side of the udder and working to the near side. The teat was taken between the index finger and the thumb, approximately 10mm above the teat canal opening and was gently squeezed to open the teat orifice slightly. The teat orifice and surrounding area were thoroughly cleaned with the moistened cotton wool swab. Milk samples were collected in sterile, marked containers starting at the teats closest to the operator. The caps of the sample container were twisted open with the left hand and then held firmly with the open end facing down. Holding the open end of the sample container at a 45° angle to the floor in order to prevent dust or material from falling into it, the container was then filled with milk, closed immediately afterwards and placed on ice in a cooler box ([Giesecke et al.,1994](#)). All trial samples, except for those taken 24 hours prior to drying off, were processed in the laboratory within 8 hours after sampling.

3.2.3.2.2 Collection of bulk tank milk samples

Sampling only commenced after all cows were milked in the parlour and after the temperature of the milk in the tanks was below 7°C. The milk in the tanks was stirred for 5 minutes before the milk was collected with a special sterile sample collector. The milk was immediately transferred from the collector to a marked, sterile 100ml container with a screw cap. A second person opened the container and held the cap in his hand facing down to prevent dust or material from falling into it and closed it again immediately after the milk was transferred. The containers were placed on ice and transported to the laboratory and processed within 6 hours after collection.

3.2.3.2.3 Collecting samples for the National Milk Recording Scheme

Samples were collected during the morning milking with a special in-line apparatus that collects a fraction of the total milk production of the cow by the farm manager. Milk was then transferred to a 50ml marked sample container containing Bronopol as preservative. These samples were transported by road to the laboratory of the National Milk Recording Scheme at Irene within 48 hours of sampling where samples were analysed.

3.2.4 Clinical procedure

All udders were examined clinically prior to drying off thus after the cow was milked out for the last time. Thereafter they were examined weekly during their dry period and post calving (after milking) by the trial veterinarian. Due to physiological oedema, peripartal udders were examined weekly post-calving until oedema had subsided and a proper examination could be performed. The manager observed the animals on a daily basis for abnormalities or other suspicious conditions.

The mammary glands were observed prior to palpation for milk dripping from the teats, lesions on the teats or udder skin and asymmetry of the udder. An inspection and palpation of the mammary gland was then performed.

3.2.4.1 Clinical examination of the teat orifice

Short-term changes in teat condition such as colour, firmness, thickening or swelling of teat was evaluated according to recommendations of the "Teat Club International" ([Mein, Neijenhuis, Morgan, Reinemann, Hillerton, Baines, Ohnstad, Rasmussen, Timms, Britt, Fransworth, Cook and Hemling, 2001](#)).

The teat orifices were examined prior to milking. The teat tip was held between the thumb and index finger and tilted so that the teat orifice could be examined. The teat orifices were examined for the presence of lesions and evaginations of the teat canal, hyperkeratosis and petechiae around the teat tip. The condition of teat orifices was scored according to the criteria presented in table 3.1.

Table 3.1 Criteria for clinical examination of teat openings based on a teat canal score

TEAT OPENING SCORE	DESCRIPTION
Score 1	The surface around the teat opening is smooth (normal)
Score 2	A raised ring is visible around teat opening
Score 3	Small cracks are visible in a ring around the teat opening
Score 4	Large cracks and teat canal eversion are visible
Score 5	Mechanical damage to the teat tip, ulcerations, warts are present

Adapted from the University of Vermont teat skin and teat evaluation system.

The teat tip and teat canal were further examined for any irregularities such as thickening (hypertrophy) by rolling the teat tip lightly between the thumb and index finger. The teat cistern was examined for thickening or irregularities by using the same rolling action.

3.2.4.2 Clinical examination of the mammary parenchyme

Clinica; examination of the mammary parenchym was performed post milking in the lactation cows. Superficial palpation of the udder was performed after milking in lactation cows to examine the texture of the udder parenchyme and to detect attachment of the skin to the parenchyme and areas of increased temperature or pain associated with touch. This was done by lightly moving four fingers of the hand in circular movements over the surface of the udder. All lesions were described and recorded.

Deep palpation was done to detect pain and texture of the udder tissue. Chronic cases of mastitis may have hard granular tissue or may be atrophied. Two hands were used on both sides of the udder. Circular movements were executed more firmly than with superficial palpation and signs of pain and abnormalities in the texture of the tissue were recorded. Clinical mastitis was diagnosed based on the presence of pain, heat and redness of the mammary gland and/or with oedema present to a greater or lesser extent ([Giesecke et al., 1994](#)).

Table 3.2 Criteria for clinical examination of the parenchyme of the mammary gland

UDDER PARENCHYME	DESCRIPTION
0	Normal pliability and elasticity of the glandular tissue, fine and soft glandular tissue
F 1	Slight diffuse fibrosis with few slight coarse granular indurations
F 2	Distinct fibrosis with multiple coarse granular indurations
F 3	Marked fibrosis with generally coarse multiple extensive indurations
N 1	Single distinct induration 2,5-5 cm in diameter
N 2	Single distinct induration 5-7,5 cm in diameter
N 3	Single distinct induration larger than 7,5 cm in diameter
A 1	Slight atrophy of a quarter
A 2	Distinct atrophy of a quarter
A 3	Marked atrophy of a quarter

Adapted from [Giesecke and van den Heever, 1974](#). (F=Fibrosis; N=Nodule; A=Atrophy)

From the time of treatment at drying off until calving, no udder secretion was removed from experimental cows, unless mastitis was suspected. When mastitis was suspected during the dry period, one jet of udder secretion was milked into a stripcup and examined for purulent admixtures or distinct floccules. If present, a sample of the udder secretion was collected aseptically for bacteriological examination. Where bacteriological data suggested septic mastitis, the affected quarter was treated and the animal withdrawn from the trial.

3.2.4.3 Udder conformation (Udder depth)

One linear descriptive trait, udder depth, was recorded objectively. The scoring of the udder depth was done in relation to the stature of the cow using a score that ranged from 1 to 9. The distance from the floor of the udder to the ground was used as a basis for udder depth. The hocks were taken as a reference point, which contributes positively to the accuracy and objectivity of scoring ([Hamoen, 1995](#)). A score of 3 was given to udders whose udder floor was the same height as the hocks. The shallower the udder, the higher the score for the udder. Scores differ approximately 2 cm in height from each other.

3.2.4.4 Body Condition Scoring (BCS)

The animals were condition scored at drying-off and at the first examination after calving. The Mulvany scale ([Mulvany, 1981](#)), a subjective numerical condition scoring system on a scale of 1 to 5, with half scales in between, was used. A score of 1 represents an emaciated cow and 5 an obese cow. Scoring was done through observation and palpation of the ribs, loin vertebrae, hip and pin bones, as well as the area around the root of the tail. The degree of muscle and fat filling was evaluated.

3.2.5 Dry-cow treatment

3.2.5.1 Allocation of treatments

Cows due for drying off were treated in all four quarters with one of the dry-cow intramammary antibiotics. The cows were allocated randomly to one of the six products. The first, seventh, thirteenth etc. cow was dried-off with product 1; the second, eighth, fourteenth etc. cow was dried-off with product 2 and so forth. No untreated cows were included in the trial as negative controls. The objective was not to prove but to compare the efficacy of the treatments with each other.

3.2.5.2 Administration

Cows were dried off abruptly after the last milking. They were milked out completely and milk production was recorded. Udders were then wiped clean with a disposable paper towel and teat ends were thoroughly cleaned with a cotton wool swab moistened with methylated spirits, starting at the teats on the far side of the udder. The teats on the far side were cleaned first and the teats nearest to the operator were treated first. All four quarters of the mammary gland were treated with the same

product. The nozzle of the applicator syringe was gently and only partially inserted into the teat canal (not more than 4mm) to prevent damage to the teat canal and super-infection. The bulk of the preparation was then injected into the teat and massaged upwards into the udder cistern. This was followed by a gentle stripping action to leave a drop of the antibiotic product suspended from the teat opening to ensure the presence of antibiotic at the teat tip. All teats were then dipped with a registered teat dip. Cows were kept standing for at least 30 minutes afterwards to allow closing of the teat canal and to prevent bacterial introduction into the exposed teat opening.

3.2.5.3 Products investigated

One of the six products used in this trial was registered by the Medicines Control Council of South Africa and one under Act 36 of 1947 as intramammary dry-cow antibiotic preparations while the other five were registered under Act 101. The products were stored below 25 °C and transported to the farm on ice in a cooler box prior to use.

The products used in this trial were:

- Bovaclox DC, 83/32, (Centaur, P.O. Box 3166, Middelburg, 1050),
- Dispolac Dry Cow, G 797, (Intervet SA, P.O. Box 4287, Edenvale, 1610),
- Nafpenzal DC, 83/668, (Intervet SA, P.O. Box 4287, Edenvale, 1610),
- Orbenin Extra DC, 91/24.1/6, (Pfizer Animal Health, P.O. Box 783720, Sandton, 2146),
- Cepravin Dry Cow, 83/639, (Schering-Plough Animal Health, P.O. Box 46, Isando, 1600)
- Rilexine 500 DC, 83/639, (Logos Agvet (Virbac)), Private Bag X 115, Halfway House, 1685).

A summary of the products, their compositions, claims to their spectrum and effective tissue concentrations are given in table 3.3.

Table 3.3 A summary of the intramammary antibiotic products used in this comparative investigation

No.	PRODUCT NAME	COMPOSITION	Indication for use	Effective tissue concentrations*
1	Nafpenzal DC	Procaine benzylpenicillin 297,92mg (300 000 iu), nafcillin 100mg and Hydrostreptomycin 100mg.	A wide range of bacteria, including penicillin resistant staphylococci and coliforms	Up to 8 weeks
2	Rilexine 500 DC	Cephalexin 250mg and Neomycin sulphate 250mg	Subchronic and subclinical infections	4 weeks
3	Dispolac Dry Cow	Procaine benzylpenicillin 4,9% m/m and dihydrostreptomycin SO ₄ 6,5% m/m	Common forms of bovine mastitis.	2 weeks
4	Orbenin Extra DC	Specially processed cloxacillin 600mg (benzathine salt) in a long-acting base with 3% aluminium mono-stearate.	Sensitive Gram-positive organisms	Up to 7 weeks
5	Cepravin Dry Cow	Cephalonium, 250mg in a long-acting base	Gram-positive and Gram-negative bacteria	Up to 10 weeks
6	Bovaclox DC	Cloxacillin (benzathine salt) 500mg, ampicillin 250mg (as the trihydrate) in a long-acting base with 3% aluminium stearate.	Gram-positive and Gram-negative organisms	Up to 4 weeks

Adapted from the [IVS Desk Reference](#), volume 6, 2001/2. (*Effective time is the time during which the tissue concentrations of the active ingredients are above the MIC most common mastitogenic pathogens)

3.2.6 Laboratory procedures

Laboratory tests on quarter and bulk tank milk samples for this trial were conducted in the milk laboratory (Milk Laboratory, Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Private Bag X 04, Onderstepoort, 0110) and SCC on cow milk samples at the laboratory of the National Milk Recording Scheme: Lacto Lab (P.O. Box 326, Irene, 0062).

3.2.6.1 Clinical inspection of milk samples

Quarter milk samples were inspected visually to supplement findings of clinical udder examination and to assess whether the udder function was normal. Milk samples were inspected for discolouration (blood), changes in consistency (flocules) and in composition (watery or serum like).

The manager collected the cow milk samples for the National Milk recording Scheme. These milk samples were discoloured due to the preservative in the sample container and were not examined for clinical abnormalities.

3.2.6.2 Microbiology ([Sandholm et al., 1995](#)) on quarter milk samples

Milk samples were cultured on bovine blood tryptose agar (Columbia Blood Agar Base, CM331 from Oxoid plus defibrinated bovine blood), which supports the growth of most mastitogenic pathogens. Inoculated agar plates were incubated for 18-24 hours at 37°C ±1°C and then evaluated for growth and re-incubated and read again for a further 24 hours if no growth was present. Colonies were tentatively identified based on colony morphology, appearance and haemolysis.

The catalase test was performed and bacterial isolates were Gram-stained to distinguish between Gram-negative and Gram-positive micro-organisms. Gram-positive cocci that were catalase negative were tested by means of the CAMP /aesculin hydrolysis test (Columbia Blood Agar Base, CM331 from Oxoid with ferric citrate and aesculin plus defibrinated bovine blood). The diagnosis of streptococci was confirmed by means of the Streptococcal Grouping Kit (Latex agglutination test) from Oxoid (CA Milsch, P.O. Box 943, Krugersdorp, 1740).

Gram-positive cocci which tested catalase positive were tested for coagulase by means of the Staphylase Test from Oxoid. Gram-negative organisms were identified using the API 20E from bioMérieux (Omnimed, P.O. Box 4328, Honeydew, 2040).

No microbiological examinations were performed on the cow samples. These samples were preserved with bronopol, which inhibits growth of micro-organisms.

3.2.6.3 Somatic cell count (SCC) determination

After milk was cultured, the remainder of each quarter sample was fixed with potassium dichromate for 18 hours. Milk samples were preheated in a water bath at 37°C (Van den Heever, Katz, Prinsloo, Giesecke, Rawlings and Jones, 1983) and then counted with a Fossomatic 90 counter (The Rhine Ruhr Group, P.O. Box 76167, Wendywood, 2144). Internal standard samples were used weekly to assess the repeatability of the counts.

Cow milk samples were collected on the farm in containers that already contained a preservative, bronopol. At Lacto Lab the milk samples were preheated at 40 °C for at least 5 minutes and then analysed with a Fossomatic 4000 (The Rhine Ruhr Group, P.O. Box 76167, Wendywood, 2144) or a Fossomatic 6000 (The Rhine Ruhr Group, P.O. Box 76167, Wendywood, 2144) for SCC, protein, lactose, butterfat and MUN. External standard samples were used daily to assess the accuracy of the counts.

3.2.6.4 Laboratory procedure on bulk tank milk samples

Quantification of the bacterial colonies present per ml of bulk tank milk was determined by means of the dehydrated film method (3M Petrifilm Aerobic Count Plate, 6400). The presence of *Escherichia coli* type 1 was determined and coliforms quantified with the dehydrated film method (3M Petrifilm *E. coli* / Coliform Count Plate, 6404).

3.2.7 Data management

Relevant data from clinical examinations and observations per cow and laboratory results from both Lacto Lab and the milk laboratory at the University of Pretoria were entered, stored and analyzed in the Milk Sample Diagnostic (MSD) computer program (Udder Health Program, Abaci Systems, P.O. Box 12995, Sinoville, Pretoria, 0127) and in Microsoft Excell.

Basic definitions of udder health are given in table 3.4.

Table 3.4 Diagnosis at calving

Diagnosis	Clinical examination of quarter and / or milk	Bacteriology	Somatic cell count (SCC)
Septic mastitis (M)	Positive	Positive with the same bacteria in one or two of the samples	400 000 or more cells per ml milk in one or both of the samples
Non-specific disturbance (NSD)	Positive	Negative	400 000 or more cells per ml milk in one or both of the samples
Mastitis suspicious	Negative	Inconsistent	Inconsistent
Mastitis negative (N)	Negative	Negative	Fewer than 400 000 cells per ml milk
Teat canal infection (TCI)	Negative	Positive	Fewer than 400 000 cells per ml milk

Data was also entered into a spreadsheet (Excel, Microsoft Corporation) and the MSD Udder Health software program (Abaci Systems). Statistical analysis was done using a commercial statistical software package (NCSS 2001, NCSS, Kaysville, UT, USA).

3.2.7.1 Criteria for assessing efficacy

A quarter was considered infected at drying off when a specific udder pathogen was isolated from the milk sample collected immediately prior to treatment. A quarter was regarded as cured when no pathogen, or a different pathogen to the pathogen isolated at drying off, was isolated at calving.

A new quarter infection acquired during the dry period was recorded when a quarter that was not infected at drying off or a quarter infected with a different pathogen yielded a pathogen at calving. It was thus possible that the same quarter could be cured and may have contracted a new IMI during the dry period.

3.2.7.2 Statistical analysis

Statistical analysis was done using a commercial statistical software package (NCSS 2001, NCSS, Kaysville, UT, USA).

The chi-squared test was used to test the significance of the association between various factors and the two outcomes, i.e. cure rate and new infection rate. Results with a P-value of greater than 0,05 were been regarded as significant. The factors examined were type of micro-organism isolated at drying off, teat canal score at drying off and after calving, udder parenchyme score at drying off and after calving, udder depth at drying off and after calving, change in udder depth from drying off to calving, milk production at drying off, lactation number, length of the dry period and total rainfall during the dry period. All this information was recorded during the trial. For this purpose, the daily milk production at drying off was classified as high if it exceeded 18 litres, otherwise low; lactation numbers were grouped into first lactation and 2nd+ lactation; the dry period was classified as normal if it was 60 days or less, long if it was 61-80days, otherwise very long; and the environment during the dry period of the cows was classified as dry if total rainfall was 0mm, medium if rainfall was 1-150mm, otherwise wet.

The Kruskal-Walles Multiple analyses were used to analyse SCC results of cow milk samples to determine if treatment groups differed significantly.

Comparison of treatment groups

Each unique pair of treatments was compared using the Fisher exact test in order to determine whether there was a significant difference in the outcome (i.e. a significant association between treatment and outcome). For the comparison of cure rates only those quarters classified as infected at drying off were included. New intramammary infections were those cases were uninfected quarters at drying were infected at calving and infected quarters at drying off that were subsequently cured but that were reinfected with a different pathogen during the dry period.

Due to the random selection of cows the percentage intramammary infections differed for each product at the start of the trial. To compensate for this initial variation, percentage point changes from drying off until calving were calculated for each antimicrobial product, taking both the cure-rates and new intramammary infections into account.

CHAPTER 4 : RESULTS AND DISCUSSION

4.1 Initial Herd survey

4.1.1 Herd prevalence of mastitis

The initial herd survey prior to this study of the 305 lactating cows revealed a prevalence of mastitis (M) of 6,42% and teat canal infection (TCI) of 6,91%. There were 68,96% normal (N) quarters and 17,71% cases of non-specific disturbance (NSD). Of the NSD cases diagnosed, 56,2% were diagnosed in one quarter, 28,8% in two, 11,8% in three and 3,2% in all four quarters per cow. The bacterial profile revealed a total quarter infection rate of 13,33%.

In most countries the prevalence of infection of mastitogenic pathogens in dairy herds is approximately 50% of cows and 10 to 25% of quarters (Radostits et al., 2000). The prevalence of 13,33% of quarters observed in the trial herd therefore compares favourably with the literature. The 17,7% cases with NSD however, was substantially higher than the ideal (National Mastitis Council, 2001; Smith, 1996). NSD was present in only 2,7% of udders in three and four quarters, indicating mainly a non-physiological cause (see 4.1.2).

Of micro-organisms isolated, 69,4% were CNS, 24,5% non-agalactiae streptococci, 4,3% STA, 1,2% *E. coli* and 0,6% other bacteria. This bacterial profile differed substantially from results published by the National Veterinary and Food Research Institute in Finland ([Honkanen-Buzalski and Myllys, 1996](#)), which found a substantially lower percentage of CNS and non-agalactiae streptococci, and more intramammary infections with STA and coliforms.

4.1.2 Milking machine analysis

Pulsograph results measured with a Westfalia Separator Pulsotest II, revealed a work:rest ratio of 65:35, a 15% D- phase, a 12,2% A- phase, a 8,0% C- phase and 60,1 pulses per minute. There was a 3 percentage point difference between channels of the alternative pulsation and a system vacuum of 43,2kPa. Especially primiparous cows were showing discomfort towards the end of milking and had an average teat canal score of 2,8 indicating excessive teat canal damage. Pulsograph results were in accordance with ISO standards, except for the C phase and the percentage point variation between channels for the alternative pulsation at cluster units. In time the short C phase could be responsible for low-grade teat-end damage due to the rapid closing movement of the liner on the teat. It is believed that the high prevalence of

NSD in one or two quarters per udder was probably due to a combination of factors. These include a too high teat-end vacuum during milking of medium and low producing cows, a too large variation between the channels of the alternative pulsation and a too slow response time of automatic cluster removers (ACR).

The milking machine plays a very important role in udder health of the modern dairy cow. It is now acknowledged that even less visible damage caused by the milking machine can be associated with an increased risk for mastitis ([Davis, Reinemann and Mein, 2000](#)).

The system vacuum was lowered to 41,6kPa four weeks prior to commencement of this trial, resulting in an average second minute teat-end vacuum of 36,9kPa (33,1kPa in high producers), while the response time of the ACRs was decreased by 5 seconds from 25 seconds to a 20 seconds response time, assisting in the minimizing of overmilking. After the system vacuum was lowered and the ACRs response time decreased, the teat-end vacuum was stable with only a 3,6kPa variation between minimum and maximum levels. First lactating cows were noticeably calmer towards the end of the milking process. The system and teat-end vacuum was monitored regularly during the trial.

4.2 Clinical mastitis during the dry period

Two cows (1,03%) participating in the trial contracted clinical mastitis during their dry periods. One cow was dried off with Cepravin Dry Cow and one with Bovaclox DC 7 and 8 weeks post-treatment. Both cows were withdrawn from the trial.

The number and percentage of clinical cases present in this trial compared favourably to those found in other studies performed during dry periods. Bradley and Green ([2001](#)) reported 3,12% of clinical cases, Williamson et al. ([1995](#)) less than 3,9% while Berry and Hillerton ([2002](#)) found no clinical cases in treated, and 9% in untreated cows. Both clinical mastitis cases in this study occurred towards the end of the dry period, while Williamson et al. ([1995](#)) found that 83% occurred within 21 days of drying off. Clinical cases of mastitis during the dry period could amongst other be positively associated with factors such as reduced feeding prior to drying off, milk yield at drying off, dry-off protocol and pathogens isolated at drying off ([Bramley and Dodd, 1984](#); [Anderson, 1976](#)). Due to the small number of cases in this study no conclusion could be made.

The use of dry-cow therapy is usually associated with fewer cases of clinical mastitis during the dry period ([Berry and Hillerton, 2002](#); [Anderson, 1996](#)). The control of

clinical mastitis during the dry period involves a range of management practices that need to be used in conjunction with dry-cow therapy ([Schukken et al., 1993](#)).

4.3 Overall quarter cure-rate and new intramammary infections (IMI)

In total 162 individual cow records, or 648 quarters samples were available for analysis at drying off and at calving. All cows with clinical mastitis and cows from which milk samples were contaminated were excluded from the trial. The bacteriological results from quarter milk samples, prevalence of IMI at drying off and at calving, cure-rates and new IMI are shown in table 4.1. The prevalence of IMI at drying off and post partum was 29,78% and 22,22% respectively, resulting in a nett reduction of 7,56% during the dry period. The overall cure-rate was 83,94% of quarters and the rate of new IMI during the dry period was 17,44% (table 4.1). Most (78,47%) of the IMI present at calving were new infections.

Table 4.1 Overall quarter intramammary cure-rate and new infection rate

	At drying off	At calving
Total number of quarters tested	648	648
Number of IMI (%)	193 (29,78%)	144 (22,22%)
Number of quarters which remained uninfected for the trial (%)	368 (56,79%)	368 (56,79%)
Number of IMI not cured (%)		31 (16,06%)
Number of IMI cured (%)		162 (83,94%)
Number of IMI cured but re-infected (%)		26 (13,47%)*
Number of new IMI (%)		113 (17,44%)

* These 26 cases were cured and became re-infected. They are both included in with the quarters cured and new IMI.

The recorded prevalence of intra-mammary quarter infections at drying off in this study was higher than in other studies. The prevalence of IMI at the time of drying off has been reported to vary from 5 to 28% of quarters ([Dingwell, 2002](#); [Schukken et al., 1993](#); [Hogan, Smith, Hoblet, 1989](#); [Oliver, 1988](#); [Eberhart, 1986](#)). This wide variation could be due to difference in sampling and schedules ([Eberhart, 1986](#)). Some variation could also be due to differences in the definition of IMI in different studies ([Eberhart, 1986](#)) or in differences in laboratory technique, or in the interpretation of results. The sensitivity of bacteriological culture increase when three or more consecutive milk samples are collected ([Dingwell, Duffield, Keefe, Descoteaux, Kelton, Lissemore, Schukken, Dick and Bagg, 2002](#)) or when the volume of the milk inoculant is increased. Godden, Jansen, Leslie, Smart and Kelton ([2002](#)) isolated more STA organisms from samples that were pre-frozen at -20° C for 14 days than from fresh milk samples.

The primary goal of the dry period, from an udder health perspective, is to minimize the number of quarters infected at the next lactation. This goal could be achieved through the elimination of existing infections (cure-rate) and the prevention of new IMI. Administration of dry-cow therapy at drying off offers the opportunity to eliminate existing IMI and also prevent new IMI occurring during the dry period. Blanket antimicrobial intramammary dry-cow therapy remains a fundamental part of a successful mastitis control program to eliminate mainly existing and prevent new IMI ([Ruegg, 2001](#); [Eberhart, 1986](#)).

The overall cure-rate of 83,94% of IMI during the dry period in this study is in agreement with other studies ([Ruegg, 2001](#)). Generally the reported cure-rates for existing IMI have been relatively high during the dry period. It has been estimated that between 70% and 90% of infections present at drying off can be eliminated with dry cow therapy ([Ruegg, 2001](#)).

New IMI during the dry period have been studied more extensively than the prevalence of IMI at drying off and the cure-rates. The recorded prevalence of new IMI during the dry period in this study is higher than recorded in most other studies. Smith et al. ([1985](#)) recorded a prevalence of between 4% and 12%. This is consistent with other studies that have observed a higher new IMI rate during the dry period of between 13,1% and 24,0%, which is in agreement with the findings of this study. Reasons for variations of results amongst studies can again be due to variation in sampling schedules, differences in the definition of IMI, laboratory technique and in the interpretation of results ([Eberhart, 1986](#)).

The rate of new IMI is many times higher during the dry period compared with the new infection rate during lactation ([Neave et al., 1950](#)). It is well known that the beginning and end of the dry period are the highest risk periods for new IMI ([Bradley and Green 2001](#); [Bradley and Green, 2000](#); [Radostits et al., 2000](#); [Todhunter, Smith, Hogan and Weiss, 1995](#); [Sandholm et al., 1995](#); [Giesecke et al., 1994](#)).

The expected rate of new IMI in bacteria free quarters that did not receive dry cow therapy was reported by Eberhart ([1986](#)) to vary between 8 and 12%. Berry ([2000](#)) however reported new infection rates during the dry period of 34,4% in untreated cows compared to 10,3% in cows that were treated with dry cow therapy. The reduction of new IMI during the dry period with dry cow therapy has been estimated at between 50% and 80% ([Ruegg, 2001](#); [Eberhart, 1986](#)).

The cure-rate and prevalence of new IMI should always be evaluated within the multifactorial context of the total dry and periparturient period. Important factors that may

influence the cure-rate and the susceptibility to new IMI in the dry period have been well documented in the literature ([Dingwell, 2002](#); [Smith and Hogan, 1995](#); [Schukken et al., 1993](#); [Enevoldsen and Sorensen, 1992](#); [Oliver, 1988](#); [Natzke et al., 1975](#)). Amongst others it includes the causative organisms, dry-cow therapy and environmental factors.

4.3.1 Micro-organisms

The prevalence of IMI caused by major and minor micro-organisms, both at drying off and at calving, cure-rates and new IMI that occurred during the dry period are presented in table 4.2. The prevalence of major pathogens at drying off and calving was 7,87% and 4,47% and for minor pathogens 21,91% and 17,75% respectively. There was a significant difference ($p < 0,05$) between the cure-rate for major and minor pathogens during the dry period (table 4,2) as well as the prevalence of new IMI with major and minor pathogens post calving. The most common bacterial pathogen observed after calving in cows with new IMI were CNS, isolated from 70,8% of the cases.

Table 4. 2 Bacteriological results at drying off and post calving, cure-rates and new IMI rates (n=648)

Micro-organism	Prevalence of quarters with IMI at drying off (%)	Cure-rates of IMI during the dry period (%)	Prevalence of quarters with IMI at calving (%)	Prevalence of quarters with new IMI at-calving (%)
Major pathogens				
STA	18 (2,78%)	17 (94,4%)	15 (2,31%)	14 (2,16%)
SAG	23 (3,55%)	23 (100,0%)	11 (1,70%)	11 (1,70%)
SDY	10 (1,54%)	10 (100,0%)	3 (0,46%)	3 (0,46%)
Subtotal	51 (7,87%) ^a	50 (98,0%) ^a	29 (4,47%) ^a	28 (4,32%) ^a
Minor pathogens				
CNS	137 (21,14%)	107 (78,1%)	110 (16,98%)	80 (12,34%)
EFA	2 (0,31%)	2 (100,0%)	2 (0,31%)	2 (0,31%)
Other	2 (0,31%)	2 (100,0%)	2 (0,31%)	2 (0,31%)
ECO	1 (0,15%)	1 (100,0%)	0	0
SUB	0	0	1 (0,15%)	1 (0,16%)
Subtotal	142 (21,91%) ^b	112 (78,9%) ^b	115 (17,75%) ^b	85 (13,12%) ^b
Total	193 (29,78%)	162 (83,9%)	144 (22,22%)	113 (17,44%)

Values within a column with differing superscripts a & b vary significantly ($p < 0.05$)

The dry period offers a valuable opportunity for elimination of existing IMI. The cure-rate of IMI during the dry period was found to vary significantly between major and minor pathogens ([Eberhart,1986](#); [Smith et al.,1985](#)).

This study found that only 10,7% of the major and 5,9% of the minor pathogens that were responsible for new IMI during the dry period were environmental pathogens. This finding differs radically from that of Oliver ([1988](#)) who found up to 95% of new IMI caused by major pathogens during the dry period were caused by environmental pathogens, while only 1% of quarters became infected with contagious pathogens. The significant lower percentage of environmental pathogens that were present in new IMI in this study may be due to well-managed dry cows in an environment with a low potential challenge of bacterial organisms. Trial cows were kept on large well-maintained grass camps on balanced nutrition during their dry period.

4.3.1.1 Cure-rate of major pathogens

The high cure-rate of 100% (n=23) for SAG in this study is consistent with other studies which recorded cure-rates of between 60 and 100% ([Radostits et al.,2000](#); [Sandholm et al. 1995](#)).

The cure-rate of STA intramammary infections of 94,4% (n=17) found in this study was substantially higher than reported in literature. Compared to other pathogens, dry cow treatment is generally acknowledged to be less successful at eliminating existing IMI caused by STA.

There is currently no consensus in literature as to the exact cure-rate of STA intramammary infection during the dry period which has been reported to vary between 20% and 80%. Radostits et al.([2000](#)) reported bacterial cure-rates during the dry period for STA to vary from 25% to 75%. Sol, Harink and Van Uum H ([1992](#)) reported cure-rates varying between 23 - 72%, Erskine et al.([1998](#)) of 21,2% and Nickerson et al.,([1999](#)) of 78,0%. Other authors ([Leslie and Schukken,1999](#); [Hamann, Funke and Schlote,1998](#); [Sandholm et al.,1995](#), [Williamson et al.,1995](#)) reported rates varying between 55% and 61%, while Ziv et al., ([1981](#)) reported cure-rates of more than 80%.

Numerous factors can influence the cure-rate of STA. Newly acquired (< 2weeks durations) STA intramammary infections were found to have a cure-rate of 70%, compared to chronic infections (> 4 weeks duration) of 35% ([Owens et al., 2001](#); [Sol, Sampimon, Barkema and Schukken, 2000](#); [Sandholm et al, 1995](#)). Sandholm et al., ([1995](#)) reported that each month a STA infection persisted in the udder, the prognosis

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worsened by 20%, if the original cure was 100%. The cure-rate of STA is said to decrease with age of the cows (from 81% for cows <48 months to 55% for cows > 96 months) and with the number of infected quarters (from 73% for 1 infected quarter to 56% for 4 infected quarters) ([Radostiits et al.,2000](#); [Sandholm et al.,1995](#); [Sol et al.,1992](#)).

During this study the prevalence of chronic infection with STA in the trial animals was low. STA was isolated from only 0,58% of quarters in the initial herd survey, and had not been isolated from this herd in more than 2% of quarters in any complete herd survey since 1997. Criteria for trial admittance also selected against chronic IMI by eliminating cows with clinically damaged udder parenchyme and/or a history of re-occurring mastitis. STA was isolated from twelve cows (18 quarters) at drying off, of which two cows were infected in two quarters, two in three and eight in a single quarter. Six of these cows were younger than 48 months, while five were older than 96 months. The single STA intramammary infection that was not cured during the dry period was isolated from a cow that was older than 96 months and that was infected in two quarters.

4.3.1.2 Cure-rate of minor pathogens

The cure-rate of 78% for CNS in this study proved to be significantly ($p < 0,05$) lower than the cure-rate for major pathogens during the dry period. In contrast, Sandholm et al. ([1995](#)) and Eberhart ([1986](#)) found significantly higher cure-rates of between 90% and 100%.

CNS were the most prevalent micro-organisms isolated from milk samples in herds using currently recommended control measures and may infect 10%-20% of quarters in well managed herds ([Jones,1998](#)). Aarestrup et al. ([1995](#)) isolated CNS from 70% of heifers prior to calving. Historically, CNS have been referred to as minor pathogens based on observations that they caused only modest increases in SCC and were infrequently associated with clinical mastitis.

Cure and re-infection during the dry period of CNS cases, which was the dominant mastitogenic pathogen during this trial, could not be ruled out as milk samples were only collected at drying off and at calving.

The cure-rate during the dry period of non-agalactiae pathogenic streptococcal IMI was reported to be between 77% and 90% ([Wilson, et al.,1996](#); [Sol et al.,1992](#);

[Eberhart 1986](#)) which correlated with the high cure-rate found in this trial. However, the sample size was very small.

4.3.1.3 New IMI during the dry period with major pathogens

The rate of new IMI with major pathogens for the study varied from 0,46% for SDY to 2,16% for STA. Almost all (96,6%) major pathogens isolated post calving were derived from new IMI (table 4.2).

Although the infected mammary gland of the lactating cow is the main source and reservoir of STA, it may also be present on the skin of teats and external orifices and lesions of cows, bedding, insects and the water supply ([Radostits et al., 2000](#)). STA present in the upper respiratory tract or ears of humans in close contact with the dairy cows can also be a source of infections for IMI in dairy cows (Petzer, unpublished).

A possibility exists that some of the IMI at calving were not new infections, but rather existing infections that were not identified at drying off.

4.3.1.4 New IMI during the dry period with minor pathogens

The rate of new IMI with minor pathogens for the study varied from 0,16% for SUB to 12,34% for CNS. Of the minor pathogens isolated post-calving, 73,9% were new infections (table 4.2).

CNS are amongst the most opportunistic skin flora pathogens ([Smith and Hogan, 1995](#)). The dry period appears to be the origin of many new IMI with CNS ([Harmon, Crist, Hemken, 1986](#)). The pathogenicity of this group of organisms still requires further investigation.

Mastitis control schemes for major pathogens are less effective against CNS, and most developed countries now report CNS as an important cause of IMI ([Smith and Hogan, 2000](#)). Teat dipping during the dry period seems to be ineffective in controlling colonisation of the teat skin with CNS ([Schultze, 1983](#)).

Much is debated about the possible protective role that CNS, when present in the teat canal, may play to prevent IMI with major pathogens. Of quarters infected with minor pathogens at drying off 10,0% became infected with major pathogens during the dry period, while only 3,7% of uninfected quarters became infected with major pathogens. No protective benefit of CNS was therefore found during this trial. Many studies ([Radostits et al.,2000](#); [Østeras et al.,1999](#); [Wilson et al.,1996](#); [Nickerson and](#)

[Boddie,1994](#); [Rainard and Poutrel,1988](#)) documented a protective role of CNS against IMI with major pathogens, while Berry and Hillerton,([2002](#)) found no benefit. Hogan and Smith ([1998](#)) found that quarters infected with CNS were more susceptible to infections with SUB and coliform bacteria.

4.3.2 Efficacy of antimicrobial treatments.

The primary objective of this study was to compare and evaluate the efficacy of six registered intramammary dry-cow products in the elimination of existing and prevention of new IMI during the dry period.

The cure-rates for the different products varied between 72,4% and 93,9% (table 4.3) with an average overall cure-rate of 83,9%. The best cure-rates of 93,9% and 91,5% were reported with Cepravin Dry Cow and Orbenin Extra DC respectively, which were significantly better than those of Dispolac Dry Cow, Bovaclox DC and Nafpenzal DC (table 4.3).

The rate of new infection for quarters that received intramammary dry cow treatment varied between 13,4% and 24,1% for the different products (table 4.3) with an overall rate of new IMI of 17,4%. The intramammary dry cow products that were consistently associated with the lowest percentage of new IMI were Cepravin Dry Cow and Rilexine 500 DC.

Table 4.3 Comparative results of cure-rates and new IMI during the dry period for the various products (n=648)

Products (active ingredients)	Number of IMI at drying off	Number of IMI cured (%)	Number of new IMI at calving (%)
Nafpenzal DC (procaine Benzylpenicillin /nafcillin / Hydrostreptomycin) (n=108)	24	19 (79,2%) ^b	22 (20,4%) ^b
Rilexine 500 DC (cephalexin / neomycin) (n=108)	28	24 (85,7%)	15 (13,9%) ^a
Dispolac Dry Cow (benzyl-Penicillin /dihydrostreptomycin) (n=100)	29	21 (72,4%) ^b	16 (16,0%)
Orbenin Extra DC (cloxacillin) (n=112)	47	43 (91,5%) ^a	27 (24,1%) ^b
Cepravin Dry Cow (cephalonium) (n=112)	33	31 (93,9%) ^a	15 (13,4%) ^a
Bovaclox DC (cloxacillin / ampicillin) (n=108)	32	24 (75,0%) ^b	18 (16,7%)
Total	193	162 (83,9%)	113 (17,4%)

Values within a column with different superscripts vary significantly (p<0.05)

Factors which affect the cure-rate and prevention of new IMI during the dry period have been extensively studied ([National Mastitis Council Factsheet](#), [Bradley and Green, 2001](#); [Østerås et al., 1999](#); [Hamann and Funke, 1999](#); [Williamson et al, 1995](#); [Østerås et al., 1994](#)).

4.3.2.1 Composition of products

Antimicrobial products used in this trial differed substantially in their composition and efficacy in curing existing and preventing new IMI. It should be kept in mind that almost all micro-organisms that were isolated were Gram-positive.

Cure rates during the dry period were the highest with cephalonium and the lowest with a combination of cloxacillin / ampicillin. The lowest percentage of new IMI was observed with cephalonium and a combination of cephalixin / neomycin (table 4.3). Of interest was an observation that the two products with the highest overall cure-rates both contained only one antimicrobial agent, compared to the other 4 products which were combinations of two or more antimicrobials.

Consistent with literature differences in cure- and prevention rates of new IMI were observed in cows treated with various intramammary dry cow products. Fox and Robertson ([1999](#)) found the cure-rate for STA intramammary infection to vary during the dry period from 45% with a penicillin / dihydro-streptomycin based product to 87% with a cephalosporin based product. In contrast, Ziv et al.,([1981](#)) found no differences in the overall efficacy among three products (procaine benzylpenicillin / nafcillin / dihydrostreptomycin, cloxacillin and cephalonium) while the differences amongst herds were large. They found cure-rates greater than 80% in 7 herds after treatment with the combination of procaine benzylpenicillin, nafcillin and dihydrostreptomycin, 8 herds after treatment with cloxacillin and 4 herds after treatment with cephalonium. Ziv et al.,([1981](#)) also found 7,8%, 6,9% and 6,7% new STA intramammary infections in cows treated with cloxacillin, procaine benzylpenicillin / nafcillin / hydrostreptomycin and cephalonium respectively. Da Fonseca, Dos Santos, Pereira and Dos Santos ([2000](#)) found cure-rates of IMI treated with gentamycin to be significantly higher ($p < 0.05$) compared to those treated with cloxacillin, but found no difference in the new infection rates between the two treatment groups during the dry period.

4.3.2.2 Duration of effective therapeutic levels (persistence)

The two products in this trial that contain cloxacillin differed significantly ($p < 0,05$) in their cure-rates i.e. 91,5% and 75,0%. The difference in these results may be as a result of different antimicrobial composition, duration of effective antimicrobial levels and pathogens. The more successful product of the two had a higher concentration of cloxacillin, 600mg compared to 500mg per dose and claimed a longer withdrawal period, i.e. 7 weeks compared to 4 weeks. No significant relationship was however found between the overall cure-rates of IMI and the withdrawal periods (table 4.3). This finding is supported by other studies ([Radostits et al, 2000](#)).

The product which claimed the longest active therapeutic level in the udder, Cepravin Dry Cow, as was the case in efficacy in intramammary cure-rates, was also the most effective in preventing new IMI. There was no significant association found between new IMI during the dry period and withdrawal periods of the products. The most to least successful product in preventing new IMI during the dry period claimed effective therapeutic levels for 10 weeks, 4 weeks, 2 weeks, 4 weeks, 8 weeks and 7 weeks respectively. Other than for Cepravin Dry Cow with a 10 weeks action, it was the short acting products that were the most effective in preventing new IMI. This finding corresponds with the findings of Radostits et al.([2000](#)) and Østerås et al.([1994](#)). Radostits et al.([2000](#)) reported, contrary to expectation, that where the efficacy in cure-rates with long-acting and short-acting dry cow antimicrobial intramammary products were compared, the short-acting intramammary preparations proved to be more effective. Østerås et al.([1994](#)) also found that short-acting, compared to long-acting preparations, had a significantly better effect in preventing new infection with STA and SDY in quarters. Bradley and Green ([2001](#) and [2000](#)) found quarters treated with an extra long-acting (14 weeks) intra-mammary product reduced new coliform IMI by 52%, while Smith et al.([1985](#)) used short-acting intramammary products (3 weeks) and reported that antibiotic dry-cow therapy could not play a role in the control of clinical coliform mastitis post-calving. The latter could be explained by the fact that coliform infections which occurred at the end of the dry period mainly led to IMI post-calving ([Sandholm et al., 1995](#)).

4.3.2.3 Efficacy of various antimicrobial products against different micro-organisms

Table 4.4 provides the comparative microbiological cure-rates versus micro-organisms isolated. Due to the small sample sizes for micro-organisms per treatment group, no statistically meaningful results were obtained and only observations will be reported on.

Table 4.4 Comparative microbial cure-rates of various products

Micro-organism	Number of quarters cured per product (%cure)					
	1*	2*	3*	4*	5*	6*
MAJOR PATHOGENS						
<i>Staphylococcus aureus</i>	3 (100%)	3 (100%)	1 (50%)	4 (100%)	4 (100%)	2 (100%)
<i>Streptococcus agalactiae</i>	3 (100%)	5 (100%)	3 (100%)	3 (100%)	5 (100%)	4 (100%)
<i>Streptococcus dysgalactia</i>	1 (100%)	2 (100%)	0	3 (100%)	3 (100%)	1 (100%)
Subtotal	7 (100%)	10 (100%)	4 (80%)	10 (100%)	12 (100%)	7 (100%)
MINOR PATHOGENS						
Coagulase negative staphylococci	12 (70,1%)	13 (76,5%)	17 (70,8%)	32 (88,9%)	16 (88,9%)	17 (68,0%)
<i>Enterococcus faecalis</i>	0	1 (100%)	0	1 (100%)	0	0
<i>Micrococcus</i> spp.	0	0	0	0	2 (100%)	0
<i>E. coli</i>	0	0	0	0	1 (100%)	0
Sub-total	12 (70,1%)	14 (77,8%)	17 (70,8%)	33 (89,2%)	19 (90,5%)	17 (68,0%)
Grand total	19 (79,2%)	24 (85,7%)	21 (72,4%)	43 (91,5%)	31 (93,9%)	24 (75,0%)

* Product 1=Nafpenzal DC; Product 2=Rilexine 500 DC; Product 3=Dispolac Dry Cow;) Product 4=Orbenin Extra DC; Product 5=Cepravin Dry Cow; Product 6=Bovaclox DC.

All major pathogens were cured during the dry period, except those treated with Dispolac Dry Cow of which 80% were cured. Cure-rates for IMI with minor pathogens varied between 68,0% with Bovaclox DC to 94,0% with Cepravin Dry Cow. Although Bovaclox DC only claims efficacy against Gram-positive bacteria, this however should not have played a role in the lower cure-rate as no Gram-negative bacteria were isolated from quarters that were treated with Bovaclox DC prior drying off.

Table 4.5 illustrates the bacteriological results of new IMI for each treatment group (product). The sample size was however too small to obtain any statistically significant findings.

Table 4.5 Comparative new IMI of pathogens per treatment group

MICRO-ORGANISMS	Treatments					
	(number of quarters treated)					
	1*	2*	3*	4*	5*	6*
	(108)	(108)	(100)	(112)	(112)	(108)
MAJOR PATHOGENS						
<i>Staphylococcus aureus</i>	4 (3,7%)	1 (0,9%)	0	5 (4,5%)	2 (1,8%)	2 (1,8%)
<i>Streptococcus agalactiae</i>	0	1 (0,9%)	1 (1,0%)	8 (7,1%)	0	1 (0,9%)
<i>Streptococcus dysgalactiae</i>	1 (0,9%)	0	0	0	2 (1,8%)	0
Subtotal	5 (4,6%)	2 (1,8%)	1 (1,0%)	13 (11,6%)	4 (3,6%)	3 (2,7%)
MINOR PATHOGENS						
CNS	16 (14,8%)	13 (12,0%)	15 (15,0%)	14 (12,5%)	7 (6,2%)	15 (13,9%)
<i>Streptococcus uberis</i>	1 (0,9%)	0	0	0	0	0
<i>Enterococcus faecalis</i>	0	0	0	0	2 (1,8%)	0
<i>E. coli</i>	0	0	0	0	1 (0,9%)	0
Other micro-organisms	0	0	0	0	1 (0,9%)	0
Sub-total	17 (15,7%)	13 (12,0%)	15 (15,0%)	14 (12,5%)	11 (9,8%)	15 (13,9%)
Grand total	22 (20,4%)	15 (13,9%)	16 (16,0%)	27 (24,1%)	15 (13,4%)	18 (16,7%)

* Product 1= Nafpenzal DC; Product 2=Rilexine 500 DC; Product 3=Dispolac Dry Cow; Product 4=Orbenin Extra DC; Product 5=Cepravin Dry Cow; Product 6=Bovaclox DC.

The percentage of new IMI contracted during the dry period varied per product for major and minor pathogens. This was however not significant due to the small sample size. Dispolac Dry Cow was the most effective and Orbenin Extra DC the least effective in preventing IMI with major pathogens, while Cepravin Dry Cow was the most and Nafpenzal DC the least effective in preventing new IMI with minor pathogens (table 4.5).

Rating of the six antibiotic dry cow products in relation to cure-rates and prevention of new IMI showed qualitative differences between them (table 4.6).

Table 4.6 Rating of efficacy of products for overall cure-rate and prevention of new IMI

	Cure-rate		Preventing new IMI		Overall rating
	Major pathogen	Minor pathogens	Major pathogens	Minor pathogens	
Nafpenzal DC	1	5	5	6	5
Rilexine 500 DC	1	3	2	2	2
Dispolac Dry Cow	2	4	1	5	3
Orbenin Extra DC	1	2	6	3	3
Cepravin Dry Cow	1	1	4	1	1
Bovaclox DC	1	6	3	4	4

1= most effective and 6 = least effective

Dispolac Dry cow was the most effective in preventing new IMI with major pathogens and Cepravin Dry Cow in preventing new IMI with minor pathogens. Cepravin Dry Cow, Nafpenzal DC, Rilexine 500 DC, Orbenin Extra DC and Bovaclox DC were equally effective, while Cepravin Dry Cow was the most effective in curing IMI with minor pathogens and in the overall cure-rate of IMI during the dry period.

The percentage point changes from drying off till calving was calculated for each antimicrobial product (table 4.7). Both the cure-rates and new IMI rate were taken into account.

Table 4.7 Percentage point improvement in IMI from drying off to post-calving for the various products

PRODUCTS	SAMPLING STAGE	% IMI	% POINT IMPROVEMENT IN IMI from drying off to calving
Nafpenzal DC	At drying off	22,22	- 12,5%
	At calving	25,00	
Rilexine 500 DC	At drying off	25,93	23,14%
	At calving	19,45	
Dispolac Dry Cow	At drying off	29,00	20,69%
	At calving	23,00	
Orbenin Extra DC	At drying off	41,96	34,03%
	At calving	27,68	
Cepravin Dry Cow	At drying off	29,46	51,49%
	At calving	14,29	
Bovaclox DC	At drying off	29,63	16,21%
	At calving	24,07	

The overall percentage point improvement in this study varied from an increase in IMI post-calving of –12,50% with Nafpenzal DC to a decrease of 51,49% IMI at calving with Cepravin Dry Cow.

4.3.3 Cow factors

The host defence mechanism is important in affecting the susceptibility of the dairy cow to udder infection ([Nickerson, 1989](#)). Cow factors are associated with the development of specific immunity and with non-specific host defence mechanisms (general resistance).

Cow factors that may influence the cure-rates and the susceptibility to new IMI in the dry period were investigated. These factors were parity, SCC at drying off, milk yield prior to drying off, length of the dry period, udder depth at drying off and teat canal score at drying off and quarter site.

4.3.3.1 Parity at drying off

4.3.3.1.1 Parity and IMI

The percentage of cows that were either ending their first, second or third and further lactations were 54,9%, 21,6% and 23,5% respectively (table 4.8). More than half of the trial cows were thus in their first lactation when they entered the trial. There were no significant differences in cure rates of primiparous cows and multiparous cows (86,8% and 81,4% respectively) (table 4.8). In this trial we particularly selected against cows with possible chronic udder infections (see 3.2.2).

The rate of new IMI (table 4.8) was significantly lower in cows completing their first lactation than in those completing their second and further lactations (12,6% and 23,3% respectively; $p < 0,05$). Multiparous cows were more likely to develop new IMI than younger cows. This finding corresponds with those of Oliver,(1988) who found 2,6% new IMI in cows completing their first or second dry period compared to 23,8% completing more than two lactations.

Table 4.8 Cure- and new IMI rate of primiparous and multiparous cows

Lactation number (% of cows)	IMI at drying off (% of group)	Cured (%)	New IMI (%)
Primiparous: 89 cows (54,9%)	91 (14,0%)	79 (86,8%) ^a	45 (12,6%) ^b
Multiparous: 73 cows (45,1%)	102 (15,7%)	83 (81,4%) ^a	68 (23,3%) ^c

Values within a column with differing superscripts vary significantly ($p < 0.05$)

A relationship between the parity of a cow at the time of drying off and the development of new IMI during the dry period has been well documented ([Radostits et al.,2000](#); [Oliver, 1988](#); [Todhunter et al.,1995](#); [Smith et al.,1985](#)). Increased parity in cows has been described as a risk factor for an increased incidence of clinical mastitis, as well as an higher prevalence of sub-clinical IMI ([Radostits et al.,2000](#); [Dohoo, Meek and Martin, 1984](#); [Schukken et al.,1993](#)). Younger cows have more effective host defence systems through better polymorphonuclear leukocyte function. The higher risk of IMI with increased parity is probably due to the increased opportunity of IMI over time, especially a herd without an effective mastitis control program ([Radostits et al.,2000](#)). With increased parity cows have more damaged teat canals and thus a less effective first line of defence against the intrusion of pathogens ([Lacy-Hubert and Hillerton,1995](#); [Oliver,1988](#); [Smith et al.,1985](#)). Udder depth also increases linearly with parity leaving the udder more vulnerable to injury the more the udder protrudes from the udder shelter ([de Jong,1997](#); [Giesecke et al., 1994](#)) (see 2.2.3.2).

4.3.3.1.2 Parity and quarter somatic cell counts at drying off

As parity increased, so did the level of SCC at drying off. More than half of the first lactation cows (51%) dried off with a quarters SCC of below 250 000 cells/ml , compared to only 21,6% of cows in their second lactation and 6,3% of cows in their sixth lactation (table 4.9).

Table 4.9 Association between parity and quarter SCC at drying off (individual SCC values per quarter)

Lactation No	SCC ranges x percentage (%) distributions				
	1-249 999 cells / ml	250 000 – 499 999 cells / ml	500 000 – 749 999 cells / ml	750 000 – 999 999 cells / ml	999 999+ cells / ml
1 st lactation (356 quarters)	51.0	16.8	5.8	4.7	21.7
2 nd lactation (140 quarters)	21.6	20.0	16.9	6.9	34.6
3 rd lactation (76 quarters)	23.7	7.6	8.7	15.0	45.0
4 th lactation (48 quarters)	8.3	12.5	14.6	8.3	56.3
5 th lactation (12 quarters)	0.0	8.3	8.3	0.0	83.4
> 5 lactations (16 quarters)	6.3	18.7	6.3	0.0	68.7

This study found a positive correlation between cows with a low SCC (individual SCC values per quarter) at drying off (<250 000cells/ml) and parity. This finding is supported by Radostits et al. (2000). SCC normally increases with advancing age and stage of lactation, however little change is observed in uninfected quarters (Eberhart, Gilmore, Hutchinson and Spencer,1976). Younger cows have a decreased susceptibility to mastitis, but the highest incidence of clinical mastitis can be found in primiparous cows compared to multiparous cow (Radostits et al.,2000). Work by Sheldrake, Hoare and McGregor (1983) and Eberhart et al. (1976) showed that if cows were separated into groups by infection status, there was little difference in SCC of uninfected cows, either as a cow aged or was in late lactation. Harmon (1998) suggested that the modest rise in SCC in uninfected quarters at the end of lactation is in fact due to a dilution effect. However, the major influence of parity and stage of lactation on SCC is still related to IMI status (Harmon, 1998).

4.3.3.1.3 Parity and the number of infected quarters per cow

A marked increase was found in the number of quarters infected per cow from the

third dry period. Only 3,33% and 2,86% of quarters of first and second lactation cow respectively were infected in three or four quarters, while 29,1% of quarters of cows in their third or further lactations were infected in three or four quarters (table 4.10).

Table 4.10 Association between parity and the number of infected quarters per cow at drying off (n=648 quarters)

Number of infected quarters per cow	Lactations and totals of quarters tested x number of infected quarters (%) per cow at drying off		
	1 st lactation (%) n=356	2 nd lactation (%) n=140	≥ 3 lactations (%) n= 152
Totals of uninfected quarters	266 (74,72%)	96 (68,57%)	93 (61,18%)
1 quarter	25 (27,78%)	12 (27,27%)	7 (11,86%)
2 quarters	32 (35,56%)	8 (18,19%)	24 (40,68%)
3 quarters	21 (23,33%)	12 (27,27%)	24 (40,68%)
4 quarters	12 (13,33%)	12 (27,27%)	4 (6,78%)
Totals of infected Quarters	90 (25,28%) ^a	44 (31,43%) ^a	59 (38,82%) ^b

Values within a row with differing superscripts vary significantly (p<0.05)

At drying off first lactation cows had 25,3% infected quarters compared to 31,4% and 38,8% quarter infections of cows in second or later lactations respectively. These infections occurred in three to four quarters per udder in 36,6% of first lactation cows, 54,5% of second lactation cows and 47,5% of cows in later lactations (table 4.10). This finding is supported by Sol et al.,(1992) who found that the number of infected quarters per cow increased with age while the cure-rate of IMI decreased. This can be mainly due to a less patent teat canal in older cows and an increased risk of chronic IMI.

4.3.3.2 The position of the quarters versus IMI cure and new IMI

Throughout this study front quarters had fewer new IMI (16,4%) than hindquarters (18,5%). This finding was however, insignificant (p= 0,20). The cure-rates observed in front and hind quarters were almost similar (table 4.11).

Hamann and Funke (1999) found higher cure-rates (72,3%) in front quarters compared to those in hind quarters (61,5%). It could be speculated that the cows front teats milked on a carousel or quick exit system are at a higher risk of teat canal damage when cluster units are removed incorrectly. Milking parlour layout should be taken into consideration when studying quarter-site differences.

Table 4.11 Dynamics of quarter-site IMI

Diagnoses	Front quarters (324)	Hind quarters (324)
IMI at drying off	101 (31,2%) ^a	92 (28,4%) ^a
IMI cured during the dry period	84 (83,2%) ^b	78 (84,8%) ^b
New IMI during the dry period	53 (16,4%) ^c	60 (18,5%) ^c
No IMI throughout the trial	184 (56,8%) ^d	184 (56,8%) ^d
	Left quarters (324)	Right quarters (324)
IMI at drying off	106 (32,7%) ^e	87 (26,9%) ^e
IMI cured during the dry period	89 (84,0%) ^f	73 (83,9%) ^f
New IMI during the dry period	63 (19,4%) ^g	50 (15,4%) ^g
No IMI throughout the trial	172 (53,1%) ^h	196 (60,5%) ⁱ

Values within a row with differed superscripts vary marginally significantly (p<0.06)

This study found that 32,7% of IMI at drying off were contracted in the left and 26,9% in the right side of udders, while 31,2% of front and 28,4% of hind quarters were infected. None of the differences in the quarters-site infections were found to be significant, although right quarters had marginal significantly less IMI throughout the trial period. Østerås et al., (1999) found a highly significant correlation ($p < 0.001$) between the right hind quarters infected with major pathogens at drying off and the lack of successful treatment. Sol et al.,(2000) and Barkema, Galligan, Schukken, Lam, Beiboer and Brand (1997) also reported a quarter-site difference in mammary gland infections. Hind quarters were found to have more infections than front quarters and right front quarters more than left quarters (Østerås et al.,1999). Arave and Walters (1980) reported that cows had a tendency to lie more on their one side and that the incidence of sub-clinical mastitis was higher on the side nearest to the ground. It can be concluded that the environment of the cow, milking system layout, management and mastitogenic pathogens have to be taken into consideration when evaluating quarters-site intramammary infections.

4.3.3.3 Association between quarter somatic cell counts at drying off, cure-rates and new IMI during the dry period.

Quarters that dried off with SCC of more than 750 000 cell/ml milk (42,5%) had more than double the IMI rate at drying off compared to those that dried off with a SCC of below 125 000 cells/ml (20,1%). Quarters with SCC of more than 750 000 cells/ml and IMI at drying off had significant lower cure-rates ($p < 0,05$) than the lower SCC cell count groups (table 4.12).

No statistically significant correlation was found between new IMI and SCC at drying off (table 4.12).

Table 4.12 Association between quarter SCC at drying off, IMI cure-rate and new IMI during the dry period.

SCC at drying off X 1000 (cells/ml)	IMI at drying off (% for group) N=193	IMI cured per SCC group during the dry period (%) n= 162	New IMI at calving per SCC group (%) n = 113
0-125 (n=149)	30 (20,1%)	28 (93,3%) ^a	21 (14,1%) ^a
126-250 (n=85)	25 (29,4%)	22 (88,0%) ^a	12 (14,1%) ^a
251-500 (n=102)	14 (13,7%)	12 (85,7%) ^a	18 (17,6%) ^a
501-750 (n=58)	16 (27,6%)	14 (87,5%) ^a	13 (22,4%) ^a
750+ (n=254)	108 (42,5%)	86 (79,6%) ^b	49 (19,3%) ^a

Values within a column with differing superscripts vary significantly (p<0.05)

In the context of the findings above (table 4.12) one should note that the SCC has pivotal diagnostic, regulatory, breeding and selection implications.

The upper limit for acceptable bulk SCC differs worldwide. The E.U., New Zealand, Australia, Switzerland and Norway all accept 400 000 cells per ml, Canada 500 000 and U.S. 750 000 cells per ml milk. The E.U. is already discussing decreasing the regulatory SCC limit to 300 000 or perhaps even 250 000 cells per ml. ([Smith and Hogen,2000](#)). The International Dairy Federation (IDF) changed the criteria for quarter milk SCC in the diagnosis of mastitis. The SCC was lowered from 500 000 cells/ml milk to 400 000 cell/ml milk as cut-off point for mastitis and non-specific disturbances. The old and new SCC criteria were used and diagnoses were compared in table 4.13 to evaluate the effect thereof in the diagnosis of mastitis. Data of all quarter milk samplings that were taken during was used in table 4.13. This includes the two samplings prior to drying off and the two samplings after calving of the trial cows.

Table 4.13 Old versus new IDF mastitis diagnosis (n=2540 individual quarter milk samples)

	Mastitis (%)	TCl (%)	NSD (%)	N (%)
Old IDF (SCC ≤ 500 000 cells/ml)	349 (13,74)	378 (14,88)	536 (21,10)	1277 (50,27)
New IDF (SCC ≤ 400 000 cells/ml)	373 (14,68)	354 (13,92)	607 (23,89)	1206 (47,48)
Difference (%) New IDF	+ 0,94%	- 0,94%	+ 2,79%	- 2,79 %

Surprisingly no major differences were found in the diagnosis of udder health comparing the cut-off point of SCC of 400 000 and 500 000 cell/ml milk in 2 540 quarter milk samples that were examined. Actually 0,9% more cases of mastitis were

diagnosed and 2,8% more NSD cases at the lower SCC level. The lowering of the SCC will however have diagnostic and regulatory implications.

Selection indices that include SCC, udder depth and clinical mastitis will diminish the rate of increase in mastitis by 20-25% (Radostits et al.,2000). It has been found that daughters of bulls with a high predicted transmitting ability (PTA) for SCC have a higher incidence of mastitis (Radostits et al.,2000). The finding of better cure in low SCC cows is in agreement with work done by Sol et al, (2000). Bodoh, Battista, Schultze and Johnston (1976) found a rise in SCC at the end of lactation, after production had dropped to below 4kg per day.

4.3.3.4 Milk yield at drying off

No significant difference was found between the cure-rates for cows with low milk yields compared to cows with high milk yields at drying off ($p = 0,241$) (table 4.14).

The rate of new IMI at drying off was significantly ($p < 0,05$) higher in cows with high milk yield at drying off (table 4.14). This finding is consistent with other reports, which demonstrates the association with milk yield at drying off and the rate of new IMI (Dingwell 2002; Schukken et al.,1993; Natzke et al.,1975).

Table 4.14 The milk yield at drying off compared to the IMI cure-rate and new IMI during the dry period.

Milk production	IMI at drying off (% of group)	Cured (%)	New infections (%)
≤18 kg (n=342)	106 (31,0%)	87 (82,1%) ^a	40 (13,5) ^b
>18 kg (n=206)	87 (42,2%)	75 (86,2%) ^a	73 (22,5) ^c
Total	193	162	113

Values within a column with differing superscripts vary significantly ($p < 0.05$)

At the time of this study the trial herd was one of the herds with the highest daily milk yield per cow in South Africa. Despite this fact, little preparation was done prior to drying off in lowering the nutritional status. Feed intake was only restricted from one day prior to drying off. The highest milk yield, measured at drying off, during the trial period was 36 kg, and only 5,5% of cows dried off with a milk yield less than 5 kg. More attention should be given to management strategies that decrease milk production prior to drying off. Regardless of the management approach, there is still great variation in the actual milk yield on the day of drying off. Further research in this area is warranted.

The optimal level of production at drying off remains to be determined. The underlying hypothesis is that high intramammary pressure just after drying off leads to of

increase in new IMI ([Dingwell, 2002](#); [Natzke et al.,1975](#)). One of the risk factors an increased intramammary pressure can lead to leaking of milk, a higher risk of bacterial penetration of the teat canal and lower concentrations of lactoferrin, immunoglobulin and phagocytic cells ([Dingwell,2002](#); [Schukken et al., 1993](#); [Natzke et al.,1975](#)).

Dingwell ([2002](#)) and Williamson et.al.,([1995](#)) found an association between milk production at drying off and closure time of the teat canal (teat plug formation). Dingwell ([2002](#)) found that 47% of cows that produced more than 20kg and 19% of cows that produced less than 21 kg at drying off, still had open teat canals 6 weeks into the dry period.

It is speculated that long-term selection pressure for milk production may have had a negative effect on the polymorphism of genes linked to the major histo-compatibility complex (BoLA) in dairy breeds. The genetic correlation between milk yield and mastitis is approximately 0,2 to 0,3, which suggests that animals with an above average milk yield are relatively more susceptible to mastitis ([Radostits et al.,2000](#)). This low positive correlation implies that the selection for high yield is accompanied by a slow decline in genetic resistance to mastitis. High yielding cows have on average shorter teats and higher milk flow. A high milking rate and short teats have been associated with an increased risk of new IMI an increased SCC ([Mein et al.,2001](#); [Hamann, 1996](#)).

4.3.3.5 Length of the dry period

The average length of the dry period of all cows participating in this trial was 73,5 days, exceeding the ideal of 60 days and varying from 21 days (an abortion) to 201 days (one cow that was not pregnant). There was no significant difference between the length of the dry periods between the treatment groups (average ranging from 71,3 to 77,2 days).

Table 4.15 Association between the length of dry period, cure-rate and new IMI during the dry period

Length of dry period (n=quarters)	IMI at drying off (% of group)	Cured (%)	New infections (%)
< 60 days (n=212)	44 (20,8%)	36 (81,8%) ^a	38 (17,9%) ^b
61-80 days (n=296)	83 28,0%	71 (85,5%) ^a	42 (14,2%) ^b
> 80 days (n=140)	66 (47,1%)	55 (83,3%) ^a	33 (23,6%) ^c

Values within a column with differing superscripts were marginally significantly (p<0.06)

The cure-rate of IMI was not influenced by the length of the dry period of the cows (table 4.15). The percentage of new IMI however, was marginally (p<0,06) higher for cows with dry periods of longer than 80 days than those with shorter dry periods

(table 4.15). These findings are consistent with findings of Enevoldsen et al. (1992). The length of the dry period has been documented as an important factor in the development of new IMI (Enevoldsen et al.,1992). The primary source (Smith and Hogan.,1995; Browning et al.,1990; Oliver,1988) of new intramammary infections in the dry period is from the environment. Therefore attention should be paid to optimise the dry period and the exposure of teat ends to environmental organisms by keeping cows in dry areas. Bradley and Green (2000) found that new IMI that established later during the dry period, are more likely to persist and result in clinical mastitis than those acquired during mammary gland involution.

4.3.3.6 Udder depth

A significantly ($p < 0,05$) lower cure-rate was observed in cows that dried off with udder depth scores of 1 and 2, compared to higher udder depth scores (table 4.16). It was also found that cows with udder depth scores of 1 and 2 contracted significantly ($p < 0,05$) more new IMI during the dry period than those with higher udder depth scores.

Cows with low pendulous udders (scores of 1 to 3) may show a higher incidence of teat and udder damage and are more likely to develop new IMI during the dry period compared to shallow udders.

Table 4.16 Udder depth score at drying off compared to the percentage cure and new IMI at calving (n=193)

Udder depth score at drying off	IMI at drying off (% of group)	IMI cure-rate during the dry period	New IMI contracted during the dry period
1+2 (n=56)	25 (44,6%)	17 (68,0 %) ^a	21 (37,5%) ^c
3 (n=69)	39 (56,5%)	32 (82,1 %) ^b	12 (17,4%) ^d
4 (n=150)	40 (26,7%)	33 (82,5 %) ^b	31 (20,7%) ^d
5 (n=178)	44 (24,7%)	41 (93,2 %) ^b	26 (14,6 %) ^d
6 (n=171)	40 (23,4%)	34 (85,0 %) ^b	21 (15,4%) ^d
7 (n=24)	5 (20,8%)	5 (100,0 %) ^b	2 (8,3%) ^d

Values within a column with differing superscripts vary significantly ($p < 0.05$)

De Jong, (1997) evaluated data from more than 500 000 cows to compare udder depth and culling rate and data from 400 000 primiparous cows to compare udder depth with SCC. He found that udder depth scores of 1 to 3 and 9 resulted in culling rates in excess of 40%, and that udder depth scores of less than 3 in primiparous cows, were associated with increased SCC.

Results from this study are in agreement with those of De Jong (1997) who found udder depth scores of 5 and 6 to be optimal for herd life due to higher cure-rates and fewer new IMI. Although the cure-rate was not significantly better or new IMI were not

significantly fewer in cows with udder depth scores of less than 5 there was a trend for better cure and fewer new IMI with an increasing udder depth score. These trial results showed that cows with an udder depth score of 7 had even lower cure-rates and fewer new IMI than cows with udder depth scores of 5 and 6.

Breeding efficiency should be combined with functional conformation. For the modern, high-yielding dairy cow a sound udder is an absolute prerequisite for a long, productive life ([Hamoen,1995](#)). Functional conformation contributes significantly to the ultimate goal as it supports production, improves longevity and increases the cow's workability. A variety of morphological and immunological factors contribute to a cow's resistance or susceptibility to mastitis ([Radostits et al., 2000](#)). According to Radostits et al. (2000) selection for low SCC, udder depth and a low incidence of clinical mastitis will diminish the rate of increase in mastitis by 20-25%. Udders with a distance of more than 40 cm between the tips of the hind teats and the ground have better natural protection being within the udder shelter than those closer to the ground ([Radostits et al.,2000](#); [Giesecke et al., 1994](#)) and also contribute to ease of milking. The teat-end-to-floor distance of the udder has a heritability of 0,2 and is also a risk factor for clinical mastitis ([Radostits et al.,2000](#)). Udder depth therefore forms part of the selection criteria for the M-index which was developed to improve the genetic potential for udder health. It is considered to be one of the most important udder traits ([Radostits et al.,2000](#); [De Jong, 1997](#); [Hamoen, 1994](#)).

4.3.3.7 Teat-end integrity and teat-canal keratin plug

Quarters with teat canal scores of 4 at drying off had marginally significantly ($p = 0,08$) better cure-rates than those with teat canal scores of 2 and 3 (table 4.17). This finding was unexpected and the reason is unclear, but due to the small sample size ($n=42$) this finding may be insignificant.

The percentage of quarters with teat canal scores of 2 and 3 at drying off had significantly ($p < 0,05$) fewer new IMI at calving than those with a teat canal score of 4 (table 4.17). This finding is in agreement with many other studies ([Mein et al.,2001](#); [Radostits et al.,2000](#); [Sandholm et al.,1995](#); [Giesecke et al.,1994](#); [Bramley and Dodd,1984](#); [Comalli et al., 1984](#); [McDonald,1975](#)).

Table 4.17 Association between teat canal score at drying off, cure-rate and new IMI during the dry period

Teat canal score prior to drying off	IMI at drying off (% of the group)	Cured (%)	New IMI (%)
2 (n=278)	86 (30,9%)	75 (87,2%) ^a	39 (14,0%) ^b
3 (n= 328)	89 (27,1%)	69 (77,5%) ^a	61 (18,6%) ^b
4 (n=42)	18 (42,9%)	18 (100%) ^a	13 (31,0%) ^c

Values within a column with differing superscripts vary significantly (p<0.05)

Deficiencies in characteristics of the teat and teat canal can be predisposing factors to mastitis. A positive association between teat canal damage and new IMI has been well researched and documented ([Fox, Nagy and Hillers,1986](#); [Agger and Willenberg,1986](#)). Dingwell ([2002](#)) demonstrated that teat ends with cracks were 1,7 times more likely to develop new IMI during the dry period than teat ends without cracks. Some inconsistencies on the association between teat end lesions and new IMI do exist in literature and may be attributed to the lack of a uniform classification used across the various studies reported. The system used in this study is similar to the system proposed by the Teat Club International ([Mein et al., 2001](#)).

Classification of bovine teat condition can be used to assess the effects of milking machine management, milking equipment or environment on teat tissue and the risk of new IMI ([Mein et al., 2001](#)). A predisposition for bacterial colonisation of keratinized teat end lesions predisposing to IM has been well documented ([Timms, Van Der Maaten and Kehrl, 1998](#)). The normal teat canal represents a physical barrier to the penetration of bacteria and is the single most important barrier to udder infection ([Sandholm et al.,1995](#); [Giesecke et al.,1994](#); [Bramley and Dodd,1984](#)). Resistance to bacterial penetration of the teat canal can be related, in part, to the presence of keratin ([Murphy,1959](#)) and the diameter of the teat canal ([Mein et al.,2001](#); [Radostits et al.,2000](#); [McDonald,1975](#)). Resistance also decreases when the pliability of the teat tissue changes due to congestion, oedema and epithelial hyperplasia, as is found immediately pre- and post-partum, or with incorrect use of the milking machine ([Bulletin of the IDF,1994](#)). When the teat canal is dilated, the risk of ascending infection is high ([Sandholm et al., 1995](#); [Comalli et al., 1984](#)). Hyperkeratosis of the teat canal is a common occurrence in high yielding cows and increases with parity. No significant relationship has however been shown between herd SCC and hyperkeratosis (roughness, cornification or callosity) of teats ([Radostits et al.,2000](#)). Factors linked with the openness of the teat orifice include high milking vacuum, overmilking, design of the liner, unusually heavy weight milking cluster unit, or high liner mounting tension ([Mein et al.,2001](#)).

A keratin plug closing the teat canal to bacterial invasion during the mid dry period develops as the dry period progresses ([Radostits et al.,2000](#); [Giesecke et al.,1994](#)).

A study done by Williamson et al. (1995) showed a distinct difference in the length of time it took for the formation of the teat plug to be completed at the start of the dry period, varying from 10 to 50 days. The formation of the teat plugs was twice as fast in cows which received intramammary treatment with a long-acting cephalonium product at drying off, compared to cows left untreated during the dry period (Williamson et al., 1995).

4.3.4 Environmental factors

An important environmental factor that influences the susceptibility of cows to new IMI in the dry period is the environmental bacterial challenge. The cleanliness and dryness of the environment influences the number of bacteria present in the bedding and season may thus have an influence on new IMI. Other herd-level factors include the management of general herd health, the overall prevalence of udder infection in a herd and meeting the nutritional needs of the dry cow. In this study, only rainfall was monitored.

4.3.4.1 Rainfall during the dry period

No significant difference in cure-rates was observed between cows that were exposed to rain during their dry period and cows that experienced no rain (table 4.18).

Rainfall was however observed to significantly ($p < 0,05$) influence the rate of new IMI. Almost double the percentage of new IMI occurred when rain fell during the dry period of cows (table 4.18).

Table 4.18 Rainfall during the dry period compared to cure-rate and new IMI

Rainfall during the dry period	IMI at drying off (% of group)	Cured (%)	New infections (%)
0 mm (n=150)	41 (27,3%)	35 (85,4%) ^a	16 (10,7%) ^b
1-150 mm (n= 330)	99 (30,0%)	80 (80,8%) ^a	63 (19,1%) ^c
>150 mm (n = 168)	53 (28,0%)	47 (88,7%) ^a	34 (20,2%) ^c

Values within a column with differing superscripts vary significantly ($p < 0,05$)

It can be hypothesised that any relationship between rainfall and new IMI may be a function of general management practices.

Somatic cell counts are generally lowest during the winter and highest during the summer (Wells and Ott, 1998; Smith et al, 1985; Paape, Schultze, Miller and Smith, 1973). The high temperature and humidity per se does not cause increased SCC. The increase is rather due to greater exposure of teat ends to pathogens, resulting in

more new infections and clinical cases during the wet summer months. The findings of Hogan et al (1989) supported the concept that temperature stress per se is not the cause of increased SCC, but the increased SCC is a result of greater exposure of teat ends to pathogens resulting in more new infections and clinical cases during the summer months.

Factors such as climate, housing systems and rainfall interact to influence the degree of exposure of the teat end to mastitogenic pathogens. The quality of housing management has a major influence on the types of mastitogenic pathogens that infect the mammary gland. Few environmental mastitogenic pathogens were isolated from mammary glands in this study, mainly due to the fact that dry cows were kept in well established kikuyu grass camps during both phases of the dry period.

4.4 The influence of intramammary treatment on the individual cow SCC during early lactation

Table 4.19 summarizes cow somatic cell counts per treatment group during the first three 5 weekly samplings of Lacto Lab for each cow participating in the trial. Cows treated with Rilixine 500 DC had a significantly lower SCC (< 400 000 cells per ml) at the first and second sampling post-calving than cows treated with the other 5 products (table 4.19).

Table 4.19 Association between the initial three cow somatic cell counts post calving and the product

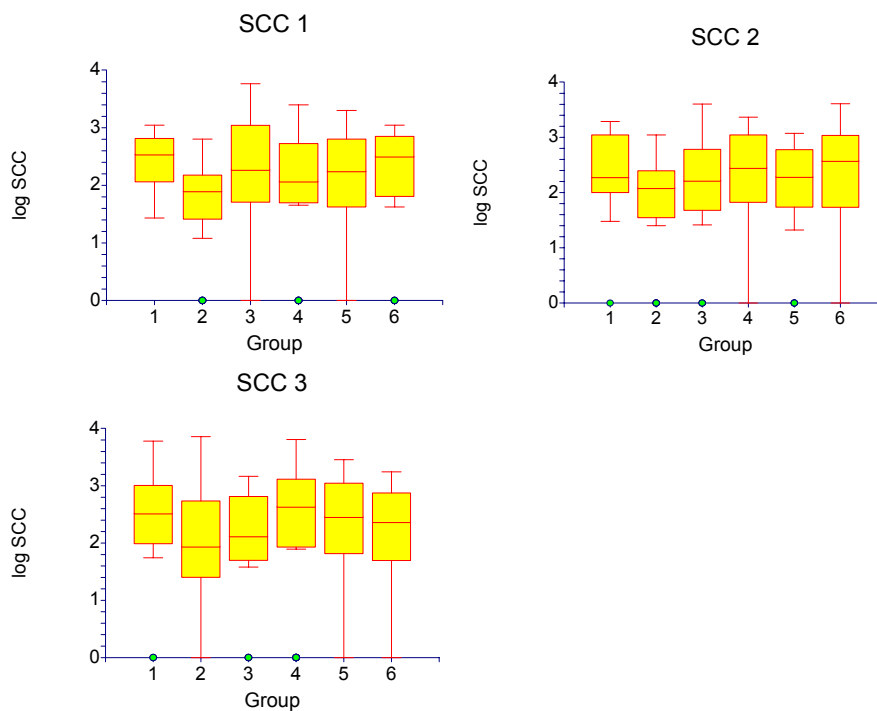
Product	First SCC (%) (SCC ≤400 00 cells/ml milk)	Second SCC (%) (SCC ≤400 00 cells/ml milk)	Third SCC (%) (SCC ≤400 00 cells/ml milk)
Nafpenzal DC	18 (64,3) ^b	17 (63,0) ^b	14 (53,8) ^b
Rilixine 500 DC	21 (84,0) ^a	21 (84,0) ^a	14 (56,0) ^b
Dispolac Dry Cow	15 (57,7) ^b	15 (57,7) ^b	16 (69,6) ^b
Orbenin Extra DC	15 (62,0) ^b	13 (54,2) ^b	8 (33,3) ^b
Cepravin Dry Cow	16 (64,0) ^b	16 (64,0) ^b	12 (50,0) ^b
Bovaclox DC	17 (60,7) ^b	13 (46,4) ^b	11 (45,8) ^b
	102 (66,2)	95 (62,1)	75 (52,1)

Values within a column with differing superscripts vary significantly (p<0.05)

Similar results were obtained with the Kruskal-Walles Multiple analyses presented in figure 4.1. Cows that were dried off with Rilixine 500 DC had significantly lower SCC at the first SCC (SCC1) sampling (Z-value = 2,8133) post-calving than those cows dried off with the other five products in this trial. For the second sampling (SCC2) the

SCC of cows dried off with Rilexine 500 DC remained significantly lower than those cows dried off with Bovaclox DC (Z-value = 2.1700) while no significant differences were present between products in the third sampling (SCC3) post-calving.

Figure 4.1 National Milk Recording SCC (logarithmic values) for three samplings post-calving



Product 1= Nafpenzal DC, product 2= Rilexine 500 DC, product 3= Dispolac Dry Cow, product 4= Orbenin Extra DC, product 5= Cepravin Dry Cow and product 6=Bovaclox DC

The somatic cell count (SCC) is a very sensitive indicator of udder health. Increased SCC values signal udder disease, decrease in milk production, change in milk composition, increase in cost of production and less profit. According to Barkema et al. (1998) and Renau (1986) quarter milk somatic cell counts, as of day 3 after calving, can be used to give an indication of IMI. The SCC from uninfected cows should be less than 300 000 by day 5 post-partum (Renau, 1986). The major factor affecting SCC in milk is IMI (Dohoo and Meek, 1982). Other factors are often implicated in increased SCC, but few have a significant impact (Radostits et al., 2000; Laevens, Deluyker, Schukken, De Meulemeester, Van der Meersch, De Muelenaere and De Kruif, 1997; Harmon 1998; Eberhart, 1986). A normal variation in SCC occurs between the milk fraction throughout milking. The highest SCC is present in strippings and lowest in foremilk. The difference in high and low SCC varies from 4- to 70-fold for individual quarters.

The number of quarters infected, the severity of the infection in those quarters and the respective milk production of each quarter further influence SCC of a composite cow sample. There is a high correlation ($r=0.86$) between SCC of quarter milk samples and those of composite (bottle) milk (Harmon,1998). The dilution of the high SCC milk from infected quarters with low SCC is an important consideration in the interpretation of the cow sample SCC.

It has become increasingly important for the South African milk producer to produce milk with a low SSC, not only from an udder health point of view, but also due to severe penalties imposed on poor quality milk. Criteria for poor quality used to only include a standard bacterial plate count (SPC) of above 50 000 colony forming units per ml milk and the presence of inhibitory substances in milk. Penalties are now also imposed for SCC above 250 000 cells per ml milk by many large milk buyers. It is therefore to the advantage of the producer to maintain a low herd SCC and have cows calving down with low SCC.

4.5 Conclusions

Conclusions from the literature review:

Administration of intramammary therapy at drying off appears to be efficacious to cure existing infections. However, results also illustrate that despite the advantages, there are shortcomings.

- Most of the available dry cow products do not persist late into the dry period and leave the udder unprotected against new IMI during the peripartal period.
- Both the spectrum of activity and persistency of available intramammary dry-cow preparations result in limited effectiveness against new IMI.
- The spectrum of activity of many products is limited to Gram-positive organisms, limiting their effectiveness against new IMI.

Conclusion from this research

This study highlighted the importance of a holistic approach to udder health during the dry period. The importance in the use of blanket dry-cow antibiotic therapy as well as the importance of identifying risk factors and managing the environment of dry cows has been demonstrated.

Due attention should be given to aspects such as:

- Cow factors prior to drying off e.g. as parity, udder conformation, TCS, milk yield, SCC and the number of infected quarters.
- Drying off protocol and effective identification and management of risk factors for new IMI prior and during the dry period.

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- Providing the cleanest and driest environment possible.
- Nutritional management during the dry period should aim to minimise the risk of a negative energy balance and other metabolic disorders post calving (sub-clinical ketosis, hypocalcaemia).
- Calving management and hygiene.

These results emphasise the variability of the response to different drugs. The emphasis must focus on the multifactorial nature of IMI and the adoption of a holistic method to control IMI. Although dry-cow therapy is necessary, it is also necessary to manipulate indirect factors. If key components such as the primary and secondary defence mechanisms of cows and bacterial challenges of the cow are controlled, the prevalence of IMI can be minimised, the success of dry-cow treatment will be improved and the losses due to mastitis will be limited.

Future Research

The spectrum of mastitogenic pathogens in South Africa is changing, as is observed in the rest of the world. At present STA from human origin, SAG and CNS and are being isolated in increasing numbers. SUB is also becoming a problem abroad. More cases of *E. coli* are also reported as dairy herds are becoming larger, have better management and lower SCC. During this study almost no Gram-negative bacteria were isolated. It may become necessary to investigate dry-cow management in cows infected with Gram-negative organisms as most dry-cow products target Gram-positive organisms.

The prophylactic use of antibiotics in food-producing animals is likely to be restricted in future due to public concerns (antibiotic resistance and residues in the food chain).

There are growing requirement for effective alternatives to antibiotic treatments such as:

- Optimising of management (Holistic approach)
- Teat sealants (internal and external)
- Vaccines (*E.coli*; *Staphylococcus aureus*, streptococci)

CHAPTER 5

5.1 SUMMARY

EFFICACY OF DIFFERENT DRY-COW INTRAMAMMARY ANTIMICROBIAL PRODUCTS
ON THE PREVALENCE OF MASTITIS IN A HIGH-PRODUCING DAIRY HERD.

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The objectives of the study were to compare the efficacy of six intramammary dry-cow antibiotic preparations for curing existing IMI and for preventing the introduction of new IMI during the dry period. The effect of factors such as parity, milk yield, udder depth, teat canal integrity, number of infected quarters per cow, quarter-site infections and somatic cell count at drying off on cure-rates and new IMI as well as the length of the dry period and rainfall during the dry period were examined. The possible influence of treatment on the SCC during the subsequent lactation was also examined.

Prior to the study a herd survey was performed to determine the basic udder health status of the trial herd.

Cows due for drying off were clinically evaluated for enrollment and randomly allocated to receive one of the six dry-cow intramammary products under

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investigation. The six products that were studied were Nafpenzal DC (procaine benzylpenicillin, nafcillin and dihydrostreptomycin), Rilexine 500 DC (cephalexin and neomycin sulphate), Orbenin Extra DC (cloxacillin), Cepravin Dry Cow (cephalonium), Bovaclox DC (cloxacillin and ampicillin) and Dispolac Dry Cow (procaine benzylpenicillin and dihydrostreptomycin).

Quarter milk samples were collected at drying off, (prior to treatment), and 1 to 6 days after calving for determination of the SSC and presence of micro-organisms in the milk.

Cows were closely observed during the dry period and udders were clinically examined on a weekly basis and post-calving till udder oedema disappeared.

Data from 162 cows and 648 quarters are summarized. The following were determined

- Prevalence of pathogens at drying off and post-calving
- Overall cure-rate and new intramammary infection rate.
- Comparative efficacy of dry-cow treatment on cure-rate and prevention of new IMI.
- Effect of parity, milk yield at drying off, udder depth, teat canal integrity, SCC, quarter-site infection, number of infected quarters at drying off, length of the dry period and rainfall during the dry period.
- Effect of treatment on three SCC post calving during the subsequent lactation.

Clinical mastitis developed in two quarters of two cows (1,03%) during the dry period which was less than described in literature, and they were removed from the trial.

The prevalence of pathogens at drying off was 29,78%, of which 7,87% and 21,91% were due to major and minor pathogens respectively. The prevalence of pathogens post-calving was 22,22%, a net reduction of 7,56%, of which 4,47% and 17,75% were due to major and minor pathogens respectively.

The overall cure-rate was 83,94% for quarters and varied between 72,3% and 93,9% for the various products.

The overall difference in the percentage of cases cured, compared to the different micro-organisms, was found to be only marginally significant ($p < 0,057$). High cure-rates were observed for STA, SAG and non-agalactiae pathogenic streptococci compared to studies previously done, while lower cure-rates than described were observed with CNS.

Antimicrobial products used in this trial differed substantially in their efficacy to cure Gram-positive IMI. Cure rates for Cepravin Dry Cow was 93,9%, Orbenin Extra DC 91,5%, Rilexine 500 DC 85,7%, Nafpenzal DC 79,2%, Bovaclox DC 75,0% and Dispolac Dry Cow 72,4%. Interestingly, but not of any significance, was an observation that the two products with the highest overall cure-rates both contained only one antimicrobial agent compared to the other 4 products which were combinations of two or more antimicrobials.

There was no significant association between parity, milk yield, teat canal integrity, number of infected quarters per cow, quarter-site infections at drying off, length of the dry period and rainfall during the dry period and the cure-rate. However, there was a significant association between udder depth ($p < 0,0056$) and SCC ($p < 0,005$) at drying off and cure-rate. The cure-rate was significantly less in cows with udder depth scores of 1 and 2 or SCC of more than 750 000 cells per ml milk at drying off.

The overall rate of new IMI during the dry period was 17,44%. The new intramammary infection rate for quarters which received dry-cow treatment varied between 13,4% and 24,1% for the different products.

The majority (70,8%) of new IMI were caused by CNS during the dry period. Almost all (96,6%) major pathogens isolated post-calving were new IMI, while 74,1% of minor pathogens were new IMI.

Antimicrobial products differed in their efficacy in preventing new IMI during the dry period. The percentages of new IMI observed in cows treated with the six products were: 13,2% for Cepravin Dry Cow, 16,3% for Rilexine 500 DC, 16,7% for Dispolac Dry Cow, 17,3% for Bovaclox DC, 21,4% for Nafpenzal DC and 25,9% for Orbenin Extra DC.

The probability of quarters developing new IMI during the dry period was significantly increased when cows were dried off with milk yields higher than 18kg ($p < 0,0037$) or had low udder depth (scores of 1 or 2) ($p < 0,0003$). Higher parity cows ($p < 0,005$) and those that had a teat canal score of 4 ($p < 0,039$) and above at drying off were also at an increased risk for new IMI. Marginally significantly ($p < 0,06$) more IMI were contracted on the left side of the udder than the right side of the udder and cows at drying off and cows with dry periods longer than 80 days had marginally ($p < 0,06$) more new IMI during the dry period. A positive correlation was found between low SCC ($< 250\ 000$ cell per ml milk) and number of infected quarters per cow and parity

(less than 3 lactations) at drying off. There was no significant association between the SCC at drying off and new IMI during the dry period.

Significantly fewer new IMI ($p < 0,05$) were observed when no rain fell during the dry period, compared to cows that experienced rain during their dry period.

A comparison between the six antibiotic intramammary dry-cow products in relation to their efficacy in curing existing IMI and preventing new IMI showed qualitative differences between them, ranked as follows:

Dispolac Dry Cow was the most effective and Orbenin Extra dry the least effective in preventing new IMI with major pathogens and Cepravin Dry Cow was to most and Nafpenzal DC the least effective in preventing new IMI with minor pathogens. Cepravin Dry cow was the most effective in the overall prevention of new IMI during the dry period.

Due to the random selection of cows the percentage of IMI differed for each product at the start of the trial. To compensate for this initial variation, percentage point improvement from drying off until calving was calculated for each antimicrobial product, taking both the cure-rates and new IMI into account. The efficacy of antimicrobial products during the dry period differed substantially when percentage point improvements were utilised: from an increase in IMI post-calving of 11,73% with Nafpenzal DC to a decrease of more than 50% in IMI at calving with both Cepravin Dry Cow and Rilexine 500 DC.

Cows dried off with Rilexine 500 DC had a significantly lower SCC for the first two months post-calving than those dried off with the other five products in this trial.

It is concluded that a substantial difference in efficacy exists between antimicrobial intramammary dry-cow products in their ability to cure and prevent new IMI during the dry period. Dry cow therapy should form part of a holistic approach towards the dry period, which also includes cow factors, dry-cow management, micro-organisms and the environment of the dry cow. Cow factors (milk yields, udder depth, parity, TCS and quarter-site infections) mainly have an influence on new IMI but a few cow factors (udder depth and SCC) are associated with the cure-rate of IMI. Management and rainfall during the dry period mainly affected new IMI rather than the cure-rate of existing IMI.

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