

# DRY-COW THERAPY FOR CONTROL OF MASTITIS IN COWS

By

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## **THESIS**

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requirement for the degree

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**1994**

## DECLARATION

I hereby declare that the thesis entitled "DRY-COW THERAPY FOR CONTROL OF MASTITIS IN COWS" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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P.R. PRADEEPKUMAR

**CERTIFICATE**

Certified that the thesis entitled "DRY-COW THERAPY FOR CONTROL OF MASTITIS IN COWS" is a record of research work done independently by Sri. P.R. Pradeepkumar, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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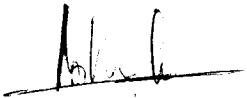
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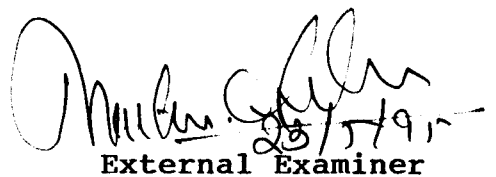
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# Introduction

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## INTRODUCTION

The Indian economy has its strong footholds on agriculture and animal husbandry and will continue to be so in the years to come. During the year 1991-92, the share of total state income from agriculture was 33.8 per cent.

In 1982 there was 3690.31 lakh heads of livestock in India and the milk production during 1988-89 was 487 lakh tonnes which increased to 937.2 lakh tonnes during 1990-91 (Farm Information Bureau, Government of Kerala).

In India animal husbandry is generally an enterprise of the poorer sector of the society providing them ample job opportunities and a livelihood to most of them.

In Kerala the number of heads of livestock was 56.45 lakh in 1982. It became 54.71 lakh in 1987 among which 17 lakh were adult female cattle. The production of milk increased from 14.6 lakh tonnes in 1988-89 to 17.85 lakh tonnes in 1991-92. The milk production in Kerala is mainly by cattle (Farm Information Bureau, Government of Kerala).

Crossbreeding programme widely employed in the state has helped to increase production of the animals. For maximum production, health of the individuals is of the most important

concern and mastitis is a major health problem affecting milk production of the animals. In Kerala 2.8 per cent of all cases treated in veterinary hospitals during 1990 was mastitis of cows (Animal Disease Surveillance Report (1990) which constitutes mainly the clinical cases.

Apart from the clinical cases, a large number of subclinical cases of mastitis escapes diagnosis and treatment under field condition, reducing the milk production, depending on the cause, severity and extent of affection. The economic losses are further enhanced by the discarding of milk due to residual antibiotic content, cost of the treatment, extra labour and in some peracute clinical cases loss of animal itself.

The term mastitis refers to inflammation of the mammary gland and is associated with varying degrees of physical, chemical and microbiological changes in the milk. This is a complex disease that results from the interaction of the cow, the environment and the pathogen. Injury of any type to mammary tissue may be expected to induce an inflammatory response or mastitis. However the udder disease of major concern is the one associated with microbial infection. Bovine mastitis is a complex disease in view of the complexity of the causes, pathogenesis, intensity, duration, residual effect, immunity, therapy and control.

Usually mastitis begins with the penetration of pathogenic bacteria into the interior of the gland. If the internal environment is favourable for the survival and multiplication of the invading organism, the by-products of its growth and metabolism may irritate the delicate mammary tissue and induce inflammatory reactions. The clinical signs produced are an expression of the host defenses intended to destroy the invader and to make way for reparative process to regain normalcy. The severity of mastitis is primarily determined by the nature of the invading pathogen, natural resistance mechanisms available to the cow and to some extent by managerial practices and environmental factors.

In practice bovine mastitis is not an eradicable disease in a herd or area except for the possible exception of that caused by Streptococcus agalactiae. Hence the importance is at maintaining the disease incidence at acceptably low levels without compromising the milk production.

Mastitis is caused by a variety of organisms including bacteria, spirochetes, mycoplasmas, yeasts and fungi (Watts, 1988). Among these Streptococcus agalactiae (Str. agalactiae), Staphylococcus aureus (S. aureus), and Corynebacterium pyogenes (C. pyogenes) cause specific mastitis in the cow and they are considered as the major mastitis pathogens of cow. Infected animals act as source of infection to other cows.

Udders free of infection at calving are expected to produce maximum amount of milk in the subsequent lactation, and in the absence of an effective control programme during dry periods more quarters will be infected at calving than at drying off. This change in the prevalence over the dry period is the result of two factors-elimination of existing infection which tends to lower the prevalence and establishment of new infections which tend to increase it. Development of a strategy for improving udder health over the dry period requires consideration of ways to eliminate those infections already present and to reduce the rate of new infections (Eberhart, 1986). These two goals could be achieved by treatment of the udder with a suitable preparation, and hence this study was undertaken.

# Review of Literature

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## REVIEW OF LITERATURE

### 2.1 History

Nocard and Mollereau (1884) reported an infectious form of mastitis in cows. They isolated a pure culture of streptococcus from the secretions of a cow that had mastitis and reproduced the disease by injecting the culture through the milk duct of a normal heifer. Kitt (1885) had similar observations with mastitis.

Nocard and Mollereau (1884) succeeded in overcoming a high incidence of chronic mastitis in a herd by insisting that the sick cows should be milked last and sound glands in the sick cows milked before the affected ones, that the secretions of the diseased should be collected separately and used as pig feed and that the milk maid should wash her hands as well as the teats of the cow with a 3 per cent phenol solution before each milking.

Nocard and Mollereau (1887) attempted on treatment of mastitis in a cow. They infused 4 per cent solution of boric acid into the infected quarter. Subsequently many antibacterial preparations were tried by different people. With the advent of antibiotics and sulphonamides the treatment of mastitis became more effective.



Heidrich and Renk (1967) presented 25 genera and 46 species of bacteria associated with the disease from a historic perspective.

Philpot and Pankey (1975) listed 84 microorganisms which reportedly caused mastitis.

## 2.2 Etiology

El-Far et al. (1987) isolated Cryptococcus neoformans from nine cases of mastitis. He also isolated Candida albicans, Torulopsis spp. and Rhodotorula spp. from cases of mastitis.

Kapur et al. (1989) isolated Pseudomonas aeruginosa from a case of mastitis in a heifer.

Owens (1987) isolated bacterial L-forms from milk of dairy cattle inoculated with S. aureus.

Sears et al. (1987) found an unstable L-form of S. aureus in milk samples from three quarters of two cows after treatment with cloxacillin.

Thomson et al. (1988) reported acute clinical mastitis with Mycobacterium smegmatis after intramammary treatment of subclinical mastitis caused by streptococci with cloxacillin.

Vecht et al. (1988) reported isolation of an unidentified microaerophilic coccus from cases of summer mastitis in association with Actinomyces pyogenes and Peptococcus indolicus.

Wani et al. (1988) isolated C. bovis from a clinical case of mastitis.

Watts (1988) listed a total of 137 microbial species, subspecies and serovars which had been isolated from bovine mammary gland, including bacteria, spirochetes, mycoplasmas, yeasts and fungi.

Blood and Radostits (1989) enlisted the most common pathogens as S. aureus, Str. uberis, Str. agalactiae, Str. dysagalactiae other streptococci and coliforms. Mastitis may be associated with many other organisms including Corynebacterium pyogenes (C. pyogenes), Psuedomonas aeruginosa, Nocardia asteroides, Clostridium perfringens, Mycobacterium spp., Mycoplasma spp., Pasteurella spp., yeasts and Prototheca spp.

Prabhakar et al. (1989) isolated Mucor spp. from culture of pus collected from clusters of thick walled cutaneous nodules on the udder of cows.

Francis (1991) isolated anaerobic bacteria from 295 (23.3%) out of 1266 samples of udder secretions collected aseptically from quarter samples of 347 culled cows slaughtered, but no sample yielded anaerobic bacteria alone.

Jackson and Boughton (1991) isolated Mycoplasma bovigentialium from milk of cows with a mild outbreak of mastitis.

Sears et al. (1991) reported isolation of L-forms after intramammary treatment of quarters affected with S. aureus using sodium cloxacillin 200 mg for three days.

Todhunter et al. (1991) isolated Serratia spp. from cases of intramammary infection in cows.

### 2.3. Prevalence

Packer (1952) reported that among 15,693 samples of milk examined, the percentages of infection were S. aureus - 72.60, Str. agalactiae - 7.78, Str. dysagalactiae - 4.4, Str. uberis - 5.5, Escherichia coli (E. coli) - 4.25 and Pseudomonas pyocyanae - 2.75.

Krishnanunni (1963) found that haemolytic streptococci was the most predominant organism responsible for mastitis among cows at Mannuthy.

In another study conducted at Mannuthy, Raju (1972) reported that out of 43 animals with mastitis, 29 were positive for bacterial growth, of which 18 were staphylococci, six were streptococci, four were E. coli and one was C. pyogenes.

Ferreiro et al. (1985a) in a study in Brazil found that of the 896 milk cultures mainly from subclinical mastitis 77.2 per cent were culturally positive. The family micrococcaceae (31.69%) predominated with S. aureus being isolated in 16.96 per cent of samples followed by S. epidermidis in 13.28 per cent, Str. agalactiae in 12.05 per cent, Str. uberis in 8.82 per cent, Str. dysagalactiae in 5.14 per cent and C. bovis in 9.7 per cent of the samples. There were also 12 yeasts (majority Candida albicans), 12 Nocardia spp. (mainly N. asteroides) and three mould fungi among other positive cultures.

Simaria and Dholakia (1986) isolated fungi from 7.48 per cent of 588 samples from apparently healthy animals and from 29.27 per cent of 82 milk samples from mastitis cases.

Aungier and Austin (1987) in an Irish study reported a frequency of 18 per cent S. aureus, 17 per cent Str. uberis and 12 per cent E. coli from clinical cases of mastitis. No pathogen was isolated from 39 per cent of the samples.

Subclinical infection of either Str. uberis (19.5%) or S. aureus (7%) was found in 28 per cent of the quarters. These authors had also reported that 1.4 per cent of the quarters was non-functional due to previous incidence of summer mastitis.

Handique et al. (1988) isolated 41 different organisms from 39 mammary glands which included S. epidermidis (24), C. bovis (3), Str. uberis (3), Str. bovis (2), E. coli (2), Klebsiella aerogenes (2), Citrobacter spp. (2), Bacillus spp. (2) and unidentified Streptococcus spp. (1).

Blood and Radostits (1989) stated that in most countries survey on the incidence of mastitis irrespective of the cause showed comparable figures of about 40 per cent morbidity among dairy cows and a quarter infection rate of about 25 per cent.

#### 2.4 Spread of infection

Blood and Radostits (1989) recorded that infection of each mammary gland occurs via the teat canal. The infection originates from two main sources - the infected udder and the environment. The same authors also stated that the frequency of occurrence of different etiological agents depends on the ability of the organism to set up infection of the mammary tissue which again is dependent on two important groups of

factors - 1. Bacterial characteristics namely ability of the organism to survive in the cow's immediate environment, ability to colonise the teat duct, ability to adhere to the mammary epithelium and set up a mastitis reaction and its resistance to antibiotic therapy. 2. Transmission mechanisms which depend on the bulk of infection in the environment including the infected quarters, efficacy of the milking personnel and milking machine including high milking speed, hygiene in the milking parlour and susceptibility of the cow. Susceptibility of the cow to infection is related to stage of lactation, age of the cow, level of inherited resistance possibly related to teat shape and anatomy of the teat canal, lesions on the teat skin especially at the orifice and finally the immunological factors.

#### 2.4.1 Effect of environmental factors

Hogan et al. (1990) reported that streptococcal teat swab counts did not differ between cows bedded on chopped newspaper and pelleted corn cabs. Cows bedded on chopped newspaper and wood shavings had similar gram negative bacteria, coliform and Klebisiella spp. and teat swab counts. Streptococcal and staphylococcal teat swab counts were greater from cows bedded on chopped newspaper than those from cows bedded on wood shavings.

Fox et al. (1991b) reported that the teat cup liner of milking machines appeared to be a significant fomite for S. aureus intramammary infection. Its significance was reduced by back flushing. Teat skin was a less significant reservoir for S. aureus intramammary infection.

Matos <sup>et al.</sup> (1991) reported S. aureus could be isolated from body sites of 163 heifers, bedding and feed stuff samples and hands and nares of the research personnel but not from flies. Nemeth et al. (1994) reported that 50 each of E. coli isolates from bovine mastitic milk, faeces of mastitic cows and faeces of healthy cows were similar with tests for biotypes. Roberson et al. (1994) suggested that primiparous cows with coagulase positive staphylococcus intramammary infection at parturition may represent significant reservoirs of infection to uninfected herd mates.

#### **2.4.2 Effect of managerial factors**

Aungier and Austin (1987) reported a high incidence of clinical mastitis in a farm which had a defective milking machine and limited control measures. Schukken et al. (1990) reported that the rate of clinical mastitis was significantly associated with some variables that increased the exposure to environmental microorganisms such as poor cubicle cleanliness and rubber mats in the cubicle. Post milking teat

disinfection was also associated with more mastitis. Schukken et al. (1991) reported teat disinfection as an important risk factor for increased rate of E. coli mastitis unlike that for S. aureus. Cleaning procedures were more important for E. coli.

Grindal and Hillerton (1991) observed an increase in new intramammary infection with greater milk flow rate during milking. Increased coliform mastitis was also associated with an increased amount of milk remaining in the udder after milking, use of free stalls, regular use of a running water wash, increased person hours spent per cow spent milking cow and poor sanitation (Bartlett et al., 1992). They have also stated that increased prevalence of environmental streptococci was associated with poor sanitation, increased number of dry days, use of tie stalls, and use of a shared wash cloth and non-use of individual dry cloth.

Enevoldsen and Sorensen (1992) found that there was little evidence of an effect of dry period on the risk of clinical mastitis and other clinical disorders around and after calving. Dry period length of approximately seven week appeared to be associated with the lowest risk of clinical health disorders.



### 2.4.3 Effect of functional status of udder

McDonald and Anderson (1981) reported that in an experimental infection of the mammary gland with E. coli during the first half of the non-lactating period, 32 per cent of the 34 inoculated glands were temporarily infected. All the intramammary infections were eradicated by the cow without therapy and no sign of mastitis was observed. During the 30 days before parturition 88 per cent of 42 inoculated glands became infected. Twenty three intramammary infections were eradicated by the cow and infection in 14 glands persisted after parturition. Peracute mastitis occurred in those cows with infected glands. Smith et al. (1985a) observed that coliform infections originated in the first half of the dry period and persisting to lactation were predominantly other than E. coli., while majority of those occurring in the latter half of the dry period and persisting to lactation were E. coli.

Eberhart (1986) had the opinion that the incidence of clinical mastitis was at least four times high during the first 15 days of lactation than in the subsequent 15 days.

Oliver (1988) found that in a herd which is free from Str. agalactiae, but with a low prevalence of S. aureus and not treated with an antibiotic at the cessation of milking, a

three fold increase in the percentage of quarters infected with major mastitis causing pathogen developed from late lactation to early involution. Coliforms and streptococci other than Str. agalactiae account for 94 per cent of major pathogen infections. The number of quarters infected with coagulase negative staphylococci increased slightly from late lactation to early involution but the number of quarters infected with C. bovis decreased greatly. Major pathogens caused 101 of 153 intramammary infections at parturition and more than 90 per cent were caused by streptococci and coliforms. At parturition 51 of 52 minor pathogen intramammary infections were caused by coagulase negative staphylococci. During early lactation there was a marked decrease in quarters infected with major pathogens. However, the number of quarters with major pathogens during early lactation was 2.3 times higher than the number of quarters infected before cessation of milking. The number of quarters with minor pathogens during early lactation was the same as at parturition but a pronounced decrease in quarters infected with coagulase negative staphylococci and a marked increase in C. bovis intramammary infection developed from parturition to early lactation. Oliver and Juneja (1990) found that mammary secretions supported the growth of C. bovis during lactation but inhibited the growth during non-lactating period. Oliver et al. (1990) reported that mammary secretions at the 14<sup>th</sup> and

28<sup>th</sup> days of involution were poor media for growth of Staphylococcus spp. and that of at the cessation of milking, parturition and early lactation supported the growth of all species studied. Todhunter et al. (1990a) suggested that the ability to overcome inhibitory properties of dry cow secretion was related to the establishment of Klebsiella pneumoniae and Klebsiella oxytoca intramammary infection in the dry period. These authors did not find any evidence of E. coli infection in dry cow secretion. Todhunter et al. (1990b) suggested that the inhibitory properties of dry cow secretion to E. coli may contribute to the low number of naturally occurring intramammary infections during the early part of the dry period.

#### 2.4.4 Effect of injuries to the udder

Seinhorst et al. (1991) reported that when teat ends of 12 dry cows were contaminated with C. pyogenus following injury, 30 of the quarters became infected and anaerobic bacteria were detected in many quarters. Capuco et al. (1992) found that partial removal of keratin from the teat canal lowered the ability of the teat to prevent passage of bacterial pathogens from the external environment into the mammary gland.

#### 2.4.5 Effect of number of previous calvings

Morse et al. (1987) inferred from their study that the incidence of clinical mastitis increased with parity. The proportion of mastitis episodes within 35 day post partum was 60 per cent after first calving but decreased from 36 per cent to 28 per cent between second to fifth parity.

#### 2.4.6 Effect of milk production

Funk et al. (1982) reported that high producing cows had fewer mastitis infections when dried off but milk production of the previous lactation had little influence on mastitis infection post calving. El-Bayoni and Mahmoud (1987) opined that subclinical mastitis was more likely to occur in older animals and those with daily milk yield less than 5 kg. But Schukken et al. (1990) found a higher incidence of mastitis in herds with high milk production. Grindal and Hillerton (1991) also had similar findings.

#### 2.4.7 Effect of infections of the udder

El-Bayoni and Mahmood (1987) reported that subclinical mastitis was more likely to occur in animals which previously had mastitis. Matthews et al. (1990) observed a reduction in infection rate of S. aureus challenge after

experimental infection with S. chromogenes, while Matthews et al. (1991) suggested that quarters harbouring a coagulase negative staphylococcus infection suppress colonisation of the mammary gland by mastitis causing pathogens. Enevoldsen and Sorensen (1992) also reported previous mastitis incidence as a predisposing cause for development of mastitis.

Scott et al. (1991) opined that except during the stress of high milk yields (27.3 kg/day) the heightened immune response against bovine Leukaemia virus indirectly, protected cows against mastitis infections.

#### **2.4.8 Effect of other concurrent diseases**

Prabhakar et al. (1988) reported isolation of same pathogens from udder and uterus of cows affected by mastitis along with metritis. Schukken et al. (1988) found that cows with retained placenta were at a higher risk of developing non-severe mastitis (relative risk = 1.5) and at a much higher risk of developing severe mastitis (relative risk = 5.4).

Schukken et al. (1990) in another study observed an increase in incidence of mastitis with cows leaking milk. Schukken et al. (1991) further reported that the percentage of cows leaking milk was a risk factor for E. coli mastitis.

#### 2.4.9 Mammary infections in primigravid heifers

Oliver (1987a) reported isolation of non-haemolytic coagulase negative staphylococci, streptococci other than Str. agalactiae, coliforms, corynebacteria, coagulase positive staphylococci and some other minor organisms from the mammary secretion of primiparous heifers in the immediate peripartum period. Trinidad et al. (1990) reported colonisation of teat canal and intramammary infections with mastitis pathogens in unbred and primigravid heifers. Roberson et al. (1991) found a two fold increase in isolation of S. aureus from body sites (muzzle, vagina, teat skin, teat orifice and lacteal secretions) of bred heifers (<18 months) compared to other rearing stages viz. preweaned (<0-2 months)", growing (2-12 months) and breeding (12-18 months). They suggested either increased exposure and/or increased susceptibility to S. aureus colonisation and intramammary infection during this stage. Pankey et al. (1991) also found intramammary infection of primigravid heifers with mainly Staphylococcus spp. environmental mastitis pathogens, coliforms and streptococci. Roberson et al. (1994) had the opinion that although primiparous cows from high prevalence herds had higher incidence of coagulase positive staphylococcus infection at parturition than did primiparous cows from low prevalence herds, the difference was not significant.

#### 2.4.10 Anatomical factors

Lozhkin (1987) found less pathological changes in udders with a single ductus lactiferi which branched into numerous smaller ducts than in udders with numerous small lobular ducts entering the milk cistern.

#### 2.4.11 Breed factors

Morse et al. (1987) reported that incidence of clinical mastitis was higher among Holstein-Friesians than among Jerseys. Dutta et al. (1988) in their study found that the risk factors for the development of mastitis were 1.21-1.78 times greater in Jersey cows than in crossbred cows. Schukken et al. (1990) observed a lower incidence of mastitis in Holstein-Friesian than in Meuse-Rhine-Yersel breed.

#### 2.4.12 Effect of nutritional factors

Forte et al. (1987) found a reduction in udder infection by 42 per cent and clinical mastitis by 32 per cent in cows nutritionally deficient in vitamin-E and selenium when these were supplemented. Schukken et al. (1990) reported that the use of sugar beet pulp in the ration increased the rate of incidence of mastitis. Weiss et al. (1990) found that herds fed high amounts of selenium had high rates of clinical mastitis but could be avoided if high amounts of vitamin-E

were fed. These authors also reported that the rate of clinical mastitis was related negatively to plasma selenium concentration and concentration of vitamin-E in the diet. Ndiweni et al. (1991) suggested an association of incidence of subclinical mastitis or inflammation and the selenium status of cattle.

## 2.5 Diagnosis of mastitis

The clinical cases of bovine mastitis are diagnosed by the obvious clinical symptoms pertaining to the affected quarter(s) and systemic signs in peracute cases. These symptoms may give an insight into the causative agent of the condition (Blood and Radostits, 1989).

Diagnosis of subclinical cases of mastitis requires specialised tests designed for the purpose. The tests that are applied to milk to measure the extent of inflammation of the quarter of its origin fall into three major categories, namely:

- a. based on increased numbers of neutrophils, macrophages and other cell types either by direct counting methods (microscope, coulter counter etc.) or by indirect methods usually involving reaction with cellular DNA (Whiteside test, California Mastitis Test) cell enzymes (Catalase, n-acetyl glucosaminidase).



- b. based on changes in milk composition as a consequence of altered secretory activity or post secretional changes to milk components (fall in lactose concentration, degradation of caseins).
- c. based on changes in milk composition as a consequence of increased permeability of the blood/milk barrier (changes in specifications of total milk conductivity, higher concentrations of blood components such as bovine serum albumin or alpha antitrypsin in the milk) (Bramley, 1991).

#### 2.5.1 Tests employed for diagnosis of mastitis

The tests employed for diagnosis of mastitis are strip cup test (Moak, 1916), determination of pH (Baker and Breed, 1920), Whiteside test (Whiteside, 1934) and Modified Whiteside test (Murphy and Hanson, 1941). Schalm et al. (1971) described chloride test, catalase test, Field Whiteside test, Somatic cell count, California mastitis test, Brabant mastitis test and Wisconsin mastitis test for diagnosis of mastitis.

Benche et al. (1977) found that only clinical examination of the parenchyma of dry udder and its secretions as well as bacteriological tests were reliable enough for safe diagnosis in the individual animal and for routine udder health examination. Electronic cell count, cell

differentiation, measurement of electrical conductivity, pH value and chloride, sodium, potassium and lactose levels of the secretions of the dry udder were not useful for diagnosis of mastitis.

Bovine serum albumin concentration in milk increased in mastitis and is reported to be a more suitable test than CMT (Bakken and Thorburn, 1985). The lactate dehydrogenase activity determination also was reported as a good method for diagnosing early stages of inflammation in the mammary gland (Hambitzer and Sommer, 1987).

N-acetyl-B-D-glucosaminidase activity was shown to be related to the bacterial counts of milk samples (Kunkel et al., 1987).

A significant negative correlation was observed between lactose and chloride content of milk in normal quarters and in quarters with subclinical mastitis (Ahmad et al., 1988).

Hoblet et al. (1988) emphasised the role of bacterial culture even in herds where clinical signs and somatic cell count profile suggested that contagious mastitis was unlikely.

Erskine et al. (1988) reported that the bacterial culture results of herd surveys may be quite different from

results of culture of milk from cows with clinical mastitis.

El-Rasheedy et al. (1988) reported that even apparently normal quarters may reveal infection by major pathogens.

Erskine and Eberhart (1988) found that single quarter samples would be adequate for determining the status of quarter infection with Str. agalactiae and S. aureus.

Electrical conductivity of milk was found to be an economical test for detecting subclinical udder infections (Swarup et al., 1989).

Sears et al. (1990) found that S. aureus is shed in a cyclical manner from many glands. Therefore consecutive samples would be advisable for accurate diagnosis of infected glands.

## 2.6 Control of mastitis

Blood and Radostits (1989) described the NIRD programme developed by the National Institute of Research in Dairying at Reading, England. The basic programme is,

1. Reduction of duration of infection by treatment of all quarters of all cows at drying off, detection and treatment of clinical cases and culling of chronic cases.

2. Reduction of new infection rate by teat dipping after each milking, adequate servicing and maintenance of milking machines and hygiene at milking.

### 2.6.1 Lactating cows

Modes for control of mastitis employed in the lactating period include,

#### 2.6.1.1 Monitoring quarter infection rate

The number of cases of clinical mastitis is to be monitored in a herd to check the effectiveness of any control programme against the prevailing pathogens in the herd, and to check that the programme is being properly applied. This can be done by monitoring the number of clinical cases of mastitis treated in a herd and the number of subclinical cases of mastitis diagnosed by somatic cell counts, CMT etc. (Blood and Radostits, 1989).

#### 2.6.1.2 Teat dipping or spraying

Prevention of new udder infection in lactation is crucial in a mastitis control programme and post milking teat disinfection is a major component in this. Numerous studies have demonstrated the merits of teat dipping in the control of contagious mastitis pathogens such as S. aureus and Str. agalactiae (Milojevie et al., 1989; Pankey, 1989; Nickerson

et al., 1990). Heider and Barr (1977) reported equal efficacy for dipping and spraying of the solution because the sphincter is covered whichever method is used. Blood and Radostits (1989) recommended that the most satisfactory method of application is complete immersion of the teat and the base of the udder in the solution.

The success of teat dipping against new infections due to contagious pathogens is because transfer at milking time and growth on teat skin and lesions are crucial in the pathogenesis of such infections (Pankey, 1989). So they are less effective against environmental pathogens.

Disinfectants continue to be the basis for most teat dips and numerous compounds and elements have been formulated into commercial products (Pankey, 1989). Iodophor solution containing 1 per cent of available iodine, hypochlorite solution containing 4 per cent of free chlorine and with negligible free alkali and chlorhexidine 0.5 or 1 per cent in polyvinylpyrrolidone or as a 0.3 per cent aqueous solution have been found to be effective (Blood and Radostits, 1989).

Chapping of the teats is a common sequel to constant use of teat dips. Emollients when added to prevent this effect reduce the bactericidal effectiveness of the dip (Blood and

Rodostits, 1989). Fox et al. (1991b) found that a dip of 1 per cent iodine with 10 per cent glycerine reduced S. aureus colonisation of chapped teat skin and was associated with faster healing and suggested that glycerine in teat dips may be of value in preventing colonisation by S. aureus and in promoting healing of chapped teat skin.

#### 2.6.1.3 Udder washing, teat stripping and teat sanitisation

It has been shown that the new infection rate is reduced significantly if the teats are stripped before premilking washing rather than after. Before milking and after-milking strippings tend to reduce the new infection rate (Blood and Radostits, 1989).

The highest number of environmental mastitis pathogens are found on teats immediately prior to milking. The degree of contamination depends on the cleanliness of cows' environment between milkings. The lowest bacterial counts in milk were obtained when only the teats were wetted and cleaned with either hosed water, a wet towel or a premilking dip in disinfectant followed by manual drying with single use paper towel (Pankey, 1989).

Blowey and Collins (1992) reported that pre-milking teat disinfection with an iodophor reduced the incidence of clinical mastitis by 57 per cent.

#### 2.6.1.4 Milking management

This includes maintenance of proper vacuum, ideal pulsation rate, management of teat cup liners, teat cup disinfection and back flushing and maintaining milking order wherein the infected cows in the herd are milked only last (Blood and Radostits, 1989; Bramley and Schultze, 1991). Fox and Hancock (1989) found that S. aureus intramammary infection can be controlled without segregation.

In hand milking the milker should wash his hand thoroughly after milking each cow, and apply any antiseptic cream at the end of milking (Blood and Rodostits, 1989).

#### 2.6.1.5 Drying-off

Natzke et al. (1975) found that cows dried off by intermittent milking had a similar number of quarters infected at drying off in comparison with cows dried off by the stop method. Cows dried off by intermittent milking also had fewer spontaneous recoveries, a higher rate of cure and developed fewer new infections in quarters without dry-cow treatment. The methods worked equally well in cows that had dry-cow treatment. Intermittent milking resulted in fewer infection at subsequent calving than stop milking in non-dry-treated cows.

Bushe and Oliver (1987) found that mammary secretions from cows milked intermittently and fed only hay during drying off period contained higher concentration of somatic cells, lactoferrin, IgG and bovine serum albumin, a lower citrate:lactoferrin molar ratio and were more inhibitory to in-vitro growth of E. coli and Klebsiella pneumoniae throughout the experimental period than mammary secretions from cows dried off by intermittent or abrupt milk cessation.

Under normal conditions there is little difference between the stop system and intermittent milking but care is advised when cows giving 10 kg or more milk are dried-off. In such animals the stop method will cause severe swelling and encourage the development of mastitis. This difficulty is minimised by confinement and complete restriction of food and water for 24-36 hours. Withdrawal of milk during the dry period does not appear to increase the chance of infection. Dipping of the teats for 20 seconds in 5 per cent tincture of iodine, after preliminary washing with sodium hypochlorite on several occasions at drying off effectively reduces the subsequent rate of new infections with S. aureus. Infusion of a teat-sealer is also recommended (Blood and Radostits, 1989).



#### 2.6.1.6 Nutrition

Vitamin-E supplemented with selenium is administered 21 days before the expected calving date as a prophylaxis against mastitis (Ivandija, 1984). Forte et al. (1987) reported a reduction of udder infection by 42 per cent and of clinical mastitis by 32 per cent in cows nutritionally deficient in vitamin-E and selenium, when, these were supplemented.

#### 2.6.1.7 Inheritance

Teat shape is an inherited character which may affect susceptibility to mastitis. Cows with cylindrical teats become affected, more commonly than cows with funnel shaped teats (Rathore, 1976). Those with inverted, funnel shaped teat ends, or with a recessed plate like end appear to be more susceptible (Blood and Radostits, 1989).

Genetic variations in resistance to mastitis have been proven with regard to Str. agalactiae mastitis and high milk cell counts in cows (Gootenhuis, 1981).

#### 2.6.1.8 Other factors

Use of intramammary devices have been tried. Cows with abraded intramammary device were more resistant to

experimental challenge with E. coli compared to a smooth intramammary device (Bright et al., 1987).

Blood and Radostits (1989) recommended the following points for controlling mastitis.

- (a) In a herd, known infected cows should be milked at last and in general young cows should be milked before the older ones. Newly introduced animals should be milked separately until screening for mastitis.
- (b) Greatest care is to be taken to ensure that new infections do not occur during the first few days of lactation when the udder is most susceptible to peracute mastitis.
- (c) Ensure that calves do not suck each other in early life, to reduce incidence of mastitis in the peripartum period of primiparous heifers.
- (d) Quarters which do not respond to treatment should be permanently dried up or the affected cows culled.
- (e) Teat sores increase colonisation of bacteria on teat skin and hence should be treated promptly.

### 2.6.2 Dry cows

Smith et al. (1985b) found that a period of heightened susceptibility occurs just before parturition.

Eberhart (1986) stated that exposure to contagious mastitis of mammary glands pathogens namely S. aureus and Str. agalactiae is reduced during the dry period, as they are primarily associated with the milking process but the exposure to environmental pathogens - mainly coliforms and streptococci other than Str. agalactiae - continues throughout the dry period. He also stated that during the first month of lactation a quarter newly infected in the dry period will sustain a production loss equal to that of one retaining an established infection throughout the dry period and if, the infection persists throughout the lactation, a proportional production loss would be expected to continue. He also noted that the new infection rate was more than six times higher during the first three weeks of the dry period compared to that of the preceding lactation as a whole, and during the remainder of the dry period the infection rate was low. The rates of new infection in the dry period in cows not treated with an antibiotic at the drying off range from 3.8 to 35.1 per cent.

Blood and Rodostits (1989) also suggested that the exposure to the contagious mastitis pathogens is likely to be reduced during dry period but exposure to the environmental pathogens continues throughout the dry period. They also stated that most of the dry period infections arise in the first few weeks of the dry period.

#### 2.6.2.1 Dry cow therapy

Christie et al. (1974) found that when cows infected with streptococci or staphylococci at drying off were treated with benzathine cloxacillin (500 mg) during the dry period, pathogens were eliminated in 82 per cent of the cases. Streptococci were readily removed than staphylococci. The level of milk production had little effect on cure rate, neither did the length of time the antibiotic remained in the udder before calving, provided, it was at least 21 days. Older cows were less likely to respond to treatment once the infection with staphylococci became chronic.

Merck et al. (1974) showed that when cows were infused with benzathine cloxacillin (500 mg) into each quarter at drying off, the incidence of subclinical mastitis after calving was reduced by 64 per cent, from the incidence prior to drying-off, while it increased by a half in the untreated group. The number of infected quarters was reduced by 53 per

cent by the dry period therapy and it cured 75 per cent of the quarters which had been affected with subclinical mastitis.

A decrease in S. aureus infection following the use of two doses of dry-cow benzathine cloxacillin two weeks apart has been demonstrated (Smith et al., 1975).

Natzke et al. (1975) also emphasised the role of routine dry cow therapy in decreasing the number of infections in dairy herds by preventing new infections and removing old infections.

Schulze and Mercer (1976) used a product containing 500,000 IU of procain penicillin and 600 mg of navobiocin in two per cent aluminium monostearate-peanut oil gel (10 ml dose) to infuse into all <sup>the</sup> quarters of 56 cows which were infected at least in one quarter at the final milking of lactation. Clearance rates against S. aureus, S. epidermidis, streptococci other than Str. agalactiae and coliform bacteria in treated quarters were 82, 94, 88 and 71 per cent respectively. New infection rates during dry period were 36 per cent among untreated cows and 6.3 per cent among treated cows as against 5.7 per cent and 0 per cent at the beginning of dry period.

Huber and Bonacina (1976) injected oxacillin ointment into the teat canal in 1939 quarters of 487 cows at the time

of drying off. The infection was eliminated in 80 per cent of quarters and 78 per cent of S. aureus infections were cleared up, while, only 51 per cent of the cows were producing milk with normal cytological values at drying off. Seventy seven per cent yielded normal milk 7-14 days after calving. New infections occurred in four per cent of cows.

Yghnalék and Skaloud (1976) infused 500 mg of cloxacillin by intramammary route at the start of dry period in two herds with subclinical mastitis caused by Str. agalactiae. The organism was isolated from a quarter of one cow out of 11 infected cows in the first herd at five days post-partum and from another at 47 days post-partum. Among 25 infected cows in the second herd, Str. agalactiae was reisolated from a quarter of one cow at 22 days and from that of another at 33 days after parturition.

Curtis et al. (1977) used cephalonium as a long acting intramammary cerate for dry-cow therapy. There was 81 per cent elimination of pathogenic bacteria - mainly S. aureus streptococci and E. coli - from the quarters. Ninety three per cent of quarters uninfected at drying off were free from infection four days after calving. No clinical cases of mastitis occurred during the dry period.

Heald et al. (1977) found that a combination of 400 mg of novobiocin and 400,000 IU of penicillin was effective against Str. agalactiae, other streptococcus spp. and S. aureus.

Meany and Nash (1977) found that 500 mg of benzathine cloxacillin and one megaunit of procaine penicillin plus 0.5 g of dihydrostreptomycin sulphate were equally effective in eliminating infections due to S. aureus

Merck et al. (1977) found that there was reduction in clinical mastitis and in cell count in milk samples, related to benzathine cloxacillin treatment.

Rindsig et al. (1978) reported that complete dry-cow therapy with a product containing  $10^6$  units of procaine penicillin G and 1 g of dihydrostreptomycin in a slow release base eliminated S. aureus, Str. agalactiae, other streptococci and Gram negative rods from 85.4 per cent of the infected quarters. Selective therapy eliminated infection from 88.2 per cent of the infected quarters. New infections occurred in 3.1 per cent of quarters with complete therapy and in 6.1 per cent of the quarters with selective therapy. Incidence of mastitis following dry period was less with complete therapy (4.6% of quarters) compared to selective therapy (7.8% of quarters). Selective therapy was effective as complete

therapy in eliminating existing infections and complete therapy would be the choice in situations where new infection in dry period are of concern.

Buddle and Cooper (1980) found that dry cow therapy with penicillin, novobiocin and neomycin eliminated 87 per cent of S. aureus infections from quarters. Dry-cow treatment with cephalonium was effective in eliminating infections only from quarters shedding S. aureus three or fewer of the four sampling days before drying off. The incidence of new infections in the dry period was 10 per cent after the combined antibiotic therapy and 17 per cent after cephalonium treatment. Sixty eight per cent of the untreated quarters having S. aureus infections at drying-off time were still infected at the next lactation and new infections were found in 26 per cent of the quarters. The small number of quarters persistently shedding S. aureus after calving following dry cow treatment resulted in reinfection of the herd; after seven weeks of lactation, the number of infected quarters approximately equalled that before drying off.

Malhotra et al. (1981) found upto 18.4 per cent of new infections after dry-cow treatment with benzathine cloxacillin.



Funk et al. (1982) found that cows treated with dry cow therapy drugs had more quarters cured during dry period than did untreated control for all major mastitis pathogens. A programme of dry cow treatment plus teat dipping was superior to dry-cow treatment alone, teat dipping only or neither.

Pankey et al. (1982) found that use of a dry cow preparation of novobiocin and procaine penicillin followed by a lactating form of the preparation 1-3 days prior to calving produced only a slightly better bacteriological cure rate (3.1% increase) for S. aureus than dry cow therapy alone (61.3%).

Larsen (1982) found that dry cow therapy has only a brief effect and did not reduce the overall frequency of mastitis.

Cogienard (1983) concluded that the use of whole herd therapy reduced the prevalence of clinical mastitis in herds which had a high prevalence in the first year, but in herds with a low prevalence of clinical mastitis the prevalence increased after the use of dry-cow therapy.

Robinson et al. (1983) reported that continued use of teat dipping and dry-cow therapy was associated with a high rate of coliform mastitis in herds with poor standards of hygiene and husbandry.

Smith et al. (1985a) had the opinion that all quarters of all cows should be dry-treated for maximum reduction of new streptococcal infections during the dry period. He also suggested that methods other than dry-cow therapy are required for control of coliform infections during dry period and streptococcal infections during the later half of dry period.

Smith et al. (1985b) reported that dry-cow therapy reduced the rate of streptococcal infections during the early dry period but was without effect during the prepartum period. There was no effect of dry cow therapy on coliform infection rate during the dry period.

Eberhart (1986) reported that antibiotic therapy at the end of lactation is the most effective means to eliminate existing infection and preventing new infections. He found that the rates of new infections in cows not treated with antibiotic at drying off range from 3.8 to 35.1 per cent of the quarters.

Harmon et al. (1986) found that dry-cow therapy reduced the prevalence of infections by minor mastitis pathogens like C. bovis and coagulase negative staphylococci.

Oliver (1987a) stated that dry cow therapy is not very effective against environmental pathogens.

Buddle et al. (1987) reported that cows with 3-4 quarters infected with S. aureus or streptococcus infection before dry-cow therapy had a higher susceptibility to reinfection in the following lactation. In control cows with 1-2 quarters infected before dry-cow therapy and for heifers with no previous history of infection, the susceptibility to reinfection or new infection was very low.

Cummins and McCaskey (1987) reported that multiple infusion with cloxacillin at day 0, 7, and 14 into the dry period prevented new streptococcal infections. Considering all genera of microorganisms causing mastitis, multiple dry-cow treatment with cloxacillin did not offer any advantage over single treatment.

Robinson et al. (1988) reported that in the dry and periparturient period the quarters in the full treatment group that were least susceptible to mastitis were those uninfected at drying off. With selective dry cow therapy, quarters infected with C. bovis at drying off were the least susceptible, and micrococcal infected quarters the most susceptible to mastitis. The infection status of quarters at calving had little effect on their susceptibility to clinical mastitis in the full treatment group, while in the partial treatment group quarters infected with C. bovis and micrococci were less susceptible. Withholding dry-cow therapy in

uninfected and micrococcal infected quarters resulted in an unacceptably high rate of new major pathogen infection.

Blood and Radostits (1989) stated that target of dry-cow therapy is the group of significant pathogens that reside in the udder namely S. aureus and Str. agalactiae. They also advocated use of 10,000 IU of procaine penicillin and 500 mg of furaltadone in a long acting base among many other combinations for dry cow therapy.

Sol and Balkt (1990) found that short nozzled intramammary cannulae were easy to use, and appeared to offer less stress to cow during intramammary infusion.

Smith (1990) noted that dry cow therapy of infected cattle with usually available agents will be of limited value in eliminating chronic staphylococcal infection but should be followed in all cases, because the procedure is useful in eliminating Str. agalactiae and presumably some other new infections.

Browning et al. (1990) noted that with dry cow therapy, incidence of clinical mastitis in early lactation was almost 50 per cent higher for the treated group of uninfected cows compared with the untreated groups. Incidence of clinical mastitis in early lactation was not significantly

different between treated groups of infected and uninfected animals.

Bertoldini et al. (1992) found that a combination of spramycin and neomycin was effective for dry-cow therapy.

Oliver et al. (1992) showed that prepartum antibiotic therapy was effective in eliminating many intramammary infections especially those caused by coagulase negative staphylococci.

Schukken et al. (1992) found that the quarters that were infused with an antibiotic showed a significantly lower incidence of clinical mastitis in the dry period and a reduction of minor pathogen infection at calving.

Sol et al. (1994) reported that with dry-cow therapy, the probability of cure of an infected quarter decreased when somatic cell count increased, another quarter was infected in the same cow, the infection was in a hind quarter and when the percentage of samples that were positive for S. aureus was high before drying off.

## **2.7 In vitro antibiotic sensitivity of organisms isolated from mastitis**

Raju (1972) found that Staphylococcus spp. isolated from cases of mastitis were sensitive to ampicillin (83.2%),

tetracycline (77.7%), neomycin (72.2%), streptomycin (72.2%), ledermycin (55.5%), erythromycin (55.5%), penicillin (50.0%) and sulphonamide (19.9%). Streptococci were sensitive to penicillin (100%) followed by streptomycin (83.3%), tetracycline (66.6%), ampicillin (50%), sulphonamide (33.3%), erythromycin (33.3%), neomycin (33.3%) and ledermycin (16.6%). E. coli were sensitive to ampicillin (75%), tetracycline (50%), erythromycin (50%), neomycin (40%), ledermycin (50%) and streptomycin (25%) and resistant to penicillin and sulphonamide.

Ferreiro et al. (1985b) found that among 112 S. aureus strains 37.5 per cent were completely susceptible to the 12 antimicrobial agents tested; 41.1 per cent were resistant to penicillin G; 20.5 per cent to tetracycline, 16.1 per cent to streptomycin, 13.4 per cent to ampicillin, 9.8 per cent to lincomycin and 5.3 per cent to chloramphenicol. Out of 71 S. epidermidis strains 56.3 per cent were totally susceptible while the remainder showed pattern of resistance mainly to penicillin, tetracycline, streptomycin, ampicillin and chloramphenicol. The best results were obtained with novobiocin and rifamycin.

Saikia et al. (1988) found that all S. aureus were sensitive to gentamicin, kanamycin and nitrofurantoin and

resistant to chloramphenicol (11.8%), tetracycline (41.2%), streptomycin (52.9%), chlortetracycline (58.7%) and oxytetracycline (76.5%). Coagulase negative staphylococci showed higher sensitivity to all the antibiotics tested. Str. uberis was resistant to streptomycin and one of them to kanamycin, chlortetracycline, oxytetracycline and penicillin. Str. bovis was resistant to oxytetracycline, kanamycin and sensitive to the rest of the antibiotics tested. E. coli was sensitive to all antibiotics except oxytetracycline and chlortetracycline. The decreasing order of efficacy was gentamicin, nitrofurantoin, neomycin, kanamycin, chloramphenicol and ampicillin.

Pal and Verma (1988) found that out of 45 isolates, both staphylococci and streptococci were sensitive to chloramphenicol (70.83%), kanamycin (66.67%) and tetracycline (54.17%). They were moderately sensitive to cephaloridine (41.66%) and erythromycin (33.33%). These isolates were highly resistant to sulphatriad (91.67%), penicillin (80.0%), ampicillin (66.67%) and co-trimoxazole (66.67%). E. coli was sensitive to gentamicin (77.78%) and chloramphenicol (66.6%) and moderately sensitive to cephaloridine (55.55%) and polymyxin B (44.44%). All isolates of E. coli were resistant to sulphatriad, colistin and co-trimoxazole. The drugs of

choice for the treatment of mastitis caused by Pseudomonas spp. were found to be kanamycin, streptomycin and carbenicillin.

Singh et al (1989) found that out of 174 isolates from bovine mastitis, gram-positive bacteria were sensitive to cotrimoxazole (75.88%), chloramphenicol (70.82%), kanamycin (66.66%), lincomycin (56.03%) and moderately sensitive to tetracycline (32.62%) and erythromycin (7.8%). They were resistant to carbenicillin (97.87%), penicillin (93.62%), cloxacillin (85.82%), sulphatriad (84.39%), cephaloridine (79.43%) and ampicillin (74.47%). Gram-negative bacterial isolates were sensitive to gentamicin (75.75%), co-trimoxazole (63.63%), kanamycin (60.60%) and chloramphenicol (51.51%) and moderately sensitive to streptomycin (9.09%). These were resistant to ampicillin, carbenicillin, sulphatriad, cephaloridine, colistin, polymyxin B and tetracycline.

Staphylococcal isolates from teat canal keratin and mammary secretion samples of unbred and primigravid Jersey heifers were tested in vitro for susceptibility to 12 antimicrobial agents. More than 92 per cent of the 311 isolates were susceptible to all antimicrobial agents tested. Staphylococci other than S. aureus demonstrated an overall susceptibility of 98.3 per cent to all antibiotics and S. aureus demonstrated a 97 per cent susceptibility. Across



all Staphylococcus spp., susceptibility of isolates from secretion samples was 98.1 per cent and susceptibility of isolates from teat canal keratin samples was 93.1 per cent. Differences in susceptibilities were observed among herds (Trinidad et al., 1990a).

Ali et al. (1990) tested 154 isolates from case of mastitis for their sensitivity to cloramphenicol, tetracycline, ampicillin, nitrofurantoin, streptomycin, penicillin, erythromycin and furazolidone, and found that nitrofurantoin was the most effective (86.36%) and streptomycin (38.31%) the least. The sensitivity of S. epidermidis and S. aureus to nitrofurantoin were 87.14 per cent and 44.29 per cent respectively and that of S. aureus were 44.29 per cent and 31.34 per cent respectively. Tetracycline was 85.71 per cent effective and erythromycin 14.29 per cent effective to enterobacteria. Streptococci were sensitive to erythromycin (100%) and furazolidone (100%).

# Materials and Methods

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## MATERIALS AND METHODS

### 3.1 Animals

Thirty cross bred cows in the University Livestock Farm, Mannuthy in their 2nd to 6th lactation which were in the process of drying off and in the seventh month of pregnancy were selected for the study. The animals were apparently healthy and maintained under identical conditions of feeding and management. History of each animal with respect to previous incidence of mastitis, abortion and retention of placenta was collected as far as possible. Animals were divided into three groups of ten each.

### 3.2 Treatments

On completion of seven months of pregnancy animals were examined in detail with respect to variations in size, shape and consistency of individual quarters of udder, teat abnormalities, enlargement of supramammary lymph node and for symptoms of any other diseases such as pseudocowpox etc.

The surface of the udder and teats was thoroughly washed with 1:1000 potassium permanganate solution<sup>and</sup> dried with a clean cloth. The tips of the teats were smeared with 70 per cent alcohol using a cotton swab and allowed to dry up.

Quarter samples were collected into sterile test tubes for culture and antibiotic sensitivity tests.

On day 8 clinical examination and collection of quarter samples for culture and antibiotic sensitivity test were repeated and the following treatments were done.

Group I - 500 mg of benzathine cloxacillin in a long acting base\* was infused into each quarter

Group II - Procaine penicillin 1,00,000 IU and furaltadone 500 mg in arachis oil\*\* was infused into each quarter

Group III - Untreated controls

The clinical examinations were repeated at weekly intervals till calving.

On day 22 of first infusion, quarter samples were collected for culture and antibiotic sensitivity test and the infusions were repeated.

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\* 3 g of Orbenin dry cow extra (M/s Beecham Animal Health, Brentford, England)

\*\* 10 ml of arachis oil B.P. was sterilised in hot air oven at 160°C for one hour and procaine penicillin 1,00,000 IU and furaltadone 500 mg were added and emulsified under sterile conditions and used immediately.

Clinical examinations were repeated on the day of parturition, on day 5 post partum and then at fortnightly intervals till 3 months post-partum (PP). California mastitis test, cultural examination and antibiotic sensitivity test were conducted on day 5 PP and then at fortnightly intervals till three months.

### 3.3 Cultural examination

#### 3.3.1 Preparation of culture media

##### 3.3.1.1 Trypton soya agar

Forty grams of Trypton Soya Agar\* was dissolved in 100 ml distilled water and sterilised by autoclaving at 121°C and 15 lbs of pressure for 15 minutes. It was cooled to 45°C poured into sterile Petridishes and incubated at 37°C for 18 hours to test sterility.

##### 3.3.1.2 Five per cent blood agar

Ten grams of beef extract, 10 g of peptone and 5 g of sodium chloride were dissolved in 1000 ml of distilled water by heating in a water bath. The pH was adjusted to 8.0-8.4 with 10 N NaOH and boiled for 10 minutes. The medium was filtered and pH adjusted to 7.2-7.4. Ten grams of agar was

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\* M/s Span Biologicals, Udhna

added and dissolved by heating in a water bath. The medium was distributed in 200 ml volumes and was sterilised by autoclaving at 121°C and 15 lbs pressure for 15 minutes. It was cooled to 50°C and 10 ml of fresh defibrinated blood was added aseptically to each 200 ml of the medium, mixed carefully avoiding formation of air bubbles and distributed into sterile Petridishes. It was incubated at 37°C for 18 hours to test sterility.

### **3.3.2 Inoculation of media, incubation , isolation and identification of organisms**

Immediately after collection a standard loopful of each of the quarter samples was inoculated on Trypton Soya agar and 5 per cent blood agar by streak method and incubated at 37°C for 24 hours and when negative for 48 hours before discarding as negative.

Well isolated colonies developed during incubation were selected and subjected to further studies.

Identification of the organisms was done based on grams reaction, motility, haemolysis on blood agar, pigment production, catalase test etc. (Cowan, 1974).



### 3.3.3 Antibiotic sensitivity test

In vitro antibiotic sensitivity of the organisms isolated was studied using disc diffusion technique (Barry, 1976).

#### 3.3.3.1 Preparation of culture media

Thirty eight grams of Muller Hinton agar was dissolved in 1000 ml of distilled water, sterilised by autoclaving at 121°C and 15 lbs pressure for 15 minutes, cooled to 45°C-50°C and poured into sterile petridishes upto a thickness of 4 mm. It was incubated at 37°C for 18 hours to test for sterility.

#### 3.3.3.2 Inoculation

A suspension of the organism in sterile peptone water, comparable to half the density of No.1 Mac Farland's standard was prepared and used within 15 minutes for inoculation. A sterile cotton swab was dipped in the above suspension and the excess suspension was removed by rotating the swab pressed against the sides of the test tube. This swab was smeared on Muller-Hinton agar plates rotating the plates approximately 60° each time for 2-3 times so as to ensure even distribution of the inoculum.

### 3.3.3.3 Application of antibiotic discs

Ready made discs\* incorporated with amoxicillin, ampicillin, cephaloridine, chloramphenicol, cloxacillin, cotrimoxazole, erythromycin, furazolidone, gentamicin, kanamycin, oxytetracycline, penicillin, streptomycin and sulphadiazine were used.

Using a sterile forceps, the discs were placed and gently pressed on the inoculated Muller-Hinton agar plates keeping a distance of 15 mm between the discs and between the discs and plate.

### 3.3.3.4 Incubation

The inoculated plates with antibiotic discs were incubated at 37°C for 18 hours.

### 3.3.3.5 Reading

The zone of inhibition of bacterial growth around each disc was measured using a millimeter ruler to the nearest millimeter and each was interpreted as sensitive or resistant by comparing with the ranges given by the manufacturer of the discs.

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\* M/s Span Diagnostics Pvt. Ltd., Udhna



### 3.4 California mastitis test

#### 3.4.1 Preparation of reagent

Forty grams of sodium lauryl sulphate, 150 ml of teepol and 0.1 g of bromcresol purple were added to 1000 ml distilled water and the pH was adjusted between 7.0 and 7.5.

#### 3.4.2 Procedure

Five millilitre each of the quarter milk sample and the reagent were mixed in a special paddle by rotatory motion. Formation of a gel indicated a positive reaction.

# Results

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## RESULTS

### 4.1 Intramammary infections

#### Group I

Mammary secretions collected on seven days prior to first infusion gave positive bacterial isolations in 15 of the 39 samples (38.5%) screened. The number of positive isolations increased to 17 (43.6%) just prior to first infusion. On the day of second infusion the number of affected quarters were four (10.3%) which increased to 14 (35.9%) on day 5 PP. The number of positive isolations made on day 20, 35, 50, 65, 80 and 95 PP were 14 (35.9%), 16 (41.0%), 16 (41.0%), 13 (33.3%), 12 (30.8%) and 12 (30.8%) respectively. Out of the total 133 positive cases, 134 isolations were made of which 109 (81.3%) were staphylococci and the remaining 25 (18.7%) were streptococci. None of the cultures turned out to be gram negative bacteria (Table 1).

#### Group II

Out of 39 samples collected seven days before the first infusion, 18 (46.2%) were culturally positive. The same result was repeated on the day of first infusion. On the day of second infusion the number of positive cultures ~~decreased to~~

six (15.4%) and then increased to eight (21.1%) on day five PP. On day five PP one quarter did not provide sample for culture due to clinical mastitis during the dry period. There was an increase in the number of positive samples to 13 (33.3%) on day 20 PP, 12 (33.3%) on day 35 PP, 13 (36.1%) on day 50 PP and 12 (33.3%) each for the rest of the period of study. Out of 124 isolations staphylococci predominated forming 66.1 per cent of the isolations followed by streptococci (25%) and coliforms (8.9%). From day 35 PP onwards, only 36 samples were obtained due to clinical mastitis which occurred during the dry period of one animal affecting all the four quarters. The results are presented in Table 2.

### Group III

Cultural examination of mammary secretions seven days prior to first infusion revealed, 16 (41.0%) isolates from a total of 39 samples which subsequently reduced to 15 (38.5%) on the day of first infusion and then increased to 21 (53.8%) on the day of second infusion. The results of cultural studies on day 5 PP and 20 PP were 25 (64.1%) and 17 (43.6%) respectively. Due to a case of clinical mastitis the total number of samples obtained was reduced to 36 from day 35 PP onwards. On day 35 PP there were 15 (41.7%) positive samples which remained almost comparable for the rest of the period of

the study revealing 17 (47.2%) positive samples on day 50 PP 20 (55.6%) on day 65 PP and 19 (52.8%) each on day 80 and day 95 PP. Out of 184 (49.1%) positive cases from a total of 375 samples, 18th isolations were made. Out of these 129 (69.7%) were staphylococci followed by streptococci (53; 28.6%) and coliforms (3; 1.6%) (Table 3).

#### 4.2 Subclinical mastitis

Four quarters in group I and 11 each in groups II and III had subclinical mastitis during the lactation period under study (Table 5). Staphylococcus and streptococcus were isolated from two cases each in group I. In groups I and II staphylococcus spp. were isolated from six and five cases respectively. Four cases of subclinical mastitis in group III were associated with Staphylococcus spp. and seven cases with Streptococcus spp.

#### 4.3 Clinical mastitis

There was only one case of clinical mastitis during the dry period which occurred in group II. All the four quarters of a cow which were previously positive for coliform organisms were affected. However, cultural examination after development of mastitis did not reveal any bacterial organism.

During the lactations studied, six quarters (Staphylococcus spp. isolated from one and none from five quarters) from three animals in group I, 10 quarters (Staphylococcus spp. from two and none from eight quarters) from three animals in group II and eight quarters (Staphylococcus spp. from one Streptococcus spp. from two and none from five) from four animals in group III had clinical mastitis (Table 6).

#### 4.4 Antibiotic sensitivity

In-vitro antibiotic sensitivity studies of different isolates from mammary secretions showed that staphylococci were sensitive to gentamicin (98.4%), chloramphenicol (95.6%), kanamycin (95.6%), cephaloridine (86.6%), amoxicillin (84.7%), co-trimoxazole (82.2%), cloxacillin (76.6%), furazolidone (75.9%), erythromycin (71.9%), sulphadiazine (71.6%), ampicillin (69.7%), penicillin (68.4%), streptomycin (64.3%) and oxytetracycline (55.3%). The streptococci isolated were sensitive to gentamicin (100%), cephaloridine (98.1%), chloramphenicol (98.1%), cloxacillin (96.3%), kanamycin (96.3%), amoxicillin (85.1%), furazolidone (84.1%), ampicillin (82.2%), penicillin (82.2%), co-trimoxazole (77.6%), streptomycin (57.9%), oxytetracycline (48.6%), sulphadiazine (48.6%) and erythromycin (47.7%). All the coliform isolates were sensitive to gentamicin and chloramphenicol.

Sensitivity to other drugs were kanamycin - 93.3%; co-trimoxazole - 76.9%; oxytetracycline - 61% and cephaloridine - 30%. All isolates were resistant to amoxicillin, ampicillin, cloxacillin, erythromycin, furazolidone, penicillin, streptomycin and sulphadiazine.

Staphylococcus spp. isolated from cases of mastitis were sensitive to chloramphenicol and gentamicin. Sensitivities to other drugs were cephaloridine - 70.6%; amoxicillin - 52.9%; erythromycin - 52.9%; kanamycin - 52.9%; cloxacillin - 35.3%; furazolidone - 29.4%; co-trimoxazole - 29.4% and ampicillin - 29.6%. The isolates were resistant to sulphadiazine, streptomycin, penicillin and oxytetracycline.

All the streptococcal isolates from cases of mastitis were sensitive to gentamicin, chloramphenicol, cephaloridine, cloxacillin and kanamycin. This was followed by amoxicillin (85.7%), ampicillin (85.7%), furazolidone (85.7%), penicillin (85.7%) and streptomycin (57.1%), sulphadiazine (50%), oxytetracycline (50%), and erythromycin (42.9%) (Table 7).

Table 1. Intramammary infections in the group I animals

Day of sampling	Total no. of samples	Positive Samples		Organisms isolated					
		Number	Percentage	Staphylococcus spp.		Streptococcus spp.		Coliforms	
				Number	Percentage	Number	Percentage	Number	Percentage
7 days prior to 1st infusion	39	15	38.5	9	56.3	7	43.7	-	-
Day of 1st infusion	39	17	43.6	13	76.5	4	23.5	-	-
Day of 2nd infusion	39	4	10.3	4	100.0	-	-	-	-
5 days PP**	39	14	35.9	11	78.6	3	21.4	-	-
20 days PP	39	14	35.9	13	92.9	1	7.1	-	-
35 days PP	39	16	41.0	14	87.5	2	12.5	-	-
50 days PP	39	16	41.0	14	87.5	2	12.5	-	-
65 days PP	39	13	33.3	11	84.6	2	15.4	-	-
80 days PP	39	12	30.8	10	83.3	2	16.7	-	-
95 days PP	39	12	30.8	10	83.3	2	16.7	-	-
Total	390	133	34.1	109	81.3	25	18.7	-	-

\*\* PP - post-partum



Table 2. Intramammary infections in the group II animals

Day of sampling	Total no. of samples	Positive Samples		Organisms isolated					
		Number	Percentage	<u>Staphylococcus</u> spp.		<u>Streptococcus</u> spp.		Coliforms	
				Number	Percentage	Number	Percentage	Number	Percentage
7 days prior to 1st infusion	39	18	46.2	10	55.6	4	22.2	4	22.2
Day of 1st infusion	39	18	46.2	10	55.6	4	22.2	4	22.2
Day of 2nd infusion	39	6	15.4	3	50.0	-	-	3	50.0
5 days PP**	38	8	21.1	6	75.0	2	25.0	-	-
20 days PP	39	13	33.3	11	84.6	2	15.4	-	-
35 days PP	36	12	33.3	9	75.0	3	25.0	-	-
50 days PP	36	13	36.1	9	69.2	4	30.8	-	-
65 days PP	36	12	33.3	8	66.7	4	33.3	-	-
80 days PP	36	12	33.3	8	66.7	4	33.3	-	-
95 days PP	36	12	33.3	8	66.7	4	33.3	-	-
<b>Total</b>	<b>374</b>	<b>124</b>	<b>33.2</b>	<b>82</b>	<b>66.1</b>	<b>31</b>	<b>25.0</b>	<b>11</b>	<b>8.9</b>

\*\* PP - post-partum

Table 3. Intramammary infections in the group III animals

Day of sampling	Total no. of samples	Positive Samples		Organisms isolated					
		Number	Percentage	<u>Staphylococcus spp.</u>		<u>Streptococcus spp.</u>		Coliforms	
				Number	Percentage	Number	Percentage	Number	Percentage
7 days prior to 1st infusion	39	16	41.0	13	81.3	3	18.8	-	-
Day of 1st infusion	39	15	38.5	11	73.3	4	26.7	-	-
Day of 2nd infusion	39	21	53.8	16	76.2	5	23.8	-	-
5 days PP**	39	25	64.1	15	57.7	8	30.8	3	11.5
20 days PP	39	17	43.6	10	58.8	7	41.2	-	-
35 days PP	36	15	41.7	10	66.7	5	33.3	-	-
50 days PP	36	17	47.2	12	70.6	5	29.4	-	-
65 days PP	36	20	55.6	15	75.0	5	25.0	-	-
80 days PP	36	19	52.8	14	73.7	5	26.3	-	-
95 days PP	36	19	52.8	13	68.4	6	31.6	-	-
Total	375	184	49.1	129	69.7	53	28.6	3	1.6

\*\* PP - post-partum

Table 4. Number of calvings and previous incidence of abortions, retention of placenta (ROP) and mastitis

Group I animals	No. of calvings	Previous incidence of			Group II animals	No. of calvings	Previous incidence of			Group III animals	No. of calvings	Previous incidence of		
		Abortion	ROP	Mastitis			Abortion	ROP	Mastitis			Abortion	ROP	Mastitis
1.	3	-	-	-	1	3	-	-	+	1	3	-	-	-
2.	3	-	-	+	2	3	-	-	+	2	3	-	-	+
3.	7	-	-	+	3	3	-	-	-	3	3	-	-	+
4.	2	-	-	-	4	3	-	-	-	4	3	-	-	-
5.	3	-	-	-	5	4	-	-	-	5	2	-	-	-
6.	3	-	-	-	6	7	-	-	-	6	7	-	-	+
7.	3	-	-	+	7	3	-	-	+	7	3	-	-	-
8.	3	-	-	-	8	3	-	-	+	8	3	-	-	+
9.	4	-	-	+	9	4	-	-	-	9	3	-	-	-
10.	4	-	-	+	10	6	-	-	+	10	6	-	-	-

Table 5. New incidences of sub-clinical mastitis

Day of sampling	Group I			Group II			Group III		
	No. of animals affected	No. of quarters affected	Isolate	No. of animals affected	No. of quarters affected	Isolate	No. of animals affected	No. of quarters affected	Isolate
Days 5 PP	2	2	Sta.2	2	5	Sta.4 Str.1	4	8	Sta.4 Str.4
Days 20 PP	-	-		1	2	Sta.2	1	1	Str.1
Days 35 PP	-	-		1	2	Str.2	1	1	Str.1
Days 50 PP	1	2	Str.2	-	-		-	-	
Days 65 PP	-	-		1	1	Str.1	1	1	Str.1
Days 80 PP	-	-		-	-		-	-	
Days 95 PP	-	-		1	1	Str.1	-	-	
Total	3	4	Sta.2 Str.2	6	11	Sta.6 Str.5	7	11	Sta.4 Str.7

Sta. Staphylococcus spp.

Str. Streptococcus spp.

Table 6. New incidences of clinical mastitis

Period	Group I			Group II			Group III		
	No. of animals affected	No. of quarters affected	Isolate	No. of animals affected	No. of quarters affected	Isolate	No. of animals affected	No. of quarters affected	Isolate
Prior to 15 days PP	-	-		1	4	N-4	-	-	
Within 15 days PP	-	-		-	-		-	-	
0-5 days PP	1	3	N-3	-	-		1	3	N-3
6-20 days PP	2	3	N-2 Sta.1	1	2	Sta.2	2	3	Sta.1 Str.2
21-35 days PP	-	-		1	4	N-4	-	-	
36-50 days PP	-	-		-	-		-	-	
51-65 days PP	-	-		-	-		-	-	
66-80 days PP	-	-		-	-		-	-	
81-95 days PP	-	-		-	-		1	2	N-2
Total	3	6	N-5 Sta.1	3	10	N-8 Sta.2	4	8	N-5 Sta.1 Str.2

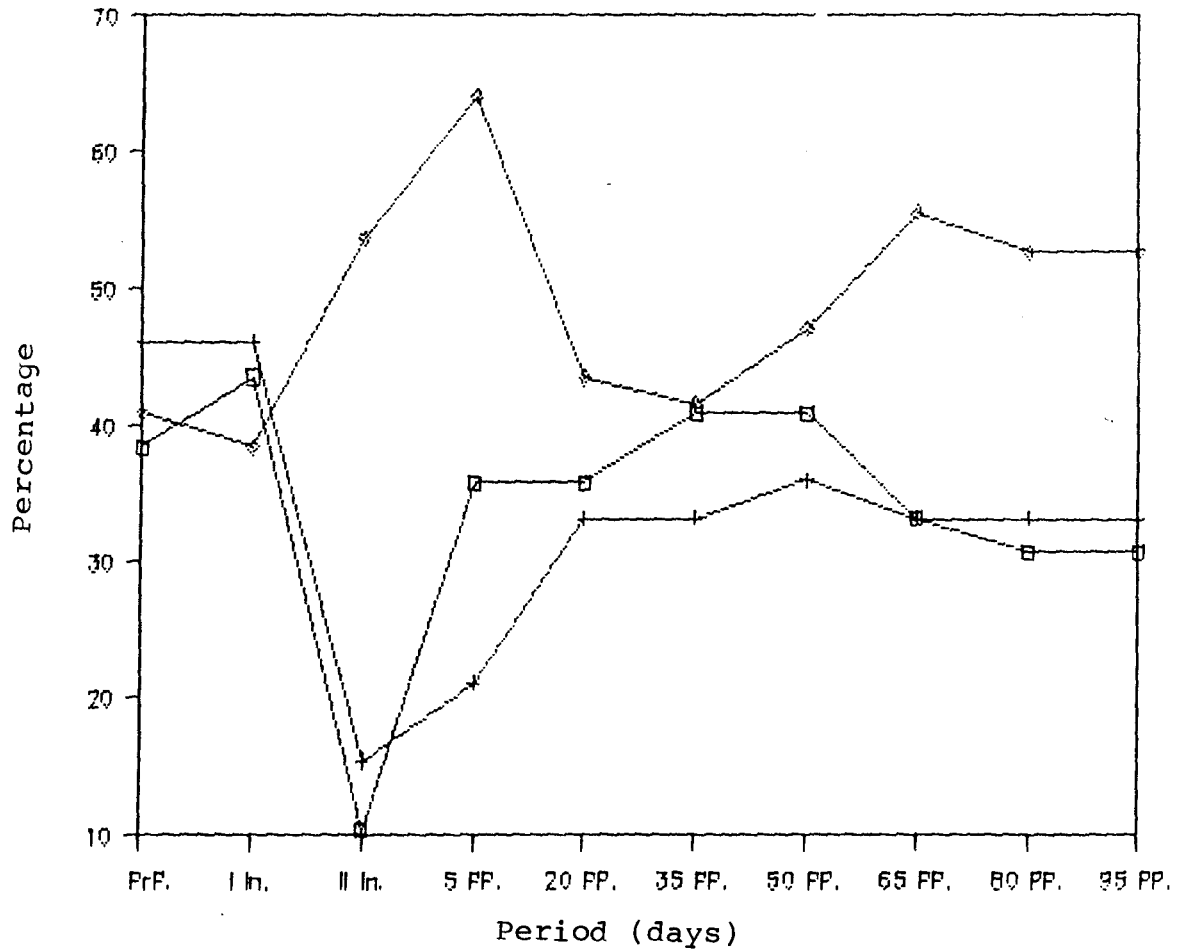
PP - Post-partum; Sta. - Staphylococcus spp.; Str. - Streptococcus spp.; N - No isolate

Table 7. Drug sensitivity of isolates

Name of drug	Intramammary infections			Subclinical mastitis			Clinical mastitis		
	Sta. (%)	Str. (%)	Coliforms (%)	Sta. (%)	Str. (%)	Coliforms (%)	Sta. (%)	Str. (%)	Coliforms (%)
Amoxicillin	84.7	85.1	0.0	46.2	100.0	-	75.0	100.0	-
Ampicillin	69.7	82.2	0.0	15.4	83.3	-	75.0	100.0	-
Cephaloridine	86.6	98.1	30.0	92.3	66.7	-	100.0	100.0	-
Chloramphenicol	95.6	98.1	100.0	100.0	100.0	-	100.0	100.0	-
Cloxacillin	76.6	96.3	0.0	23.1	100.0	-	75.0	100.0	-
Cotrimoxazole	82.2	77.6	76.9	23.1	75.0	-	50.0	100.0	-
Erythromycin	71.9	47.7	0.0	53.8	50.0	-	50.0	0.0	-
Furazolidone	75.9	84.1	0.0	15.4	83.3	-	75.0	100.0	-
Gentamicin	98.4	100.0	100.0	100.0	100.0	-	100.0	100.0	-
Kanamycin	95.6	96.3	93.3	38.5	100.0	-	100.0	100.0	-
Oxytetracycline	55.3	48.6	61.0	0.0	58.3	-	0.0	0.0	-
Penicillin	68.4	82.2	0.0	0.0	83.3	-	0.0	100.0	-
Streptomycin	64.3	57.9	0.0	0.0	58.3	-	0.0	0.0	-
Sulphadiazine	71.6	48.6	0.0	0.0	66.7	-	0.0	0.0	-

Sta. - Staphylococcus spp.

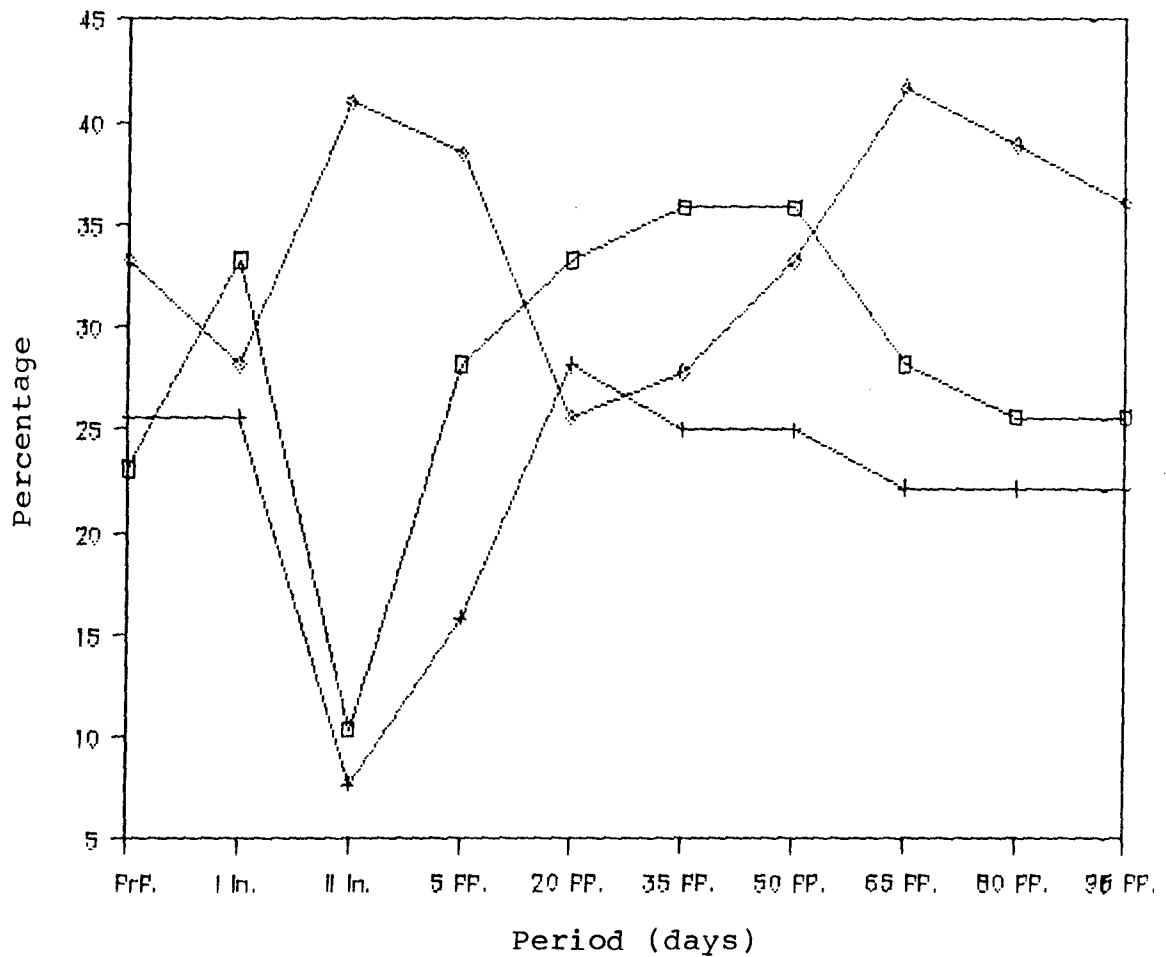
Str. - Streptococcus spp.



□ Group I                      + Group II                      ◇ Group III

PrP - Preparatory phase; In - Infusion; PP - Post partum

FIG.1 BACTERIAL ISOLATES FROM INTRAMAMMARY INFECTIONS

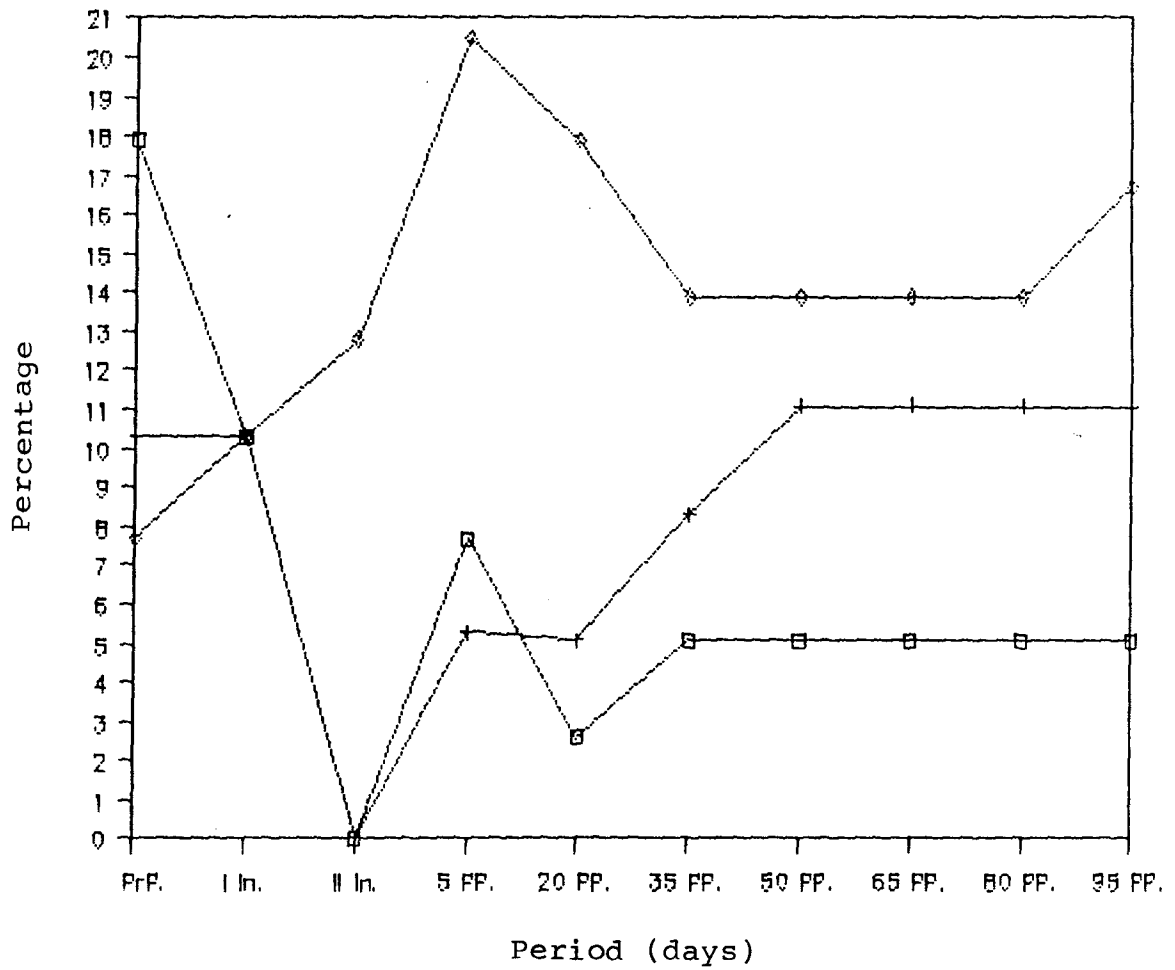


□ Group I      + Group II      ◇ Group III

PrP - Preparatory phase; In - Infusion; PP - Post partum

FIG.2 STAPHYLOCOCCI ISOLATED FROM INTRAMAMMARY INFECTIONS

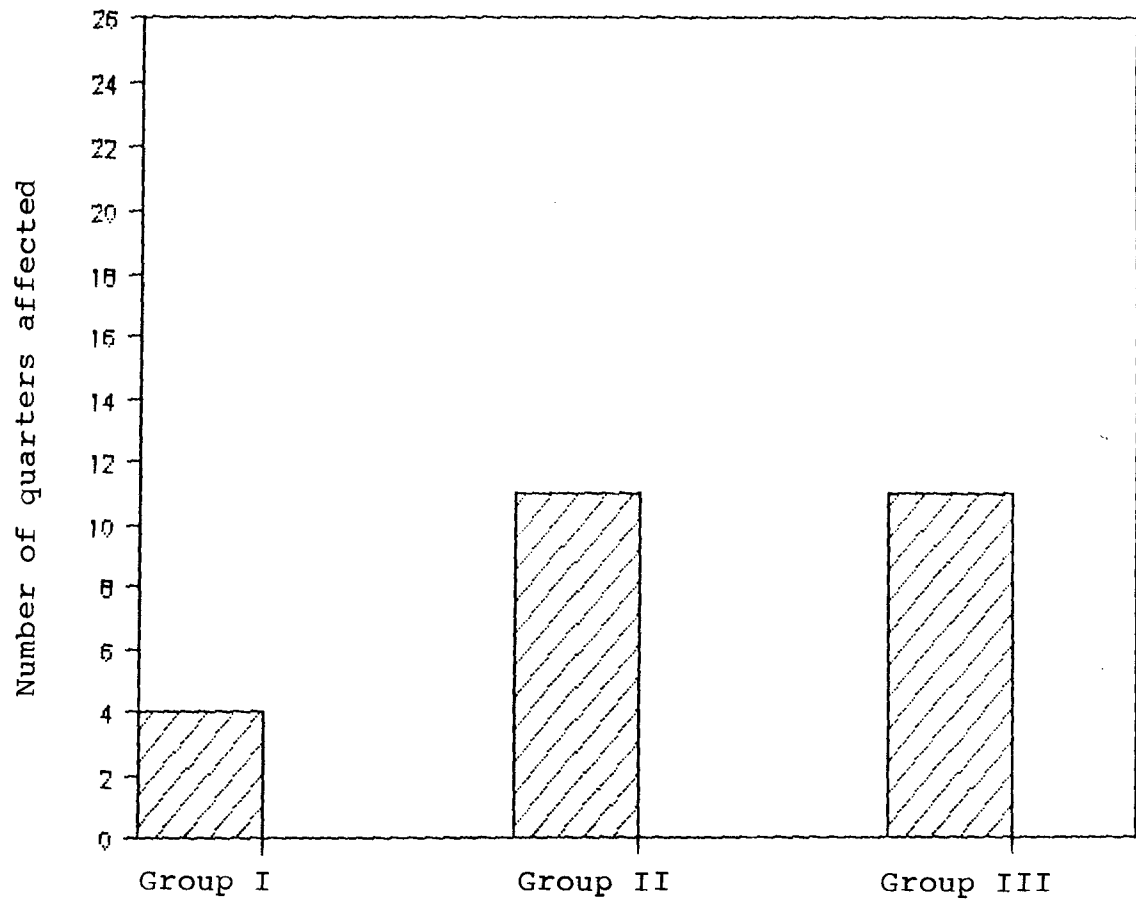




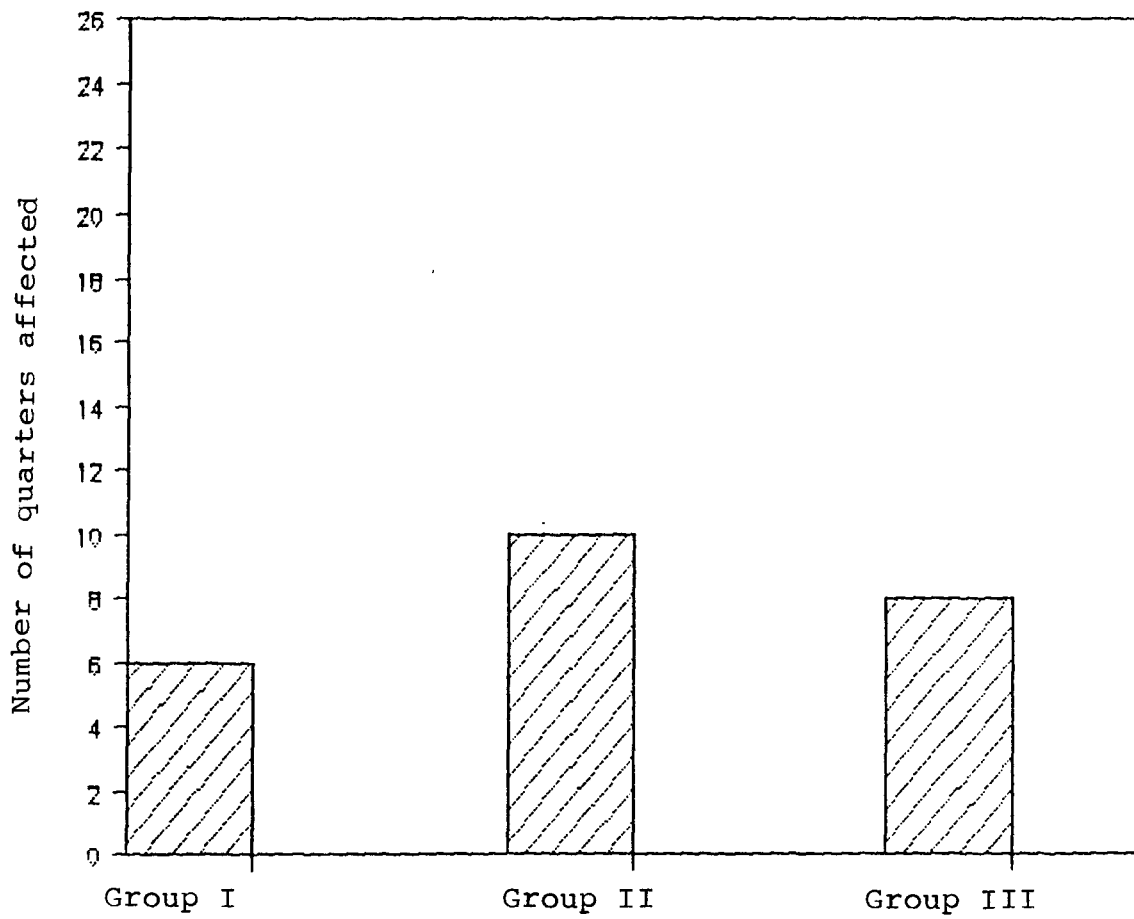
□ Group I                      + Group II                      ◇ Group III

PrP - Preparatory phase; In - Infusion; PP - Post partum

**FIG.3 STREPTOCOCCI ISOLATED FROM INTRAMAMMARY INFECTIONS**



**FIG.4 NEW INCIDENCES OF SUBCLINICAL MASTITIS**



**FIG.5 NEW INCIDENCES OF CLINICAL MASTITIS**

Plate 1 Benzathine cloxacillin



**Orbenin**  
**Extra DRYCOW**

Each syringe contains 3.5g of suspension.

Orbenin Extra DRYCOW contains 3.5g of Chloramphenicol.  
This is a broad spectrum antibiotic.  
It is effective against a wide range of bacteria.  
It is also effective against some viruses.  
It is also effective against some fungi.  
It is also effective against some parasites.  
It is also effective against some protozoa.

For animal treatment only.  
Do not use in humans.  
Keep out of the reach of children.

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# Discussion

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## DISCUSSION

Due to the advanced breeding and managerial practices widely employed, there has been increase in milk producing ability among cattle. The present Government policy envisages a great development in the dairy sector. To have maximum milk production from the available animals, they should be healthy especially with regard to the mammary glands. Mastitis is the most common affection of the mammary glands and dry-cow therapy is one of the recognised methods to control mastitis in cattle (Eberhart, 1986).

In this study, two preparations (1) Benzathine cloxacillin 500 mg in a long acting base and (2) procaine penicillin 1,00,000 IU and furaltadone 500 mg in arachis oil were tested for their efficacy in preventing clinical and subclinical mastitis during the first three months of lactation, when the drugs were infused during the dry period. Thirty cows in the process of drying off, divided into three groups of 10 each were used for the study.

There was no significant difference between the three groups with respect to parity and available data of previous

incidence of mastitis, retention of placenta and abortions (Table 4). A high incidence of quarter infection was observed in animals of all the three groups. This observation was similar to that of Oliver (1988). All the quarters which gave isolates were apparently normal; a finding which was also reported by El-Rasheedy et al. (1988). There was no significant difference between the groups with respect to intramammary infections prior to administration of the preparations. There was no significant difference between the infection rates of the replicates of the same group also. Staphylococcus spp. predominated in the positive samples (68%) followed by Streptococcus spp. (24%) and coliforms (8%). These findings are similar to that of Ferreiro et al. (1985a). Oliver (1987b) also observed a higher incidence of staphylococcal intramammary infection followed by streptococci and coliforms.

Following the first infusion of the preparations in both the groups I and II, there was drastic and significant reduction ( $P < 0.01$ ) of intramammary infection rates as revealed by the cultural examination done just prior to the second infusion (76.5% reduction in intramammary infection in group I, and 66.7% in group II). Funk<sup>et al.</sup> (1982) and Harmon et al. (1986) had findings of reduction in intra-mammary infection with dry cow therapy. Misra et al. (1984) did not observe any



new infections after treatment of dry quarters with benzathine cloxacillin, but, Malhotra et al. (1981) observed 18.4 per cent new infection after dry-cow treatment with benzathine cloxacillin.

There was an apparent higher percentage of infection in the group II attributed to three coliform isolates which did not respond to treatment, but this difference was not statistically significant.

On day 5 PP, there was an increase in the percentages of isolations in all the three groups. The increase in intramammary infection rate in groups I and II did not differ significantly but this was significantly lower ( $P < 0.05$ ) compared to that in the control group. Such a finding was reported earlier by Christie et al. (1974); Eberhart (1986) and Schukken et al. (1992). The increase in the number of isolations were apparently higher in group I compared to group II.

From day 20 PP onwards the intramammary infection levels in the groups I and II increased to levels almost comparable to those in the pretreatment samples. This pattern persisted during the rest of the period of this study. This hike may be attributed to the high prevalence of intramammary infection among the control animals which were housed along

with the treated groups, and the absence of other essential concurrent control methods such as teat dipping to prevent peripartum and subsequent infection of the quarters after the concentration of the drug infused into the udder had decreased. Buddle and Cooper (1980) also had similar findings of increased intramammary infection.

Staphylococci were the most common isolates from all the three groups uniformly throughout the study period (81.3%; 66.1% and 69.7% in the groups I, II and III respectively) followed by streptococci (18.7%; 25% and 28.6% in groups I, II and III respectively) and coliforms (0%, 8.9% and 1.6% in groups I, II and III respectively). These results were similar to that of Packer (1952), Raju (1972) and Ferreiro et al. (1985a).

Statistical analysis revealed that both the treatments were significantly ( $P < 0.01$ ) effective in controlling staphylococcal and streptococcal intramammary infections in dry cows. Paired t test revealed that staphylococcal infections are controlled more effectively by benzathine cloxacillin treatment as against the streptococcal infections which gave better results with penicillin and furaltadone.

The incidence of clinical mastitis was not significantly different among the three groups. This was

similar to the reports of Larsen (1982) and Browning et al. (1990). This finding could be directly related to the increase in the intramammary infections during the immediate post-partum period. But Merck et al. (1974) found a reduction in clinical mastitis during subsequent lactation in cows that had been treated with benzathine cloxacillin during the previous dry period.

Out of a total of 24 quarters affected among all the three groups, there was only one case of clinical mastitis during the dry period, affecting all the four quarters of a cow of group II possibly involving coliforms. No isolate was obtained from the cultures after the development of mastitis, but the quarters were previously culturally positive for coliforms. All the other clinical cases were seen during the lactation period. Six quarters (25%) (three each in group I and III) developed mastitis during the first five days PP, twelve quarters (50%) (three in group I, six in group II and three in group III) developed mastitis during the period of 6-35 days PP. Two quarters in the group III were affected during the third month of lactation. Among the isolates staphylococci (75%) were the commonest organism and 25% were streptococci. Secretions from eighteen of the affected quarters did not give any organism on culture.

Incidence of subclinical mastitis in the three groups (four (10.3%) quarters in group I and 11 (28.2%) quarters each in group II and group III) did not differ significantly ( $P < 0.05$ ) even though there was an apparent reduction in the number of quarters affected in the group I. Staphylococcus spp. were isolated from subclinical cases in 2 (5.1%) quarters in group I, 6 (15.4%) quarters in group II and 4 (10.3%) quarters in group III and these results did not differ significantly ( $P < 0.05$ ) staphylococci predominated among the isolates from subclinical cases during the first month of lactation. This was directly related to the high incidence of intramammary infection by staphylococci. It is also possible that reinfection of the glands by the pathogenic staphylococci from quarters which are persistently shedding them after calving increased the level of infection in the herd. This could happen even with the use of recommended hygienic procedures including teat spray (Buddle and Cooper, 1980). There is also chances of developing infection from L-forms, formed after treatment of intramammary infection by staphylococci with antibiotic (Sears et al., 1987).

A total of 46.2 per cent of the subclinical cases during the entire period of this study were attributed to Staphylococcus spp. and 53.8 per cent to Streptococcus spp. This is supported by the report that the bacterial culture

results of herd surveys may be quite different from result of culture of milk from cows having mastitis (Erskine et al., 1988b). So also even apparently normal quarters may reveal infection by major pathogens (El-Rasheedy et al., 1988). Staphylococci of low pathogenicity may also contribute to the higher proportion of intramammary infection in the normal quarters as against a low proportion in the diseased ones. It is also reported that S. aureus is shed in a cyclical manner from many glands which are infected (Sears et al., 1990). This will reduce the percentage of isolates from infected glands.

In vitro antibiotic sensitivity studies indicate that gentamicin, chlormphenicol, kanamycin, cephaloridine, amoxicillin, co-trimoxazole, cloxacillin, furazolidone, erythromycin, sulphadiazine, ampicillin, penicillin, streptomycin and oxytetracycline are effective against Staphylococcus spp. isolated from intramammary infections, in the decreasing order of sensitivity. Similarly streptococci causing intramammary infections were sensitive to gentamicin, cephaloridine, chloramphenicol, cloxacillin, kanamycin, amoxicillin, furazolidone, ampicillin, penicillin, co-trimoxazole, streptomycin, oxytetracycline, sulphadiazine, and erythromycin, in the decreasing order of sensitivity. Coliforms causing intramammary infection were sensitive to gentamicin, chloramphenicol, kanamycin, co-trimoxazole,

oxytetracycline and cephaloridine and resistant to amoxicillin, ampicillin, cloxacillin, erythromycin, furazolidone, penicillin, streptomycin and sulphadiazine. These results were comparable with observations of Ali et al. (1990).

Staphylococci isolated from cases of mastitis were sensitive to chloramphenicol, gentamicin, cephaloridine, amoxicillin, erythromycin, kanamycin, cloxacillin, furazolidone, co-trimoxazole and ampicillin and resistant to sulphadiazine, streptomycin, penicillin and oxytetracycline. Streptococci isolated from cases of mastitis were sensitive to gentamicin, chloramphenicol, cephaloridine, cloxacillin and kanamycin followed by amoxicillin, ampicillin, furazolidone, penicillin streptomycin, sulphadiazine, oxytetracycline and erythromycin. These results are comparable with reports of Raju (1972), Ferreiro et al. (1985a), Saikia et al. (1988), Pal and Verma (1988), Sing et al. (1989) and Trinidad et al. (1990b).

It is concluded that dry-cow therapy with both benzathine cloxacillin and procaine penicillin plus furaltadone are effective in controlling intramammary infections during the dry period. Staphylococcal intramammary infections are better controlled by benzathine cloxacillin and streptococcal infections by procaine penicillin plus furaltadone. These treatments did not control reinfection of

the glands during the peripartum period and subsequent three months of lactation. The treatments had no significant effect in controlling clinical and subclinical mastitis during the first three months of lactation, although there was an apparent decrease in the incidence of subclinical mastitis with benzathine cloxacillin treatments.

It is also concluded that gentamicin and chloramphenicol are the most effective antibiotics for the treatment of mastitis caused by gram positive cocci and oxytetracycline the least effective.

# Summary

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## SUMMARY

This study was conducted to evaluate the efficacy of dry-cow therapy in preventing post partum incidence of mastitis in cows.

Thirty cows in the process of drying off and in the 7th month of pregnancy were selected for the study. They were divided into three groups of 10 each. Mammary secretions were collected from individual quarters of all the cows for cultural examination and the animals were examined for clinical mastitis.

After seven days the animals were clinically examined for the presence of mastitis, mammary secretion were collected for cultural examination and intramammary infusions were given to all quarters of group I and group II animals as follows:

Group I : Benzathine cloxacillin (500 mg)

Group II : Procaine penicillin (100,000 IU) and  
furaltadone (500 mg)

Group III : Animals acted as untreated controls

Clinical examinations, collection of mammary secretions for cultural examination and a second infusion of

the respective preparation were done on day 22 of first infusion. Clinical examinations were repeated at weekly intervals upto calving, on the day of calving, five days post partum and then at fortnightly intervals for three months. California mastitis test and cultural examination of milk were done from day five post partum and then at fortnightly intervals for three months.

Staphylococci, streptococci and coliforms were isolated from mammary secretions during the dry period. It was found that both the treatments significantly reduced intramammary infections with staphylococci and streptococci, but not with the coliforms during the dry period. Intramammary infections with staphylococci were better controlled by benzathine cloxacillin and streptococci by penicillin plus furaltadone. The treatments had little effect on the intramammary infection levels during the subsequent lactation.

Both treatments did not have a significant effect on the incidence of clinical and subclinical mastitis, although, there was an apparent reduction in the incidence of subclinical mastitis during the first three months of lactation in those treated with benzathine cloxacillin.

Gentamycin and chloramphenicol were found to be the most effective antibiotics for the treatment of mastitis and oxytetracycline the least effective.

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\* Originals not seen

# DRY-COW THERAPY FOR CONTROL OF MASTITIS IN COWS

By

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## ABSTRACT OF A THESIS

Submitted in partial fulfilment of the  
requirement for the degree

*Master of Veterinary Science*

Faculty of Veterinary and Animal Sciences  
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## ABSTRACT

Efficacy of dry-cow therapy with benzathine cloxacillin (500 mg) and procaine penicillin (100,000 IU) plus furaltadone (500 mg) in preventing intramammary infections and clinical and subclinical mastitis during the dry period and first three months of lactation were studied.

Both the treatments were significantly effective in controlling intramammary infections during the dry period but, not during the subsequent lactation. Although there was an apparent reduction in subclinical mastitis during lactation in animals treated with benzathine cloxacillin, there was no significant difference between incidences of clinical and subclinical mastitis in treated and untreated groups.

Gentamicin and chloramphenicol were found to be the most effective antibiotics against gram-positive organisms isolated from cases of mastitis and oxytetracycline the least effective.

Intramammary infections with staphylococci are better controlled by benzathine cloxacillin as against streptococcus which is better controlled by penicillin plus furaltadone.