CLINICO-THERAPEUTIC STUDIES ON BACTERIAL MASTITIS IN GOATS

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DECLARATION

I hereby declare that this thesis entitled **"CLINICO-THERAPEUTIC STUDIES ON BACTERIAL MASTITIS IN GOATS"** 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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Certified that this thesis entitled **"CLINICO-THERAPEUTIC STUDIES ON BACTERIAL MASTITIS IN GOATS"** is a record of research work done independently by **Dr. Sreeja. S.,** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Introduction

1. INTRODUCTION

The dairy goat industry is rapidly gaining importance through out the world. In recent years the goat population and milk production from goats in India has reached 1,24,358,000 and 2,700,000 metric tones respectively. In India, the goat is reared mostly by the marginal and landless labourers as a part of their livelihood and hence called the 'poor mans cow'. Goat milk is tolerated well by the sick, elderly and infants. Therefore any factor that adversely affects the quality and quantity of caprine milk is of great economic interest and one among this is mastitis or inflammation of the mammary gland.

Mastitis is one of the costly diseases in the dairy industry but few studies deal with the condition in goats. A variety of organisms: bacteria, mycoplasma and yeasts have been incriminated as the cause of mastitis in goats. The most common bacterial agents isolated from caprine mastitis cases are *Staphylococcus aureus,* Streptococci, *Escherichia coli* and Corynebacteria. Predisposing factors like poor management and hygiene, teat injuries and faulty milking machines hasten the entry of infectious agents into the udder and leads to mastitis.

Mastitis in sheep and goats is predominantly subclinical (Contreras *et al.,* 1999) and because of this it is the financially most crippling form of disease. This goes undetected due to the normal appearance of milk and udder. The subclinical form of mastitis is 15 to 40 times more prevalent than the clinical form, is of long duration, difficult to detect, reduces the milk production and usually precedes the clinical form. When manifested clinically, mastitis in goats is more prone to become gangrenous than in cattle which may even endanger the life of the animal. The relatively poor vascularisation of goat udder together with the scarcity of anastomoses is the main predisposing factor for gangrenous mastitis in this species (Abu-Samra *et al.,* 1988).

Physical examination for signs of inflammation and culture tests of mammary secretions are the most commonly used techniques for detection of mastitis besides some indirect tests based on the cell content in milk. Indirect tests

like California mastitis test, Modified Whiteside test and Modified Aulendorfer mastitis probe test are mainly based on the somatic cell content in milk while others like NAGase and Trypsin inhibitory spot test are based on the mediators released during inflammation of the mammary gland. Rapid identification methods, in particular nucleic-acid based tests are currently gaining popularity as highly sensitive and specific diagnostic tools in mastitis. Techniques like Random Amplified Polymorphic DNA (RAPD) fingerprinting have the potential to be extremely specific and can also discriminate between closely related pathogens. It enables the sensitivity patterns of known genotypes to be compared and makes the choice of antibiotic easy.

Indiscriminate use of antimicrobials in the treatment of mastitis is common and this might generate an increase in the resistance level of many micro organisms to different drugs (Contreras *et al.,* 1995). So a thorough knowledge about the causal organisms and their profile of susceptibility to drugs is essential to initiate suitable therapy and reduce the losses. Recommended standard prophylactic and control measures of caprine mastitis closely resemble those recommended for bovine mastitis and antibiotic treatments are significant in the cure, control, and restoration of the functional capacity of the affected udder. Attention to sanitation, proper milking procedures, teat dipping and dry period antibiotic therapy is beneficial in the control of caprine mastitis.

Though much study has been conducted regarding mastitis in bovines, there is paucity of published information about appropriate treatment of clinical mastitis, dry period treatment of goats and preventive techniques in milking management of goats under Indian conditions.

Under these circumstances the present study was envisaged with the following objectives:

1. To understand the bacterial agents responsible for mastitis and their antibiogram.
2. To assess the efficacy of the two drugs used.

Review of Literature

2. REVIEW OF LITERATURE

2.1. ETIOLOGY

Ojo and Ikede (1976) induced clinical mastitis in three goats by inoculation of a local strain of *Mycoplasma agalactiae subspecies bovis* and found it to be pathogenic in the caprine mammary gland producing a disease similar to that of cattle.

Lerondelle and Poutrel (1984) reported that 7.5 per cent of non clinical intramammary infections of goats were due to major pathogens, *Staphylococcus aureus* being the most frequent isolate and 24.1 per cent was due to coagulase negative Staphylococci. Coagulase negative Staphylococci were associated with persistent infections whereas gram negative organisms were associated with intermittent infections.

Poutrel (1984) reported that *Staphylococcus epidermidis* was the most prevalent coagulase negative Staphylococci involved in infections of the caprine mammary gland and about half of the coagulase negative Staphylococcal infections gave negative CMT scores.

Bachh and Pathak (1985) opined that Staphylococci were the main etiological agents responsible for clinical and subclinical mastitis in ewes.

Staphylococcus was found to be the most commonly isolated organism from udder tissues of goats having mastitis (Dhingra *et al.*, 1985).

Kennedy-Stoskopf *et al.* (1985) in a study of virus host interaction in a goat herd noticed that all lactating does tested had virus in milk suggesting that the mammary gland may be a more important target organ for Caprine arthritis encephalitis virus.

El Hassan *et al.* (1986) isolated *Mycoplasma agalactiae* from goats in Sudan showing characteristic signs of mastitis.

A study on mastitis of goats in Iraq revealed that *Staphylococcus aureus* was the most common organism in clinical as well as in apparently normal milk samples followed by Streptococcus *spp*, Mycoplasma *spp*, *Escherichia coli*, Pasteurella and *Corynebacterium pyogenes* (Al-Graibawi *et al.*, 1986).

Examination of five goat herds to determine the cause of subclinical mastitis revealed that about 36 per cent of the halves were affected by coagulase negative Staphylococci (Manser, 1986).

In a study to determine the prevalence of intramammary infection in healthy goats, East *et al.* (1987) isolated coagulase negative Staphylococci from 17.5 per cent of the does and *Staphylococcus aureus* from 3.1 per cent.

El Sagheer (1988) reported that *Staphylococcus aureus* and *Escherichia coli* were the most prevalent causative agents in goat mastitis.

Abu-Samra *et al.* (1988) reported that coagulase positive *Staphylococcus aureus* was isolated from 60 percentage of the gangrenous mastitis in goats among the 150 cases diagnosed.

Al-Graibawi *et al.* (1989) recovered ten Mycoplasma isolates from the milk of Angora goats having firmness of udder and scanty watery milk secretions containing flakes.

Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus zooepidemicus, Streptococcus pyogenes, Streptococcus faecalis, Escherichia coli and Salmonella were isolated from goats suspected of subclinical mastitis (Mallikeswaran and Padmanabhan, 1989).

Kalogridou-Vassiliadou (1991) studied the prevalence of mastitis pathogens in 1350 normal milk samples from northern Greece and found that coagulase negative Staphylococci, *Staphylococcus aureus* and various species of the genus Bacillus predominated.

Upadhyaya *et al.* (1992) isolated *Aspergillus niger* and *Candida albicans* from milk samples of goats with mastitis and Penicillium *spp* and *Aspergillus niger* from goats without mastitis.

Escherichia coli was reported as the causative organism of gangrenous mastitis in a doe (Ameh *et al.*, 1994).

Contreras *et al.* (1995) reported that Staphylococcus (71 per cent), Corynebacteria (12 per cent), Enterobacteria (3 per cent), Pasteurella (3 per cent), Streptococci (1 per cent) and yeast (1 per cent) were the common causative organisms of mastitis in ten herds of Murciano–Granadina goats and among Staphylococci, *S. caprae, S. epidermidis* and *S. chromogenes* were the most frequent isolates.

Deinhofer and Pernthaner (1995) reported that *S. epidermidis, S. aureus, S. caprae, S. lentus, S. simulans, S. capitis, S. lugdunensis, S. xylosus, S. chromogenes, S. hominis, S. arlettae, S. warneri, S. sciuri* and *S. saprophyticus* were the Staphylococcal species isolated from goat milk.

Coagulase positive Staphylococci were the main isolates in ewes with clinical mastitis whereas coagulase negative Staphylococci predominated in subclinical mastitis of milking sheep with unilateral infections being common (Kirk *et al.*, 1996).

Bassam and Hasso (1997) experimentally induced mastitis in lactating goats with *Nocardia asteroides* of caprine origin and found that the caprine isolate produced acute clinical mastitis with systemic reaction and was more pathogenic when compared to bovine isolate.

Contreras *et al.* (1997) reported that Staphylococcus *spp* and *Pseudomonas aeruginosa* were responsible for persistent intramammary infection throughout lactation in Murciano-Granadina goats.

Udder halves of goats subclinically infected with coagulase negative Staphylococci had lower IgG concentrations in milk throughout the lactation (Ferrer *et al.*, 1997).

Fthenakis *et al.* (1998) in an investigation carried out in 335 ewes in southern Greece reported that *Listeria monocytogenes* infection led to subclinical mastitis infection in ewes which was invariably bilateral.

Tzora and Fthenakis (1998) reported *Serratia marcescens* as the causative organism of mastitis in dairy ewes.

Among the pathogens isolated from udder halves of 138 lactating goats, 95.7 per cent were Staphylococcus *spp* of which *S. epidermidis* was the most predominant (Contreras *et al.*, 1999).

Routine examination of goat milk as a part of the milk quality monitoring programme in Connecticut and Rhodeisland revealed that the most prevalent mastitis agents were non hemolytic Staphylococcus *spp* (38.2 per cent) followed by *S. aureus* (11 per cent), Streptococcus (4.1 per cent), *Escherichia coli* (1.6 per cent), Pseudomonas (1.2 per cent) and culture negative mastitis accounted for 43.9 per cent of the samples considered mastitic (White and Hinckley, 1999).

Contreras *et al.* (2000) reported that coagulase negative Staphylococci were the most prevalent pathogens involved in subclinical mastitis of goats and could even produce clinical mastitis.

Ndegwa *et al.* (2000) reported that the most prevalent bacteria in clinically normal mammary gland halves of Kenyan dairy goats were Staphylococcus *spp.* (63.6 per cent) and Micrococcus *spp* (17.7 per cent) of which the most prevalent isolates were coagulase negative Staphylococci (64.3 per cent).

According to Egwu *et al.* (2001) *Mycoplasma agalactiae* and *Mycoplasma capricolum* occurred at a significantly higher rate in udders affected by mastitis than in normal healthy udders.

A study on the prevalence of subclinical mastitis in goats and dairy ewes by bacterial isolation revealed the predominance of coagulase negative Staphylococci (McDoughall *et al.*, 2002).

Heras *et al.* (2002) isolated *Streptococcus equi subspecies zooepidemicus* from an outbreak of clinical mastitis affecting 13 of 58 lactating Awassi crossbred sheep in Spain.

Sanchez *et al.* (2002) in a study conducted in a herd of ten Murciano-Granadina goats reported that *Staphylococcus caprae* accounted for 57.7 per cent of the isolates among halves infected by coagulase negative Staphylococci.

Streptococci and gram negative bacilli are the major intramammary pathogens but were not very frequent in goats and were frequently associated with poor hygienic conditions in housing and milking parlour. Streptococci have been isolated in less than 5 to 10 per cent of the clinical cases whereas a number of epidemiological studies on subclinical mastitis reported the absence of this pathogen (Contreras *et al.*, 2003).

Sanchez *et al.* (2003) showed that *Staphylococcus aureus* and gram negative bacilli could be isolated from frozen goat milk samples which proved the utility of such samples in goat subclinical mastitis control programmes.

Coagulase negative Staphylococci were the agents most commonly involved in subclinical mastitis of goats (da Silva *et al.*, 2004).

Sanchez *et al.* (2004) reported that *Staphylococcus caprae* was the main coagulase negative Staphylococci isolated in pre and post milking samples taken from intramammary infections of seven commercial goat herds.

da Silva *et al.* (2005) reported that majority of Staphylococci isolated from mastitic goat milk were haemolytic which related to their pathogenicity in the caprine mammary gland.

2.2. EPIDEMIOLOGY

2.2.1. Prevalance

Nelson (1980) observed that with the common use of hand milking in dairy goats the comparative incidence of mastitis was lower than in dairy cattle.

Dep *et al.* (1985) reported that the prevalence of clinical and subclinical mastitis among a herd of 267 goats in Srilanka were 1.5 per cent and 12.4 per cent respectively.

Vihan (1989) reported a prevalence of 13 to 20 per cent mastitis in two herds of goats.

Maisi (1990) reported that the prevalence of infected halves in caprine subclinical mastitis was 20.2 per cent.

Ryan and Greenwood (1990) examined the prevalence of bacterial infections of udder in four commercial goat herds in New South Wales and found that coagulase negative Staphylococci were the predominant bacteria isolated from 13.3 per cent halves tested and rarely only, both halves were affected.

Incidence of major pathogens in three goat herds was 14.9 per cent to 16.4 per cent and showed no variation among herds while that of minor pathogens varied between 55 to 73.7 per cent (Kalogridou-Vassiliadou *et al.*, 1992).

The prevalence of infection in different herds ranged from 7 to 34 per cent of glands and 17 to 44 per cent of goats, most of the infections affecting a single udder half (Contreras *et al.*, 1995).

Haenlein and Hinckley (1995) stated that due to inherent differences between cows and goats, goat milk with SCC of one million cells/ml could be produced from healthy non mastitic udder and could be considered as quality milk.

According to Boscos *et al.* (1996) the prevalence of bacteria in milk samples ranged between flocks from 19 to 35.7 per cent of the glands in Saanen and autochthonous Greek goats.

Contreras *et al.* (1997) examined 1834 milk samples and reported a prevalence of mastitis in nine per cent of Murciano- Granadino goats in Spain.

In a study involving 138 lactating goats, Contreras *et al.* (1999) reported that the prevalence of bacterial intramammary infection was 34 per cent and seroprevalance of Caprine arthritis encephalitis infection was 94.3 per cent.

Sanchez *et al.* (1999) reported that the prevalence of intramammary infection in goats was 22 per cent of halves and 34 per cent of animals.

In a survey to investigate mastitis in four locations of Maiduguri, Ameh and Tari (2000) observed that out of 300 goats unilateral infections were predominant in 68 per cent of the affected animals.

The prevalence of mastitis in infected goats and halves did not change between parturition and 40 days postpartum. The number of infected halves increased with age group in goats (McDoughall *et al.*, 2002).

Gupta *et al.* (2002) reported an infection rate of 65.45 per cent in a flock of 224 goats in an organized farm with the main cultural isolates being Staphylococci, Streptococci, gram positive and negative rods, Corynebacterium, Pseudomonas and *Bacillus subtilis.*

Period prevalence of subclinical mastitis in Awassi sheep in southern Jordan was 18.3 per cent and prevalence in each flock remained relatively constant throughout the study period (Al-Majali and Jawabreh, 2003).

The most prevalent pathogens in subclinical mastitis of goats were coagulase negative Staphylococci while *Staphylococcus aureus* had little prevalence and less capacity for transmission among goats (Contreras *et al.*, 2003).

2.2.2. Managemental factors

In an experiment to determine optimum vacuum level, pulsation ratio and pulsation rate for machine milking, Lu *et al.* (1991) came to the conclusion that higher vacuum levels increased average and maximum milking rates, decreased the milking time and elevated the somatic cell counts in Alpine goats.

Contreras *et al.* (1996) opined that low rate of intramammary infections caused by gram negative bacilli in goats could be attributed to natural mammary defense and good housing management.

Lowest prevalence of intramammary infections (15 to 20 per cent) were seen in goat herds with low-line pipe line systems in which udders were washed and dried using individual paper towels (Menzies and Ramanoon, 2001).

Contreras *et al.* (2003) noticed a high prevalence of coagulase negative Staphylococci in subclinical mastitis of goats which was associated with incorrect milking machine routines, poor hygienic conditions in housing and the milking parlour.

Fthenakis *et al.* (2004) suggested a positive association between increased consumption of gossypol and high prevalence rate of mastitis (greater than 94 per cent) in a flock of dairy sheep.

2.2.3. Anatomical factors

Kirk *et al.* (1996) observed that unilateral infections of the mammary gland predominated in milking sheep.

Contreras *et al.* (1999) reported that Mycoplasmas and *Staphylococcus epidermidis* were mostly responsible for bilateral infections in goat mammary gland.

According to Ameh and Tari (2000) out of 51 goats with mastitis 16 had both halves of udder affected whereas 35 had only one half of udder affected. There

was no association between teat diameter, teat-end-to-floor distance and mastitis but teat injuries and teat end shape showed a significant degree of association.

According to Menzies and Ramanoon (2001) poor udder confirmation (supernumerary teats, unconventional teats or poorly shaped udders) had a significant but minor effect on the frequency of intramammary infections in sheep.

Presence of teat lesions had no impact on prevalance of subclinical mastitis in goats (Al-Majali and Jawabreh, 2003).

2.2.4. Risk factors

As determined by a log linear analysis, it was found that does of the Nubian breed, those with non lactating periods of more than 60 days and does in the first and last third of lactation were at higher risk for intramammary infection (East *et al.*, 1987).

Lerondelle *et al.* (1992) reported that vaccination and acidosis increased the somatic cell counts (SCC) of both halves while bacteriological infection in one half did not modify the SCC of opposite half.

Egwu *et al.* (1994) observed that does aged 2 to 5 years were more clinically affected with various types of mastitis in Sahel zone of Nigeria.

Analysis of 8403 monthly test day records from 3202 ewes revealed that flock, stage of lactation, parity and type of birth are important factors influencing milk yield, SCC and milk composition in dairy ewes (Gonzalo *et al.*, 1994).

Zeng and Escobar (1995) reported that parity of does affected milk production but did not affect standard plate count and major milk components.

Boscos *et al.* (1996) in a study of goats in Greece found that parity, breed and stage of lactation did not affect the mean CMT scores and type of bacteria isolated whereas it affected the somatic cell counts significantly.

Zeng and Escobar (1996) reported that breed or milking method had no significant effect on SCC in goat milk.

Sanchez *et al.* (1999) in a cross sectional study of 324 lactating goats of Murciano–Granadina breed reported that goats of more than six parities are at high risk for subclinical intramammary infections.

Ameh and Tari (2000) observed that goats between 2 to 5 years of age were more prone to mastitis of one form or other.

Ndegwa *et al.* (2000) observed that the risk of mastitis was more in nursing does, in advanced stage of lactation and was influenced by the type of housing and milking hygiene but had no association with parity.

Acute and chronic mastitis were more commonly observed in goats between 1 and 3 years of age (Egwu *et al.*, 2001).

McDoughall *et al.* (2002) reported that goats had a lower spontaneous cure rate of udder halves infected at parturition than sheep.

2.2.5. Spread of infection

Infection by Caprine arthritis encephalitis virus can lead to mastitis and milk and colostrums served as the primary means of transmission of the virus (Kennedy-Stoskopf *et al.*, 1985).

Wet bedding, contaminated disinfectants for teat dips or unsuitable procedures for intramammary treatment were responsible for mastitis due to Pseudomonas in goats (Smith and Sherman, 1994).

Tzora and Fthenakis (1998) observed that the teat dip cup was the source of infection in a flock of ewes leading to mastitis by *Serratia marcescens.*

Transmission of mastitis is possible by buccal carriage in lambs and may be important for Staphylococci, Pasteurella and Parapox virus (Bergonier *et al.*, 2003).

Contreras *et al.* (2003) reported that low transmission of *Staphylococcus aureus* strains among infected goat herds during milking suggested caprine strains to be less contagious than bovine strains.

2.3. DIAGNOSIS

2.3.1. California Mastitis Test (CMT)

Pettersen (1981) found a highly significant correlation between the results obtained by direct microscopic count, electronic cell count and the CMT.

El Sagheer (1988) opined on evaluating CMT score in goats a that a '3+' reaction is indicative of udder troubles while samples with score '+' should be considered suspicious and further confirmatory tests should be applied.

Kalogridou–Vassiliadou *et al.* (1992) reported that 81 per cent of udders infected with major pathogens and 65 per cent infected with minor pathogens gave CMT scores of '2' and '3'.

Caprine milk samples with coulter counter counts greater than $2x10^6$/ml or CMT greater than or equal to '2' indicated the presence of a major pathogen such as *Staphylococcus aureus* (Boscos *et al.*, 1996).

Contreras *et al.* (1996) opined that CMT scores of '2' and '3' differentiated the uninfected and infected udder glands in caprine subclinical mastitis.

Gonzalez-Rodriguez and Carmenes (1996) in a study to detect subclinical mastitis in ewes observed that CMT had a correlation of 0.82 and a positive predictive value of 89.6 per cent with SCC but the number of false positives classified with CMT increased towards the end of lactation.

McDoughall *et al.* (2001) opined that though the SCC was a better predictor of bacteriological status than either CMT score or impedence, the knowledge of CMT score and impedence increased the likelihood of predicting the presence of a bacterial pathogen compared to no testing at all.

2.3.2. Modified Whiteside test (MWST)

Manser (1986) investigated the large between herd variation in the geometric mean cell counts of uninfected milk samples and showed that WST and CMT are unreliable aids in the diagnosis of caprine mastitis.

Vihan and Sahni (1987) studied the efficacy of indirect tests for detection of subclinical mastitis in goats and found that CMT detected the highest percentage of positive cases on animal and udder half basis followed by WST and mastoid test.

Guha *et al.* (1989) found that percentage of agreement of CMT, WST and bromthymol blue card test with bacteriological examination was 80.46 per cent 89.84 per cent and 85.16 per cent respectively.

Fthenakis (1995) examined 1084 milk samples from clinically healthy ovine mammary glands and recommended score '1' for CMT and score '1+' for WST as threshold values for diagnosis of subclinical mastitis, offering at least 93 per cent accuracy of the diagnostic methods.

2.3.4. Somatic Cell count (SCC)

Pettersen (1981) observed that the geometric mean cell count of goat's milk in mid lactation was $880x10^3$ cells/ml and $690x10^3$ cells/ml as estimated by direct microscopic count and the electronic cell count respectively.

Dulin *et al.* (1983) showed that lactation number, stage of lactation and intramammary infection influenced the somatic cell counts but not the cytoplasmic particles. Infection of one udder half increased the somatic cells in the corresponding half of the same goat.

According to Hinckley (1983) elevated total levels of SCC in goat milk is seen at parturition and during late lactation.

Poutrel and Lerondelle (1983) observed that the mean cell counts in goat milk were approximately twice as high in halves affected with coagulase negative Staphylococci than in uninfected halves. Infections by major pathogens resulted in

cell counts seven times more compared to uninfected halves by coulter counter and fossomatic methods.

SCC of goat milk was higher than that of cow milk and coagulase positive Staphylococci infected halves had much higher cell counts (Hunter, 1984).

According to Lerondelle and Poutrel (1984) the arithmetic mean cell counts as determined by coultercounter for milk samples from halves infected with major pathogens were $6.77x10^6$ cells/ml and $1.78x10^6$ and $1.54\ x10^6$ cells/ ml for halves infected by coagulase negative Staphylococci and non infected halves respectively.

Atroshi *et al.* (1986) in a study in 400 Finnish goats observed that SCC was lower in goats with higher haemoglobin values.

Manlongat *et al.* (1998) concluded that increase in SCC observed in normal late lactation goat milk was due to the presence of physiologic chemotactic factors.

An investigation in five herds of Nubian goats revealed that no significant difference existed between the mean total microscopic cell count for udder halves yielding organisms and those free of infection (Siddique *et al.*, 1988).

Hinckley (1990) proposed that a new standard for goat milk was essential since dairy goats in mid lactation with no bacterial mastitis had SCC of 1 million or more.

Lu *et al.* (1991) observed that higher vacuum levels increased average and maximum milking rates, decreased milking time and elevated somatic cell counts in an experiment conducted in fifty Alpine goats.

Droke *et al.* (1993) reported that the occurrence of high somatic cell counts in bulk tank goat milk was due to the high number of neutrophils in it.

Haenlein (1993) suggested that because of the basic differences in goat and cow physiology and in the composition of their milk, SCC and CMT standards based on dairy cows were discriminatory to dairy goats and must be replaced by suitable goat milk standards.

Rota *et al.* (1993) concluded that average cell concentrations were significantly affected by stage of lactation and parity and that a simple SCC in goats had little relevance.

Deinhofer and Pernthaner (1995) observed that infection with coagulase negative Staphylococci in goats caused an increase in milk SCC and pathological changes in udder but highest SCC and highest prevalence of clinical udder alterations were associated with coagulase positive Staphylococci.

SCC in goat milk increased with intramammary infection, increasing lactation stage, increase in parity and lower milk production and 90 per cent of the difference in goats SCC was not due to intramammary infections (Wilson *et al.*, 1995).

Zeng and Escobar (1995) opined that high SCC in goat milk was natural, particularly in the later stages of lactation.

The SCC threshold for defining subclinical mastitis in the second and third month of lactation in Murciano–Granadino goats was 500 $x10^3$ cells /ml and it had a high probability of detecting accurately uninfected glands (Contreras *et al.*, 1995).

In a study conducted in three commercial dairy goat farms it was found that the overall means of SCC for a complete lactation was 9.3 $x10^5$ cells /ml and it increased as lactation advanced (Zeng and Escobar, 1996).

SCC in goat milk is apparently elevated due to the presence of nucleated cytoplasmic particles, stage of lactation, parity and Caprine arthritis encephalitis virus infection (Paape and Capuco, 1997).

According to Perrin *et al.* (1997) CMT was efficient at discriminating SCC upto and at a level of 750000 cells per ml with sensitivity of 87.6 per cent and specificity of 92.7 per cent.

Poutrel *et al.* (1997) observed that the geometric means of SCC for uninfected halves, halves infected by coagulase negative Staphylococci and those infected by major pathogens were 272x 10^3 cells/ml, 932x10^6 cells/ml and 2443x

10^6 cells/ml respectively in 1060 goats examined from eight commercial herds in France.

According to Zeng *et al.* (1997) once a month testing plan was inappropriate for Alpine goats if the SCC data were to be used as indicator of intramammary infection since the SCC was high in early and late lactations and showed marked daily variations.

In a cross sectional study in Awassi sheep it was found that the mean SCC was lower in milk samples obtained from left half compared with samples from the right half of the udder (Lafi *et al.*, 1998).

Udder halves infected by *S. epidermidis* had higher SCC ($1.8x10^6$/ml) than halves infected by other Staphylococci ($1.5x10^6$/ml) (Contreras *et al.*, 1999).

Elevated somatic cell counts alone are not a valid indication of mammary infection in goats and uninfected goat milk may contain more than $1x10^6$ somatic cells /ml (White and Hinckley, 1999).

Paape (2000) reported that mean SCC for goats were higher than cows because these are affected by physiological and environmental factors, presence of nucleated cytoplasmic particles, stage of lactation, parity and infection with Caprine arthritis encephalitis virus.

According to Haenlein (2001) variation in SCC in commercial goat herds due to non infectious factors can be as high as 90 per cent, the correlation with standard plate count of bacteria being $r = 0.44$.

A study in 159 Israeli Assaf dairy sheep revealed that the mean SCC varied significantly depending on the types of bacteria isolated, lactation number and days in lactation. In four per cent of samples SCC was over $5000x10^3$ cells although no bacteria were isolated (Leitner *et al.*, 2001).

According to Leitner *et al.* (2003) infection of udder by coagulase negative Staphylococci increased the SCC to a much greater extent in sheep than in dairy cow.

2.3.5. Other tests

The trypsin inhibitor capacity of bovine milk which increased during mastitis showed good correlation with the CMT score, somatic cell count and bovine serum albumin content (Honkanen-Buzalski and Sandholm, 1981).

Vihan and Sahni (1987) opined that NAGase activity in milk would be a more sensitive and useful diagnostic test than SCC, CMT and Whiteside test in the diagnosis of caprine subclinical mastitis.

Mattila *et al.* (1986) found that NAGase and antitrypsin were better indicators of differences between infected and non infected quarters in bovine mastitis than bovine serum albumin or SCC.

Emanuelson *et al.* (1987) compared the ability of various diagnostic tests to predict the infection status of a quarter on test day and found that predictive ability was higher for adenosine triphosphate, SCC and NAGase.

Siddique *et al.* (1988) observed that Hotis test, a routine screening test for the detection of bovine mastitis was applicable to goat milk also.

Milk NAGase, CMT and antitrypsin were elevated in infected halves when compared to healthy halves in a study conducted in 39 Finnish landrace goats during the whole lactation period (Maisi, 1990).

Grove and Jones, (1992) reported that an ELISA test could be used to identify *Staphylococcus aureus* intramammary infection in bovines with 96 per cent accuracy, 90 per cent sensitivity and 94 per cent specificity.

Cows with *Staphylococcus aureus* intramammary infection could be accurately identified by detection of specific antibody titres in milk whereas it was less accurate in identifying which quarters were infected with *S. aureus* (El Rashidy, 1992).

An enzyme linked immunosorbent assay for detecting *Listeria monocytogenes* antibodies in bovine, caprine and ovine milk samples was found to have a good specificity and sensitivity (Bourry *et al.*, 1997).

Samad and Awaz (1997) found that the trypsin inhibitor test was superior over CMT in that it could correctly diagnose 95 per cent subclinical bovine mastitis cases whereas CMT could predict only 70 per cent cases.

Bhujbal *et al.* (1999a) found that the reliability of CMT, trypsin inhibition activity, and immunoglobulin flocculation test in comparison to bacteriological examination for detection of subclinical mastitis in goats were 73.47 per cent, 100 per cent, and 83.72 per cent respectively.

Moroni and Cuccuru (2001) observed that bacteriologically negative ovine udders had higher phagocytic activity and NAGase activity.

Among CMT positive samples, Modified Aulendorfer mastitis probe test (MAMP) detected 66.79 per cent as positive for subclinical bovine mastitis (Sebastian, 2001).

Seroprevalence of *Mycoplasma agalactiae and Mycoplasma mycoides subspecies mycoides* large colony (MmmLC) was found to be high in Canary Islands by an indirect ELISA (Assuncao *et al.*, 2004).

2.3.6. Molecular techniques

Tenover *et al.* (1994) found that techniques like phage typing, plasmid DNA restriction analysis and antibiogram analysis identified 23 to 26 out of 29 isolates of *Staphylococcus aureus.*

Vandenesch *et al.* (1995) reported that ribotyping could clearly identify *Staphylococcus caprae* isolates from five human cases and goat milk from eight herds according to their species specific ribotype.

Lipman *et al.* (1996) reported that RAPD, ERICIR and ERIC primers could be used for the identification and differentiation of *Staphylococcus aureus* strains from mammary glands.

RAPD fingerprinting could be used as an accurate method to identify Streptococcus and Enterococcus species of bovine origin with a sensitivity of 90 per cent and specificity of 92 per cent (Gillespie *et al.*, 1997).

Hermans *et al.* (2001) obtained 13 different RAPD types from 59 *Staphylococcus aureus* strains from rabbit Staphylococcosis.

Riffon *et al.* (2001) found that a PCR based assay for the detection of major pathogens in cases of mastitis directly from milk samples was specific for *E coli, S. aureus, S. agalactiae, S. dysgalactiae, S. parauberis* and *S. uberis*.

Sebastian (2001) grouped 24 *Staphylococcus aureus* isolates from clinical mastitis cases of bovines into seven different genotypes using RAPD analysis.

RAPD analysis to verify antimicrobial efficacy in *Staphylococcus aureus* mastitis in cows revealed that new infections were caused by a different strain of *S. aureus* in five of the nine pairs of samples analysed from a single herd (Reeve-Johnson, 2003).

The DNA fingerprints of *Streptococcus uberis* strains from mastitis was studied using PFGE and it was found that most of the isolates were not related to each other (Khan *et al.*, 2003).

Onasanya *et al.* (2003) carried out genetic fingerprinting of 18 *Staphylococcus aureus* isolates using RAPD and out of 100 operon primers, ten showed polymorphism among the isolates.

Smyth *et al.* (2003) observed variation in the enterotoxin gene content when he screened 92 Staphylococcal isolates for enterotoxin genes *sea-sej* and the gene encoding toxic shock syndrome toxins by multiplex PCR.

Staphylococcus aureus isolated from mastitis cases in Java, Indonesia and Germany showed only minor differences in their gene patterns when analysed by PCR amplifications of genes encoding 23SrRNA, clumping factor, coagulase and gene segments encoding IgG binding region and X region of protein A (Salasia *et al.*, 2004).

Scherrer *et al.* (2004) found remarkable differences in phenotypic traits between *Staphylococcus aureus* isolates from bulk tank milk of goats and sheep and bovine milk by detection of coagulase gene (*coa*), Staphylococcal enterotoxin genes and toxic shock syndrome toxin 1(*tst*) gene by PCR.

Vancraeynest *et al.* (2004) studied the resistance to antimicrobial agents in 56 *Staphylococcus aureus* isolates from rabbits by PCR and found that antimicrobial resistance was relatively rare when compared to that of *S. aureus* isolates from other animals and humans.

El-Sayed *et al.* (2005) characterized *Staphylococcus aureus* strains from birds genotypically by PCR amplification.

2.3.7. Antibiogram

In vitro antibiotic sensitivity tests on 96 bacterial isolates from caprine mastitis revealed that chloramphenicol was the most sensitive followed by erythromycin, ampicillin, terramycin and penicillin (Venugopal, 1978).

Kapur *et al.* (1984) reported that *Staphylococcus aureus* isolated from cases of mastitis in cattle, buffaloes and goats in Hissar was found to be highly sensitive to furadantin, cloxacillin and chloramphenicol whereas *Escherichia coli* and Corynebacteria were sensitive to furadantin, neomycin and chloramphenicol.

In a study to assess the causative agents of clinical and subclinical mastitis in goats in Srilanka, 96 to 98 per cent of the pathogens isolated were sensitive to penicillin and streptomycin (Dep *et al.*, 1985).

The most effective antimicrobial agents against majority of mastitis pathogens were gentamicin, nitrofurantoin, neomycin, chloramphenicol,

erythromycin, kanamycin, tetracycline, cloxacillin and ampicillin (Al-Graibawi *et al.*, 1986).

Guha *et al.* (1989) reported that among goats in West Bengal, highest number of isolates from milk samples were sensitive to gentamicin followed by neomycin, erythromycin, tetracycline, chloramphenicol and furadantin.

Junior *et al.* (1993) reported that 80 Staphylococcal isolates from goats positive to CMT were sensitive to gentamicin and cephalothin.

Egwu *et al.* (1994) showed that tylosin had the lowest minimum inhibitory concentration out of six antibiotics tested against isolates from cases of caprine mastitis.

In vitro tests on Staphylococcus species from clinical and subclinical mastitis proved that newer antibiotics like cefoperoxone, cefuroxime, cloxacillin, methicillin, enrofloxacin and clindamycin were more effective (Fthenakis,1998).

In vitro antibiotic sensitivity tests on 56 milk samples of 36 goats indicated that cloxacillin, kanamycin, ciprofloxacin, gentamicin and chloramphenicol were the most effective antibiotics (Gupta *et al.*, 2002).

Staphylococcus aureus and coagulase negative Staphylococci were susceptible to cephalothin whereas pencillin G showed the highest *in vitro* resistance (da Silva *et al.*, 2004).

Moroni *et al.* (2004) evaluated the antibiotic susceptibility of 70 strains of coagulase negative Staphylococci and found that minimum inhibitory concentration measurements showed that all β lactams (except cefoperazone) were effective against *Staphylococcus epidermidis* and *Staphylococcus caprae* whereas other antibiotics had only minor *in vitro* efficacy.

2.4. TREATMENT

Erskine *et al.* (1995) observed that systemic administration of Ceftiofur, a third generation cephalosporin to *Escherichia coli* induced mastitis in cows helped in the treatment of the condition by prevention of tissue invasion and sepsis.

Morin *et al.* (1998) found that intramammary administration of cephapirin with intravenous administration of oxytetracycline or both and supportive treatment resulted in a better outcome for cows with clinical mastitis than did supportive measures alone.

Treatment of early and intermediate stages of gangrenous mastitis of goats with systemic and intramammary oxytetracycline in combination with diuretics and antiseptic cream was successful (Menzies and Ramanoon, 2001).

Rantala *et al.* (2002) in a study on the efficacy and pharmacokinetics of enrofloxacin and flunixin meglumine for treatment of cows with experimentally induced *Escherichia coli* mastitis found that treatment with enrofloxacin might be beneficial in the treatment of high-yielding cows in early lactation and the majority of the antimicrobial activity in milk originated from the active metabolite, ciprofloxacin.

Enrofloxacin (5mg/kg) or norfloxacin (10mg/kg) once a day by the intramuscular route has been found to be useful in the treatment of clinical mastitis in goats (Bergonier *et al.*, 2003).

Gupta and Gupta (2005) reported that ceftriaxone was 90 per cent effective in the treatment of acute mastitis of bovines under field conditions.

2.5. CONTROL

Vaccination of ewes in late pregnancy with an attenuated strain of *Mycoplasma agalactiae* gave virtually complete protection against challenge with a virulent strain (Foggie *et al.*, 1971a).

Vaccination of ewes before mating with an attenuated *Mycoplasma agalactiae* strain provided high degree of immunity to the animals when challenged during lactation (Foggie *et al.*, 1971b).

Daley and Hayes (1992) proposed that vaccines and cytokines can be used for the prevention and therapy of mastitis.

Smith and Sherman (1994) opined that the use of post milking teat dipping does not aid in the control of coliform infections in goats as these are initiated between milkings.

No difference was found for SCC in milk between dipped and undipped groups when the goats were dipped after morning and evening milkings through out the lactation with a teat dip product containing nisin (Poutrel *et al.*, 1997).

A dry cow intramammary therapy cured coagulase negative Staphylococcal infections in goats during dry period and was found to be beneficial (Menzies and Ramanoon, 2001).

Sordillo and Streicher (2002) opined that the development of successful immunomodulatory strategies can help to prevent the establishment of the disease within the mammary gland.

Dry therapy, elimination of chronically infected animals and milking time hygiene measures can be adopted as the principal control measures to decrease new infections (Bergonier *et al.*, 2003).

Chaffer *et al.* (2003) suggested that dry off treatment could reduce intramammary infection and somatic cell counts in dairy sheep.

Vaccination with an experimental vaccine against *Staphylococcus aureus* mastitis elicited a non specific health improvement of the udder in addition to specific protection against *S. aureus* infection (Leitner *et al.*, 2003).

Shrekta *et al.* (2004) concluded that DNA – protein vaccination against FnBP and *Clf* A of *Staphylococcus aureus* caused both lymphoproliferative and

humoral immune responses that provided partial protection of mammary gland from Staphylococcal mastitis.

Klinglmair *et al.* (2005) reported that the use of 1.94 per cent dodecyl benzene sulphonic acid as a teat dip did not eliminate coagulase negative Staphylococci causing subclinical mastitis in dairy ewes and therefore teat dipping cannot be recommended as a measure to control subclinical mastitis in sheep.

Materials and Methods

3. MATERIALS AND METHODS

The study was carried out in the Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary and Animal Sciences Mannuthy. The goats having mastitis which were brought to the University Veterinary Teaching Hospitals, Mannuthy and Kokkalai and from the University goat and sheep farm, Kerala Agricultural University formed the material of study.

3.1. SCREENING FOR SUBCLINICAL MASTITIS

3.1.1. Animals

The goats in the University goat and sheep farm, KAU, were screened for subclinical mastitis once in three months using California Mastitis Test (CMT).

3.1.2. Milk Sampling

All lactating does in the farm were screened by CMT to detect subclinical mastitis. The samples were collected at morning milking after discarding the first three streams of foremilk. The teat tips were carefully cleaned with cotton and 70 per cent ethanol. About 10 ml of milk from positive halves were collected aseptically in sterile vials and subjected to Modified Whiteside test, Somatic cell counts, Modified Aulendorfer mastitis probe test, Trypsin inhibitory spot test and Culture and sensitivity test. The isolates were identified by methods described by Cowan (1974).

3.2. CALIFORNIA MASTITIS TEST (CMT)

3.2.1. Reagents

CMT reagent:

Sodium lauryl sulphate	40g
Teepol	150 ml
Brom cresol purple	10 mg
Distilled water (upto)	1000 ml

3.2.2. **Procedure**

The California mastitis test was conduced as per the procedure of Schalm *et al.* (1971).

Symbol	Description of visible reaction	Mean no: of neutrophils per ml
-	No reaction	68,000
T	Slight slime, tends to disappear with continued swirling	268,000
1	Distinct slime but without gel	800,000
2	Immediate gel formation; moves as a mass during swirling	2,560,000
3	Gel develops a convex surface and adheres to the bottom of the cup	$\geq$ 10,000,000

3.3. MODIFIED WHITESIDE TEST (MWST)

3.3.1. Reagents

Sodium hydroxide (1N) solution 4 per cent

3.3.2 Procedure

Modified Whiteside test was done as per the method of Murphy and Hanson (1941).

3.3.3. Interpretation

Symbol	Description of visible reaction
-	Mixture remains opaque and free of particles
1+	Definite coagulation occurs during stirring with little or no tendency for the mass to adhere to stick. On continued stirring it separates into milky whey and well defined particles.
2+	Mixture coagulates almost as soon as stirring is started. Coagulum follows movement of stick and finally when separation occurs particulate matter is arranged in thread like whorls in clear whey.
3+	Tenacious coagulum forms immediately and adheres to stick. Upon continuous stirring, mass separates into clear whey and thready clumped opaque material.
4+	Tenacious coagulum with little or no tendency to break down into whey and particulate matter.

3.4. MODIFIED AULENDORFER MASTITIS PROBE TEST (MAMP TEST)

3.4.1. MAMP Reagent:

Sodium lauryl sulphate	40g
Urea (BP grade)	240g
Distilled water	1000ml

A few drops of phenolphthalein was added to this reagent and pH adjusted to 8 by addition of 1N NaOH solution.

3.4.2. Procedure

The test was conducted as per the method cited by Buragohain and Dutta (1991). Test tubes (10mm x 100 mm) were arranged in a test tube rack. Milk samples (2.5 ml) collected from individual halves of animals were taken in the

labeled test tubes. Equal volume of MAMP reagent was added to each test tube and mixed thoroughly. The tubes were left undisturbed for 24 hours at room temperature.

A positive reaction was observed in the form of a gelatinous product which was initially milky but became flaky later and begins to clear from bottom depending on the intensity of the reaction.

3.4.3. Interpretation

The reaction was read in centimeter units measuring from top to bottom as follows:

0 to 3 cm turbidity	: healthy udder half
3 to 5 cm turbidity	: mild subclinical mastitis
5 to 8 cm turbidity	: severe subclinical mastitis

3.5. TRYPSIN INHIBITORY SPOT TEST (TIST)

3.5.1. Milk trypsin reagent:

Trypsin (1:250)

PBS (pH 7.4)

(19 ml of 0.2 M $NaH_2PO_4.2H_2O$ + 81 ml of 0.2 M $Na_2HPO_4.12H_2O$)

Milk trypsin reagent was made by adding 530 mg trypsin (Difco) to 100 ml of PBS (pH 7.4) so that 400 BAEE units of trypsin were present in 1 ml of the solution. The reagent was stable at refrigeration temperature for a period of 1 week.

3.5.2. Procedure:

The test was conducted as per the method of Samad and Awaz (1997).

To 20 µl of milk sample taken on a glass slide 20 µl of trypsin reagent was added and mixed well. The mixture was spotted on an unexposed X ray film measuring 2.5 x 2.5 cm and allowed to stand at an ambient temperature for 10 minutes. The film was washed under running tap water with gentle rubbing of the surface of the film.

3.5.3. Interpretation:

Digestion of film	: no trypsin inhibitor	: normal milk
Absence of digestion	: presence of trypsin inhibitor	: subclinical mastitis

3.6. SOMATIC CELL COUNT (SCC)

3.6.1. Reagents

1. Broadhurst-Paley stain

Methylene blue	1.5g
Ethyl alcohol (70 per cent)	250 ml
Basic fuchsin	1.0g
Ethyl alcohol (95 per cent)	10.0 ml
Aniline	5 ml
Sulphuric acid	15 ml

Methylene blue (1.5 g) was dissolved in 250 ml hot 70 per cent ethanol. To this 10 ml of saturated alcoholic basic fuchsin solution (1g dissolved in 10 ml of 95 per cent ethanol) was added. Keeping the solution warm, 5 ml of aniline was added to it and mixed well. Then added 15 ml dilute sulphuric acid to the solution and filtered it. To every 100 ml of the filtrate 50 ml of hot distilled water was added. The stain was then stored in a glass stoppered bottle in a refrigerator.

2. Xylene

3. 95 per cent ethanol

3.6.2. Procedure

The milk smear was prepared as per the procedure of Prescott and Breed (1910) and it was stained using Broadhurst-Paley triple step procedure described by Schalm *et al.* (1971).

3.6.3. Calibration of microscope and cell counting

A monocular microscope with a microscopic factor of 566,300 was used. Microscopic factor was obtained by measuring the diameter of the microscopic field seen through the oil immersion objective using a stage micrometer and ocular micrometer. The stained milk smears were examined using this calibrated microscope. Fifty fields were counted by moving the slide horizontally from one edge of the film through the center to the opposite edge. The second count was made vertically through the film in the same manner. The working factor was calculated by dividing the microscopic factor by the number of fields counted. The total cells counted multiplied by the working factor gave the number of cells per ml of milk.

All nucleated somatic cells within a field including those at the periphery with more than 50 per cent of the cell body in view were counted. Free nuclei representing more than 50 per cent of the nuclear material were also counted. Cytoplasmic mass without nucleus and small cell fragments with little nuclear material were not counted.

3.6.4. Interpretation

Milk solids were stained pink, mononuclear cells deep blue, polymorphonuclear leukocytes pale blue and bacteria either deep or light blue. A value of more than 1×10^6 cells per ml of milk was considered to be indicative of mastitis.

3.7. ISOLATION AND IDENTIFICATION OF BACTERIA

3.7.1. Glasswares and reagents

Glasswares of Borosil brand, analytical or guaranteed grade of reagents, chemicals and culture media (Hi-media laboratories, Private Limited, Mumbai) were used for the study.

3.7.1.1. Preparation of glassware and culture media

The petriplates and test tubes were kept in 0.1 per cent hydrochloric acid overnight. They were washed in running tap water and immersed in detergent solution for a day. The petriplates and test tubes were washed thoroughly in running tap water and then in distilled water. They were then dried and sterilized in hot air oven at 160°C for 1 hour.

The culture media were reconstituted in double distilled water according to the manufacturer's instructions. It was then sterilized by autoclaving at 121°C and 15 lbs of pressure for 15 minutes. After cooling to 45°C, it was poured into sterile petriplates and test tubes and incubated at 37°C for 24 hours to test the sterility.

3.7.1.2. Blood agar

Brain heart infusion (BHI) agar supplemented with 5 to 10 per cent sterile ovine or bovine blood was used for isolation.

3.7.2. Method of isolation

A loop from each sample was inoculated into blood agar plates. The plates were examined after 24 to 48 hours incubation. Isolated colonies were selected and a representative sample was streaked on BHI agar slants for further identification. The slants were preserved by storing in refrigerator at 4°C.

3.7.3. Identification of bacteria

The isolates were stained by grams method and the preliminary tests were done based on it. The morphological, cultural, biochemical and sugar fermentation tests of the isolates belonging to different species were determined as per the methods described by Cowan (1974).

Selective media used in the present study were

1. Mannitol salt agar
2. Edwards medium

3. Eosin methylene blue agar
4. McConkey agar

The biochemical tests employed for the identification of isolates were

1. Catalase Test
2. Oxidase test
3. Oxidation – fermentation test
4. Carbohydrate utilization
5. Coagulase test
6. Indole production
7. Methyl Red test
8. Voges- Proskeur test

Rapid biochemical test kit [Hi-E coli (KB010), Himedia] was employed for the identification of *Escherichia coli.* The tests included in the kit were:

1. Methyl Red test
2. Voges-Proskeur test
3. Indole production
4. Citrate utilization
5. Glucuronidase production
6. Nitrate reduction
7. ONPG
8. Lysine decarboxylation
9. Carbohydrate fermentation

3.8. ANTIBIOGRAM

3.8.1. Materials

Mueller–Hinton agar was used to study the antibiotic sensitivity pattern of the isolates. The following antibiotic discs with known concentration as noted in micrograms (mcg) were used (Hi- media).

1. Ceftriaxone	10 mcg / disc
2. Ciprofloxacin	5 mcg / disc
3. Chloramphenicol	30 mcg / disc
4. Oxytetracycline	30 mcg / disc
5. Gentamicin	10 mcg / disc
6. Sulphadiazine	300 mcg / disc

3.8.2. Method

Antibiotic sensitivity test was done as per the standard Single disc diffusion method of Bauer *et al.* (1966).

3.8.3. Interpretation

The zone of inhibition of bacterial growth around each disc was measured and interpreted as sensitive, moderately sensitive or resistant by comparing with the ranges given by the manufacturer.

3.9. CLINICAL MASTITIS CASES

The goat mastitis cases brought to the University Veterinary Teaching Hospitals Mannuthy and Kokkalai were divided into two treatment groups. A broad spectrum antibiotic ceftriaxone and a chemotherapeutic agent ciprofloxacin were tested for their efficacy in mastitis cases.

Animals were allotted to each group randomly. On the day of presentation the milk samples were collected aseptically in sterile vials and subjected to culture and sensitivity tests. Similarly milk samples from individual halves were collected 72 hours after the antibiotic therapy and bacterial isolation was attempted to assess the efficacy of the drugs in treating the condition.

Group I: Treated with Ceftriaxone (Wocef- 0.5)[1] @ 10 mg/kg paranterally once daily for 3 to 5 days.

[1] Wocef- 0.5 : Wockhardt Limited, Mumbai

Group II: Treated with Ciprofloxacin (C Flox inj)[2] @ 10 mg/kg paranterally once daily for 3 to 5 days.

3.10. RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) ANALYSIS

3.10.1. DNA Isolation

Isolation of DNA from bacterial cultures was done using high salt method (Lahiri and Nurnberger, 1991).

3.10.1.1. Reagents

1. Tris potassium chloride magnesium chloride-1 buffer (TKM-1)

Tris HCl	10mM pH 7.6
KCl	10mM
MgCl2	10mM
EDTA	2mM

2. Tris potassium chloride magnesium chloride-2 buffer (TKM-2)

Tris HCl	10mM pH 7.6
KCl	10mM
$MgCl_2$	10mM
EDTA	2mM
NaCl	0.4M

3. Low Tris EDTA buffer (LTE) pH 8.0

Tris 1 M stock solution (pH 8.0)	1ml
Sodium EDTA 0.2 M stock solution (pH 8.0)	0.5 ml

Distilled water to make 100ml

4. Lysozyme (Sigma)

Bacterial lysozyme 5 mg/ml

[2] C Flox inj : Intas Pharmaceuticals, Ahmedabad

5. Ten per cent sodium dodecyl sulphate

6. 6M NaCl

7. Absolute ethanol

8. 70 per cent ethanol

3.10.1.2. Procedure

1. The Staphylococcal isolates were grown overnight on Mannitol salt agar plates and a pure colony was inoculated into 5ml of BHI broth and incubated at 37°C for 18 hours.

2. 2 to 3ml of the broth culture was taken in eppendorf tubes and centrifuged at 10,000 rpm for 5 minutes to form a pellet.

3. Resuspended the pellet in 0.5 ml TKM- 1.Washed the pellet twice or thrice in TKM-1.

4. Resuspended the final pellet in 0.5 ml of TKM-2, added 50µl of lysozyme (5mg/ml) and kept at 37°C in a water bath for 15 minutes.

5. To this added 50µl of 10 per cent sodium dodecyl sulphate and mixed by pipetting.

6. Added 250µl of 6M NaCl and mixed well.

7. Centrifuged at 10,000 rpm for 10 minutes. The supernatant containing nucleic acid was transferred to another eppendorf tube and two volumes of absolute alcohol was added.

8. Mixed thoroughly by inverting and centrifuged at 10,000 rpm for 15 minutes.

9. Washed the pellet with 70 per cent ethanol.

10. Centrifuged at 10,000 rpm for 10 minutes.

11. Resuspended the pellet in 15µl of LTE buffer by keeping at 65°C for 15 minutes.

3.10.2. RAPD Assay

3.10.2.1. Reagents

The DNA amplification kit and buffer for the RAPD Assay were obtained from Genei, Bangalore.

1. Primer

G3 primer used by Sebastian, (2001) was used in the assay. The sequence of the primer was as follows:

5' AGTAAGTGACTGGGGTGAGCG 3'

The primer was obtained from Genei Bangalore.

2. RAPD Reaction Buffer

This included 50mM KCl, 10mM Tris and 1.5mM $MgCl_2$

3. Taq DNA polymerase

The Taq DNA polymerase enzyme with a concentration of 1.5 U/µl was used.

4. Deoxyribonucleotide triphosphate (dNTPs)

200 micromoles each of dGTP, dATP, dTTP and dCTP (10mM) were used in the assay.

5. Magnesium chloride

$MgCl_2$ with a concentration of 25 mM was used in the assay.

6. DNA Molecular Size Markers

A 10 kilobase pair molecular marker (Life Technologies, USA) with fragment sizes of 12216, 11198, 10180, 9162, 8144, 7126, 6108, 5090, 4072, 3054, 2036, 1636, 1018, 517, 506, 396, 344, 298, 220, 201, 154, 134 and 75 base pairs was used.

3.10.2.2. Reconstitution and dilution of primers

5 µl of the primer was added to 45 µl of the buffer to make a working concentration of 25 nm.

3.10.2.3. RAPD –PCR

The RAPD–PCR reaction was carried out as per the method described by Vandenbergh *et al.* (1999).

RAPD – PCR was performed in a total volume of 30 µl reaction mixture. A mastermix was prepared before setting up the PCR reaction by combining the reagents in a 25 µl volume.

Preparation of mastermix for the reactions

Reagents	Quantity
Buffer	2.5 µl
d NTPs	2 µl
$MgCl_2$	1.5 µl
Primer	1.0 µl
Taq DNA polymerase	0.5 µl
Triple distilled water	18 µl

To each PCR tube 25 µl of mastermix was added along with 5 µl of the template DNA. The PCR amplification was carried out in an automated thermal cycler (Eppendorf master cycler, Germany).

Programme for amplification was as follows:

	First cycle	Next 34 cycles
Denaturation	94°C for 5 minutes	94°C for 5 seconds
Annealing	34°C for 30 seconds	34°C for 30 seconds
Extension	72°C for 1 minute	72°C for 1 minute
	Final extension 72°C for 5 minute	
Total number of cycles = 35		

3.10.3. Detection of PCR products by electrophoresis

The amplified RAPD products were detected by electrophoresis in 1.5 per cent agarose gel in TAE buffer (1x). A horizontal immersion gel electrophoresis tank (Genei, Bangalore) was used for electrophoresis.

3.10.3.1. Reagents

1. TAE buffer pH 8.0 (50x)

Tris base	242 g
Glacial acetic acid	57.1 ml
0.5 M EDTA	100 ml
Triple glass distilled water	1 liter

Working solution:

Stock solution (50x)	10 ml
Triple distilled water	490 ml

2. Ethidium bromide solution

A stock solution of 5mg/ml ethidium bromide was prepared using TAE working solution. Then a working solution of ethidium bromide was prepared in the concentration range of 0.5 to 1 mg/ml using TAE working solution and stored at 4°C in amber coloured bottles.

3. Agarose gel

MultiABgarose (ABgene, UK)	0.45g
TAE buffer	30 ml

4. Gel loading buffer

Bromophenol blue	0.25g
Xylene cyanol	0.25g
Sucrose	40.0g
Distilled water	100ml

3.10.3.2. Procedure

Agarose gel electrophoresis was carried out in a horizontal submarine electrophoresis unit. Agarose (1.5 per cent) was dissolved in TAE buffer by heating and cooled to 50°C. To this ethidium bromide was added at a final concentration of 0.5µg/ml. The gel was placed in the gel tray in buffer tank after a time period of 30 minutes for solidification of the gel. TAE buffer was added until it covered the top of the gel completely. Ten µl of the RAPD product was mixed with 2µl of gel loading buffer and samples were loaded into respective wells carefully and a 10 kilobase pair molecular marker was loaded into the last well.

Electrophoresis was carried out at 100V at room temperature for 30 to 45 minutes depending on the length of the gel or until the bromophenol dye had migrated more than half the length of the gel. The gel was visualized under UV transilluminator (Fotodyne, USA) and the results were documented in a gel documentation system (Bio-rad laboratories, USA).

Genetic fingerprinting and phylogenetic diversity between the different Staphylococcus isolates were determined by analyzing the RAPD data using RAPD distance software to generate a phylogenetic tree.

3.11. STATISTICAL ANALYSIS

The data obtained in the study were subjected to statistical analysis as per the procedure of Snedecor and Cochran (1994).

Results

4. RESULTS

The California mastitis test was used to screen the animals in the University goat and sheep farm, for subclinical mastitis once in three months. The positive milk samples were subjected to other tests like Modified Whiteside test, Modified Aulendorfer mastitis probe test, Somatic cell count, Trypsin inhibitory spot test, and Culture and sensitivity test. Twenty four animals with clinical mastitis brought to the University Veterinary Teaching Hospitals, Mannuthy and Kokkalai and from the University goat and sheep farm were randomly allocated into two treatment groups, the first group being treated with ceftriaxone and the second group using ciprofloxacin. The Staphylococcal isolates from both clinical and subclinical mastitis cases were subjected to RAPD analysis.

4.1. SCREENING FOR SUBCLINICAL MASTITIS

Milk samples from 642 halves of 324 crossbred does maintained at the University goat and sheep farm were screened for subclinical mastitis using CMT. 194 udder halves were found to be positive for subclinical mastitis. Screening was done thrice during the period of study. In the first screening, a total of 267 halves of 135 goats were examined and 23.97 per cent were found to be positive. The second screening detected the occurrence of subclinical mastitis as 33.82 per cent when 207 udder halves of 104 goats were screened. The occurrence of subclinical mastitis was found to be 35.71 per cent when 168 halves of 85 goats were screened using CMT in the third screening. All milk samples were classified based on CMT reaction as 'T', '1', '2' and '3' (Table 1).

4.1.1. Teat characteristics

Teat length and the distance from teat-end-to-floor were recorded in 324 goats. The results are presented in Table 2. Teat length ranged from 5.5 to 14.8 cm and distance from teat-end-to-floor ranged from 7.5 to 31.2 cm. Chi-square analysis of the results showed that there was no significant association between teat length and occurrence of subclinical mastitis. There was significant association between teat-end- to-floor distance and the occurrence of subclinical mastitis. As

Table 1. Screening for subclinical mastitis

Number of screenings	Period	Number of goats screened	Number of halves	Number of goats + ve by CMT	Number of blind halves	Number of CMT +ve halves	Number of CMT -ve halves	CMT scores			
								T	1	2	3
1	November 2004	135	267	32	3	64 (23.97)	203 (76.03)	13 (20.3)	22 (34.38)	26 (40.6)	3 (4.69)
2	February 2005	104	207	38	1	70 (33.82)	137 (66.18)	9 (12.86)	19 (27.14)	33 (47.14)	8 (11.43)
3	May 2005	85	168	33	2	60 (35.71)	108 (64.29)	7 (11.67)	27 (45.0)	21 (35.0)	6 (10)
Total		324	642	103	6	194 (30.2)	448 (69.78)	29 (14.95)	68 (35.05)	80 (41.24)	17 (8.76)

Figures in parentheses indicate percentage to total udder halves in each screening

Table 2. Subclinical mastitis reactors based on teat length and distance from floor to teat

Teat length (cm)				
	<8	8-11	11-14	>14
Culture negative	82 (78.1)	58 (77.3)	5 (55.56)	2 (40.0)
Culture positive	23 (21.90)	17 (22.67)	4 (44.44)	3 (60.0)
Total	105	75	9	5
Chi- square = 5.89			NS at 5 percent level	
Floor–teat distance (cm)				
	<14	14-21	21-28	>28
Culture negative	12 (100)	26 (52)	30 (38.96)	32 (58.18)
Culture positive	0 (nil)	24 (48)	47 (61.03)	23 (41.81)
Total	12	50	77	55
Chi- square = 17.63		P < 0.01	Degrees of freedom = 3	

Figures in parenthesis indicate percentage to total

Table 3. Udder half distribution of subclinical mastitis

No:	Description	No: +ve by CMT	Percentage	No: +ve by culture	Percentage
1	Left half	97	50	22	38.6
2	Right half	97	50	25	43.9
3	Only one half	12	6.19	4	7.02
4	Both halves	91	46.91	6	10.53

the distance increased occurrence of mastitis decreased. The highest occurrence (61.03 per cent) was observed in animals where the distance from teat-end-to-floor was between 21 and 28 cm.

4.2. CALIFORNIA MASTITIS TEST (CMT)

Six hundred and forty two milk samples were screened for the presence of subclinical mastitis using CMT. Out of these 194 (30.2 per cent) samples were positive and 448 (69.78 per cent) were negative. Among the positive samples, 29 (14.95 per cent), 68 (35.05 per cent), 80 (41.24 per cent) and 17 (8.76 per cent) halves were graded as 'T', '1', '2', and '3' respectively (Fig 1).

When 267 halves were screened in November 2004; 64 (23.97 per cent) were positive by CMT out of which 20.3, 34.38, 40.6 and 4.69 per cent were graded as 'T', '1', '2' and '3' respectively. Out of 207 halves screened in February 2005; 70 (33.82 per cent) were positive and 12.86, 27.14, 47.14 and 11.43 per cent were classified as 'T', '1', '2' and '3' respectively. In the third screening done in May 2005; 168 udder halves were examined by CMT and 60 (35.71 per cent) were positive. Among the positive cases 11.67, 45.0, 35.0 and 10 per cent samples were grouped in the respective CMT scores. The distribution of subclinical mastitis udder half-wise in CMT positive samples are represented in Table 3. Both halves were affected in 46.91 per cent cases. Left and right halves were equally affected (50 per cent) and in 6.19 per cent only one half was affected. As per bacteriological findings the occurrence of subclinical mastitis was 7.32 per cent.

4.3. SOMATIC CELL COUNT (SCC)

Somatic cell counts of 194 milk samples were noted. The appearance of somatic cells in Broadhurst-Paley stained milk smear is shown in Plate 1. Statistical analysis showed that SCC in goat milk were not normally distributed. So the actual SCC were transformed to logarithmic form for further statistical analysis. For testing significant difference between CMT and MWST scores and MAMP reactions analysis of variance (one-way ANOVA) was done by taking logarithmic values of actual counts and the results are presented in Table 4.

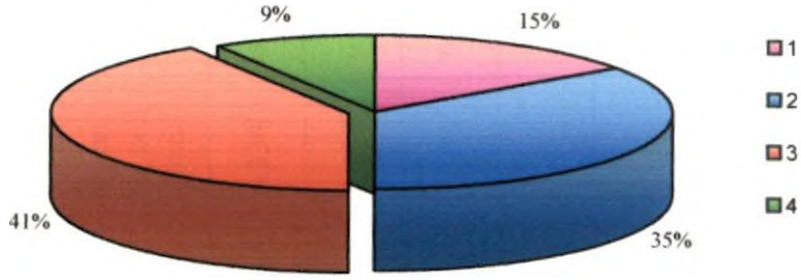

Fig 1. Grading of udder half milk samples based on CMT

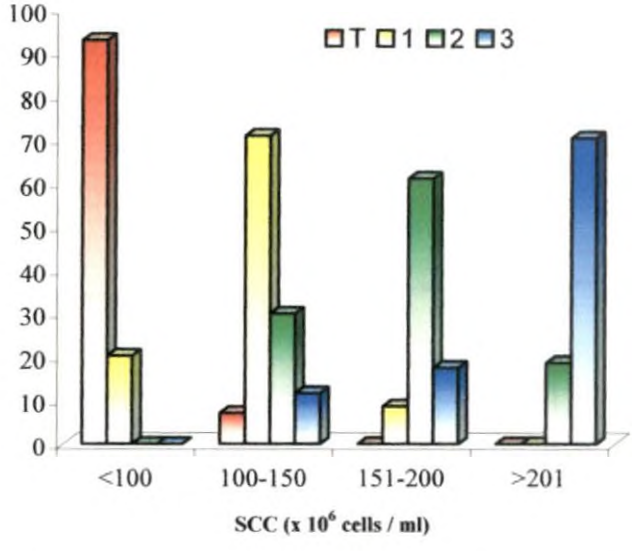

Fig 2. Distribution of CMT scores for different ranges of SCC

Table 4. Mean somatic cell counts ($\log_{10}$) for CMT and MWST scores and MAMP values

No:	CMT score	Mean log cell count	SD	Arithmetic SCC ($x10^6$)	MWST score	Mean log cell count	SD	Arithmetic SCC($x10^6$)	MAMP	Mean log cell count	SD	Arithmetic SCC($x10^6$)
1	T	6.237^a	0.04	0.736 ± 0.033	0	6.321^a	0.08	1.131 ± 0.049	0-3 cm	6.334^a	0.08	1.2 ± 0.04
2	1	6.339^b	0.05	1.199 ± 0.031	1+	6.380^a	0.07	1.432 ± 0.054	3-5 cm	6.525^a	0.32	4.055 ± 0.895
3	2	6.428^c	0.08	1.732 ± 0.086	2+	6.476^b	0.27	3.124 ± 0.760	5-8 cm	6.673^b	0.43	7.109 ± 1.723
4	3	7.325^d	0.08	20.417 ± 0.851	3+	6.601^b	0.38	5.254 ± 2.458				
5					4+	7.081^c	0.41	14.259 ± 4.285				

*$P < 0.05$. Means having same superscripts do not differ significantly

From the table it can be inferred that the mean log cell counts for each CMT score showed significant difference. The mean log cell counts for CMT scores 'T', '1', '2' and '3' were 6.237, 6.339, 6.428 and 7.325. The arithmetic mean cell counts for the CMT scores were - score 'T' had a cell count of $0.736 \pm 0.033 \times 10^6$; '1' had $1.199 \pm 0.031 \times 10^6$; '2' had $1.732 \pm 0.086 \times 10^6$ and '3' had a count of $20.417 \pm 0.851 \times 10^6$ cells/ml. The distribution of CMT scores for different ranges of somatic cell counts are presented in Fig 2.

4.4. MODIFIED WHITESIDE TEST (MWST)

Among 194 milk samples subjected to Modified Whiteside test, 72 (37.11 per cent) were classified as negative, 53 (27.32 per cent) as '1+', 55 (28.35 per cent) as '2+', 10 (5.15 per cent) as '3+' and 4 (2.06 per cent) were graded as '4+' (Fig 3). The mean logarithmic cell counts corresponding to the MWST scores '-', '1+', '2+', '3+' and '4+' were 6.321, 6.380, 6.476, 6.601 and 7.081 and the arithmetic cell counts were $1.131 \pm 0.049 \times 10^6$, $1.432 \pm 0.054 \times 10^6$, $3.124 \pm 0.760 \times 10^6$, $5.254 \pm 2.458 \times 10^6$, and $14.259 \pm 4.285 \times 10^6$ cells/ml respectively (Table 4). MWST detected 62.89 per cent milk samples as positive for subclinical mastitis out of the 194 CMT positive samples.

4.5. MODIFIED AULENDORFER MASTITIS PROBE TEST (MAMP TEST)

MAMP test performed on 194 CMT positive milk samples detected 110 (57.73 per cent) as healthy udder half, 56 (35.05 per cent) as having mild subclinical mastitis, 28 (7.22 per cent) as having severe subclinical mastitis (Fig 4). The different grades of reaction in MAMP test is presented in Plate 2. The mean logarithmic cell counts for the different grades of MAMP reaction were 6.334, 6.525 and 6.673 (Table 4). The mean SCC corresponding to the three classifications based on MAMP were $1.2 \pm 0.04 \times 10^6$ for healthy half, $4.055 \pm 0.895 \times 10^6$ for mild subclinical mastitis and $7.109 \pm 1.723 \times 10^6$ cells/ml for severe subclinical mastitis. MAMP test detected 43.29 per cent of CMT positive samples as having subclinical mastitis.

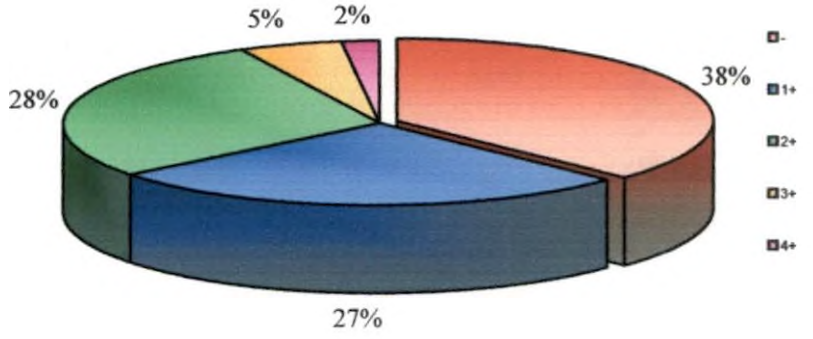

Fig 3. Grading of udder half samples based on MWST

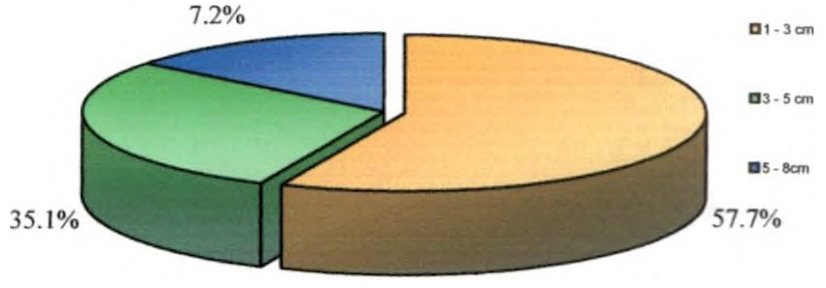

Fig 4. Grading of udder half samples based on MAMP

4.6. TRYPSIN INHIBITORY SPOT TEST (TIST)

The 194 CMT positive milk samples were subjected to TIST. The results of TIST for the corresponding CMT and MWST scores and MAMP grades are shown in Table 5. Out of the CMT positive milk samples 50 were positive for TIST (25.77 per cent) and 144 (74.23 per cent) were negative (Plate 3). TIST gave the maximum positive values (60 per cent) for CMT score '2' and maximum negative values, 57 (39.58 per cent) for CMT score '1'. Similarly maximum TIST positive and negative values were seen in MWST scores of '2+' and '0' respectively. MAMP reaction between 5 and 8 cm included the maximum TIST positive values (34.88 per cent). Maximum TIST negative values were seen in MAMP reaction between 0 and 3 cm (64.24 per cent).

4.7. COMPARISON OF SCREENING TESTS

4.7.1. Comparison among screening tests

To find out the relationship among CMT, MWST, MAMP and SCC Spearmans rank correlation was applied (Table 6). It was found that significant positive correlation existed among the four tests. The highest correlation was between CMT and SCC (correlation coefficient of 0.829). The correlation coefficient between CMT and MWST was 0.570, CMT and MAMP 0.566, MWST and MAMP 0.525, MWST and SCC 0.470 and between MAMP and SCC it was 0.443.

4.7.2. Comparison of screening tests with culture results

The results of the screening tests for the 47 culture positive subclinical mastitis cases were analysed using the test for proportion to find out their agreement with culture results (Table 7). The Chi square value showed significant variation in all the four tests namely CMT, MWST, MAMP and TIST. For CMT it was seen that score '3' detected the maximum infected (64.7 per cent) followed by score '2' (38.75 per cent). MWST score of '3+' detected the maximum positive cases (70 per cent) followed by scores '4+' and '2+' (50 and 47.17 per cent respectively).

Table 5. Results of TIST for the corresponding CMT and MWST scores and MAMP reactions.

CMT x TIST				MWST x TIST			MAMP x TIST		
No:	CMT score	TIST +ve	TIST -ve	MWST score	TIST +ve	TIST -ve	MAMP reaction	TIST +ve	TIST -ve
1	T	3 (6.0)	25 (17.36)	0	10 (22.73)	60 (40.0)	0-3 cm	12 (27.91)	97 (64.24)
2	1	10 (20)	57 (39.58)	1+	9 (20.45)	48 (32.0)	3-5 cm	16 (37.21)	43 (28.48)
3	2	30 (60.0)	51 (35.42)	2+	19 (43.18)	35 (23.33)	5-8 cm	15 (34.88)	11 (7.29)
4	3	7 (14.0)	11 (7.64)	3+	5 (11.36)	5 (3.33)			
5				4+	1 (2.27)	2 (1.33)			
Total		50	144		44	150		43	151

Figures in parenthesis indicate percentage to total

Table 6. Spearmans rank correlation for comparison of the screening tests

	CMT	MWST	MAMP
CMT	1		
MWST	0.570**	1	
MAMP	0.566**	0.525**	1
SCC	0.829**	0.470**	0.443**

**P< 0.01

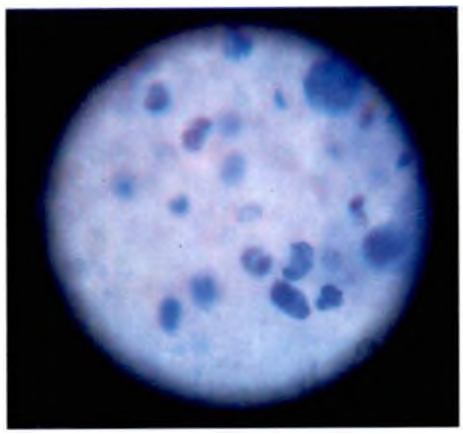

Plate 1. Milk smear stained by Broadhurst-Paley method

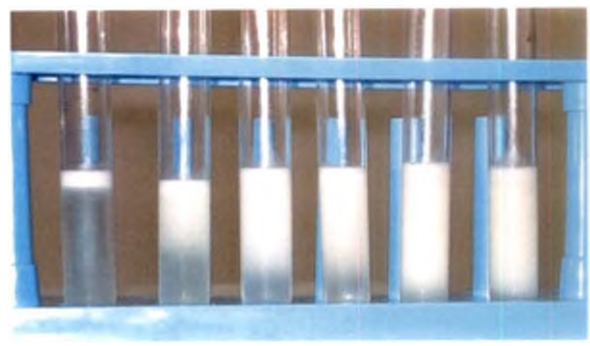

Plate 2. Different grades of reaction in MAMP test

Plate 3. Trypsin inhibitory spot test (TIST)

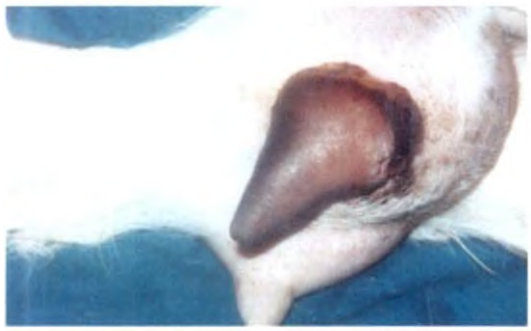

Plate 4. Gangrenous mastitis in a goat affecting one half

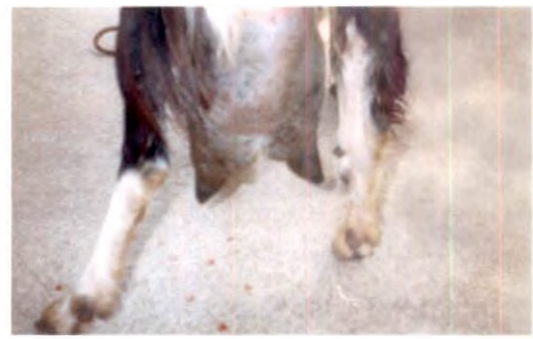

Plate 5. Gangrenous mastitis in a goat affecting both halves.

In MAMP reaction, the group in which turbidity ranged from 5 to 8 cm helped in identifying 42.86 per cent of the bacteriologicaly positive samples as true positive. Among the TIST positive samples 20 (44.44 per cent) were also bacteriologicaly positive whereas in TIST negative samples 18.12 per cent gave positive culture results. Regression performed on the CMT scores and SCC in bacteriologicaly positive samples showed significant linear relationship between the two tests (r^2 = 0.724) and it was found that for a unit change in CMT score the SCC values increased by 0.055 x 10^6.

4.8. CULTURE AND SENSITIVITY TESTS

4.8.1 Bacteriological findings

All the 194 milk samples positive by CMT from the three screenings and milk samples from 29 clinical mastitis cases were examined for the presence of bacteria by culture and isolation. Out of the samples which were CMT positive in the screenings, 47 (24.23 per cent) yielded bacterial growth while 37 out of 58 samples were bacteriologicaly positive in clinical cases.

Staphylococcus aureus predominated in both clinical and subclinical caprine mastitis occurring at a frequency of 39.7 per cent and 10.3 per cent respectively. *S. aureus* was isolated from all the seven gangrenous mastitis cases (Plates 4 and 5) encountered in the study out of which four succumbed to death during the course of treatment. Four isolates (6.9 per cent) each of Streptococci and *Escherichia coli* were obtained from clinical cases of mastitis. Bacteriological analysis of milk from subclinical cases yielded one isolate each of Streptococci and *Escherichia coli*. Two isolates of coagulase negative Staphylococci were obtained from clinical mastitis cases and 13 isolates from subclinical cases. Corynebacterium *spp* and Enterobacter *spp* were isolated only in clinical mastitis cases whereas Proteus and Bacillus *spp* were found only in cases of subclinical mastitis. Milk samples from screenings yielded only a single species of bacteria. Mixed infections were noticed in four clinical mastitis cases wherein *Escherichia coli* was isolated along with *S. aureus* from three gangrenous mastitis cases and Streptococci and Micrococcus in

Table 8. Bacterial isolates from clinical and subclinical mastitis

Culture result	Clinical mastitis		Subclinical mastitis		Classification of pathogen
	Number	Relative frequency	Number	Relative frequency	
Culture negative	21	36.2	147	75.8	No pathogen
Culture positive	37	63.8	47	24.2	
Staphylococcus aureus	23	39.7	20	10.3	Major
Streptococci	4	6.9	1	0.5	Major
Escherichia coli	4	6.9	1	0.5	Major
Corynebacterium	2	3.4			Minor
Enterobacter	1	1.7			Minor
Coagulase –ve Staphylococci	2	3.4	13	6.7	Minor
Micrococci	1	1.7	7	3.6	Minor
Proteus			2	1.0	Minor
Bacillus			3	1.5	Minor
Total halves	58		194		

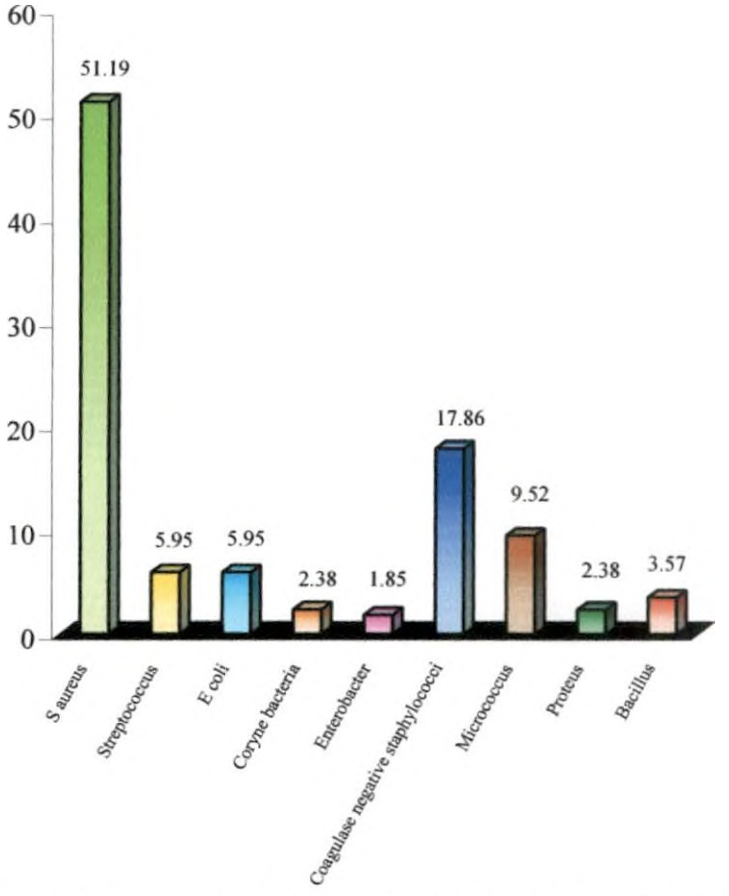

Fig 5. Percentage distribution of bacterial isolates from clinical and subclinical caprine mastitis cases

Plate 6. *Staphylococcus aureus* on
Mannitol Salt agar

Plate 7. Hemolytic colonies of *Staphylococcus aureus* on
Blood agar

Plate 8. Streptococcus *spp.* on
Edwards medium

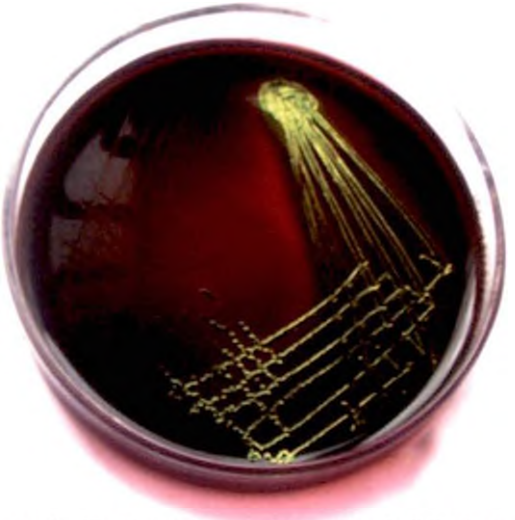

Plate 9. *Escherichia coli* on Eosin Methylene Blue agar

Plate 10. *Escherichia coli* on McConkey agar

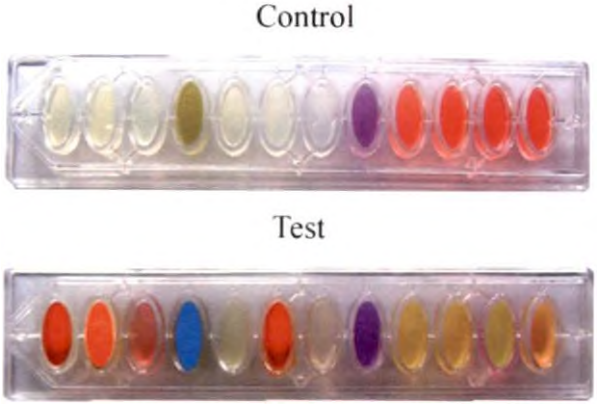

Plate 11. Hi-E. coli biochemical test kit

another case. All the bacterial isolates were classified as major and minor pathogens based on their frequency of isolation and severity of clinical signs. *S. aureus*, Streptococcus and *E. coli* were grouped as major pathogens and Enterobacter, Corynebacterium, coagulase negative Staphylococci, Bacillus, Proteus and Micrococcus were included as minor pathogens. The percentage distributions of bacterial isolates are depicted in Fig 5. The different bacterial isolates from clinical and subclinical cases are given in Table 8.

Majority of the *S. aureus* isolated in the study were hemolytic and were identified by hemolysis in blood agar (Plate 6) and yellow colonies on Mannitol salt agar (Plate 7). The Streptococcal isolates gave transparent hemolytic colonies on Edwards medium (Plate 8). Characteristic metallic sheen on Eosin Methylene Blue agar (Plate 9) and growth on McConkey agar (Plate 10) detected *E. coli* organisms. The Hi-E coli biochemical test kit (Hi-Media) was used for further confirmation of *E. coli* (Plate 11).

4.8.2. Antibiogram

In vitro antibiotic sensitivity studies were carried out on all bacterial isolates from subclinical cases and clinical caprine mastitis cases where treatment was carried out. In clinical mastitis cases the sensitivity tests were done on the whole udder microflora instead of taking individual isolates for finding out whether the antibiotic administered was effective or not (Plate 12).

All the *Staphylococcus aureus* isolates were sensitive to chloramphenicol whereas only 97.14 per cent were sensitive to ceftriaxone, 88.57 per cent to ciprofloxacin, 51.53 per cent to oxytetracycline and 25.71 per cent to sulpha. All the four isolates of Streptococci obtained in pure culture were sensitive to ceftriaxone and chloramphenicol whereas only 75 per cent were sensitive to ciprofloxacin. These pathogens were resistant to oxytetracycline and sulpha.

None of the antibiotics were effective against the *Escherichia coli* isolated in the study. Fifty per cent of the *E. coli* isolates were sensitive to ceftriaxone,

ciprofloxacin, chloramphenicol, gentamicin and sulpha. All the isolates were resistant to oxytetracycline. The Corynebacterium *spp* isolated from a clinical mastitis case showed sensitivity to ciprofloxacin, ceftriaxone, chloramphenicol and oxytetracycline and were resistant to gentamicin and sulpha.

All the coagulase negative Staphylococci were sensitive to ceftriaxone, ciprofloxacin, chloramphenicol and gentamicin whereas only 60 per cent of the isolates were sensitive to oxytetracycline and 46.67 per cent to sulpha. A single isolate of Enterobacter *spp* was sensitive to all the six antibiotics *in vitro*.

Micrococci isolates were sensitive to ceftriaxone, ciprofloxacin, and gentamicin and chloramphenicol but majority of the isolates were resistant to oxytetracycline and sulpha. Isolates of the genus Bacillus also showed sensitivity to ceftriaxone, ciprofloxacin, chloramphenicol and gentamicin. *In vitro* sensitivity analysis on Proteus isolates from cases of subclinical mastitis revealed that they were resistant to oxytetracycline and sulpha while ceftriaxone, ciprofloxacin and chloramphenicol were sensitive.

Sensitivity analysis of mixed cultures of *S. aureus* and *E. coli* revealed that chloramphenicol is the antibiotic of choice and sulpha drug is not a treatment preposition. Antibiogram of mixed culture of Streptococci and Micrococcus showed that all antibiotics were equally effective. Results of the *in vitro* antibiotic sensitivity tests in terms of sensitive and intermediate or resistant is presented in Table 9.

The *in vitro* antibiotic sensitivity patterns of caprine mastitis cases revealed that chloramphenicol is the most sensitive antibiotic followed by ceftriaxone, ciprofloxacin and gentamicin.

Table 9. *In vitro* antimicrobial sensitivity patterns of bacterial isolates from clinical and subclinical caprine mastitis cases

Bacterial isolate	No of isolates	Sensitive						Intermediate/Resistant					
		Ci	Cf	C	G	O	Sz	Ci	Cf	C	G	O	Sz
S. aureus	35	34 (97.14)	31 (88.57)	35 (100)	31 (88.57)	18 (51.53)	9 (25.71)	1 (2.94)	4 (11.76)	0 nil	4 (11.76)	17 (48.57)	26 (74.29)
Streptococci	4	4 (100)	3 (75)	4 (100)	1 (25)	0 nil	0 nil	0 nil	1 (25)	0 nil	3 (75)	4 (100)	4 (100)
E. coli	2	1 (50)	1 (50)	1 (50)	1 (50)	0 nil	1 (50)	1 (50)	1 (50)	1 (50)	1 (50)	2 (100)	1 (50)
Corynebacterium	2	2 (100)	2 (100)	2 (100)	0 nil	2 (100)	0 nil	0 nil	0 nil	0 nil	2 (100)	0 nil	2 (100)
Coagulase -ve Staphylococci	15	15 (100)	15 (100)	15 (100)	15 (100)	9 (60)	7 (46.67)	0 nil	0 nil	0 nil	0 nil	6 (40)	8 (53.33)
Enterobacter	1	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0 nil	0 nil	0 nil	0 nil	0 nil	0 nil
Micrococci	7	7 (100)	7 (100)	6 (85.7)	7 (100)	3 (47.86)	1 (14.29)	0 nil	0 nil	1 (14.29)	0 nil	4 (57.14)	6 (85.71)
Bacillus	3	3 (100)	3 (100)	3 (100)	3 (100)	2 (66.67)	1 (33.33)	0 nil	0 nil	0 nil	0 nil	1 (33.33)	2 (66.67)
Proteus	2	2 (100)	2 (100)	2 (100)	1 (50)	0 nil	0 nil	0 nil	0 nil	0 nil	1 (50)	2 (100)	2 (100)
S. aureus + *E. coli*	3	2 (66.67)	1 (33.33)	3 (100)	2 (66.67)	1 (33.33)	0 nil	1 (33.33)	2 (66.67)	0 nil	1 (33.33)	2 (66.67)	3 (100)
Streptococci + Micrococci	1	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0 nil	0 nil	0 nil	0 nil	0 nil	0 nil

Figures in parentheses indicate percentage of sensitive or resistant pathogens

Ci : Ceftriaxone Cf : Ciprofloxacin C : Chloramphenicol G : Gentamicin O : Oxytetracycline Sz : Sulpha

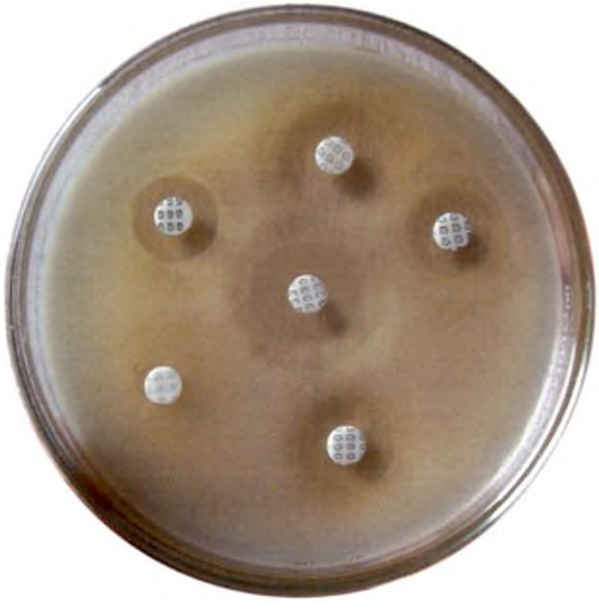

Plate 12. Antibiogram of *Staphylococcus aureus*

Ci- Ceftriaxone - sensitive
Cf- Ciprofloxacin - sensitive
C- Chloramphenicol - sensitive
G- Gentamicin - sensitive
O- Oxytetracycline - sensitive
Sz- Sulphadiazine - resistant

4.9. TREATMENT TRIALS WITH CEFTRIAXONE AND CIPROFLOXACIN

The clinical mastitis cases brought to the hospital were allocated randomly into two groups, each group being treated by ceftriaxone and ciprofloxacin respectively. Out of the 29 cases, treatment could be completed only in 24 cases which were allotted to the two treatment groups at random. Five cases were excluded from treatment trials due to various reasons. Three animals succumbed to death due to gangrenous mastitis and in two cases treatment had to be discontinued as the animals showed severe anaphylactic reactions to ciprofloxacin. Twenty six goats had a history of recent kidding and three were in their dry periods. The parity of the animals ranged from one to five. The details of the clinical cases treated with the two drugs are given in Tables 10 and 11.

Treatment with ceftriaxone was carried out for five days in 12 animals in Group I. Five animals had both clinical and bacteriological cure. Four cases did not show either clinical or bacteriological cure and *S. aureus* was isolated after the completion of treatment. In one case no bacteria could be isolated from the post treatment milk samples though the animal was not clinically cured.

The outcome of treatment with ciprofloxacin could be assessed in 12 animals in Group II. Only five of the 12 treated animals showed both clinical and bacteriological cure in this group. Two cases in this group were presented with gangrenous mastitis. Since there was no clinical improvement, in the first case the drug was changed to chloramphenicol after two days as per the antibiogram. In spite of this, the animal died after a treatment period of seven days. In the second case there was no bacteriological cure though the animal showed clinical cure. In two animals clinical cure was seen but bacteria could be isolated from these animals even after the completion of antibiotic therapy. There were two cases which showed neither bacteriological nor clinical cure. No bacteria could be isolated from a case of Streptococcal mastitis after the completion of treatment though the animal did not show clinical cure. Mild anaphylactic reactions were shown by one animal in this group but treatment could be completed. The comparative efficacies of the two treatment trials are presented in Table 12. Five cases each in the ciprofloxacin

Table 10. Caprine clinical mastitis cases treated with Ceftriaxone

Number	Characteristic of milk	Bacterial isolate	Days treated	Clinical cure	Bacteriological cure
1	Pale watery milk with flakes	*S. aureus*	5	No	Yes
2	Serosanguineous secretion	*S. aureus*	5	No	No
3	Watery milk with clots	*S. aureus*	5	Yes	No
4	Yellow curd like milk	*S. aureus*	5	No	No
5	Cream coloured milk with clots	*S. aureus*	5	Yes	Yes
6	Serosanguineous secretion	*S. aureus* + *E. coli*	5	No	No
7	Straw coloured watery milk	*S. aureus*	5	No	No
8	Curd like milk with clots	*S. aureus*	5	Yes	Yes
9	Straw coloured watery milk	Streptococci	5	Yes	No
10	Rose coloured milk with clots	*S. aureus*	5	Yes	Yes
11	Yellow creamy milk with clots	*E. coli*	5	Yes	Yes
12	Apparently normal milk	*S. aureus*	5	Yes	Yes

Table 11. Caprine clinical mastitis cases treated with Ciprofloxacin

Number	Characteristic of milk	Bacterial isolate	Days treated	Clinical cure	Bacteriological cure
1	Yellow creamy milk	Enterobacter	5	Yes	Yes
2	Pale watery milk	Streptococci	5	Yes	No
3	Straw coloured watery milk	*S. aureus*	5	Yes	Yes
4	Pale watery later serosanguineous	*S. aureus* + *E. coli*	5	Drug changed, animal died	
5	Straw coloured watery milk	*S. aureus*	5	No	Yes
6	Slightly blood tinged milk	Corynebacterium	5	No	No
7	Apparently normal milk with flakes	Coagulase –ve Staphylococci	5	Yes	Yes
8	Serosanguineous secretion	*S. aureus* + *E. coli*	5	Yes	No
9	Watery milk	Coagulase –ve Staphylococci	5	Yes	Yes
10	Straw coloured watery milk	*S. aureus*	5	Yes	Yes
11	Pale watery milk	Streptococci + Micrococci	5	No	Yes
12	Straw coloured watery milk	*S. aureus*	5	Yes	No

and ceftriaxone treated groups showed both clinical and bacteriological cure. But on considering the clinical and bacteriological cure of individual animals it was found that Group I had 58.33 per cent and 50 per cent cure rates whereas Group II had 66.67 per cent and 58.33 per cent cure rates respectively. Hence clinical and bacteriological cure was found to be better in the case of ciprofloxacin.

5.0. RAPD ASSAY FOR *Staphylococcus aureus*

Eighteen Staphylococcus isolates from clinical mastitis cases wherein treatment was initiated and 23 Staphylococcus isolates from subclinical cases were typed by RAPD fingerprinting. The different isolates were grouped into 12 different genotypes arbitrarily designated as a, b, c, d, e, f, g, h, i, j, k, and l based on their band patterns. Among a total of 41 isolates 40 yielded banding patterns using the 21 mer arbitrary primer. Electrophorogram revealed that all isolates showed bands with molecular weights ranging from 6108 to 220 base pairs (Plates 13 to 20).

Genotype c occurred at frequency of 17.5 per cent with a maximum of seven isolates followed by genotype i with five isolates and genotypes d, k and l with four isolates each. Genotypes b, e and f included three isolates each while genotypes a, g and j had two isolates each. There was only one isolate belonging to RAPD genotype h.

Staphylococcal isolates from clinical mastitis cases was grouped under seven genotypes. Genotype c predominated with a frequency of 29.41 per cent. Genotypes a, b, g, h and j were absent. In subclinical mastitis genotype b and i predominated with a frequency of 13.04 per cent. Genotypes d and h had the lowest frequency (4.35 per cent) each. Out of the four isolates from the gangrenous mastitis cases encountered in the study, two belonged to genotype d and the others to genotypes c and f respectively. Frequencies of occurrence of the different RAPD types of Staphylococcus are shown in Table 13.

Table 12. Comparative efficacy of two treatment trials.

Group	Drug	No: of goats	Clinical cure	Cure rate per cent	Bacteriological cure	Cure rate per cent
I	Ceftriaxone	12	7	58.33	6	50
II	Ciprofloxacin	12	8	66.67	7	58.33

Table 13. RAPD types of Staphylococcus isolates from clinical and subclinical caprine mastitis

Types of cases	RAPD genotype												
	a	b	c	d	e	f	g	h	i	j	k	l	Total
Clinical mastitis	-	-	5 (29.41)	3 (17.65)	2 (11.76)	1 (5.88)	-	-	2 (11.76)	-	2 (11.76)	2 (11.76)	17
Subclinical mastitis	2 (8.70)	3 (13.04)	2 (8.70)	1 (4.35)	1 (4.35)	2 (8.70)	2 (8.70)	1 (4.35)	3 (13.04)	2 (8.70)	2 (8.70)	2 (8.70)	23
Clinical & subclinical mastitis	2 (5.0)	3 (7.5)	7 (17.5)	4 (10.0)	3 (7.5)	3 (7.5)	2 (5.0)	1 (2.5)	5 (12.5)	2 (5.0)	4 (10.0)	4 (10.0)	40

Figures in parentheses indicate frequencies of occurrence

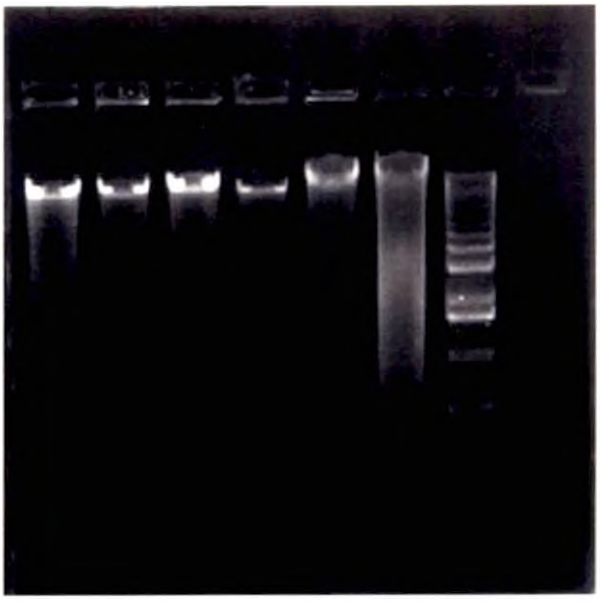

Plate 13. Electrophorogram showing DNA extracted from bacterial isolates

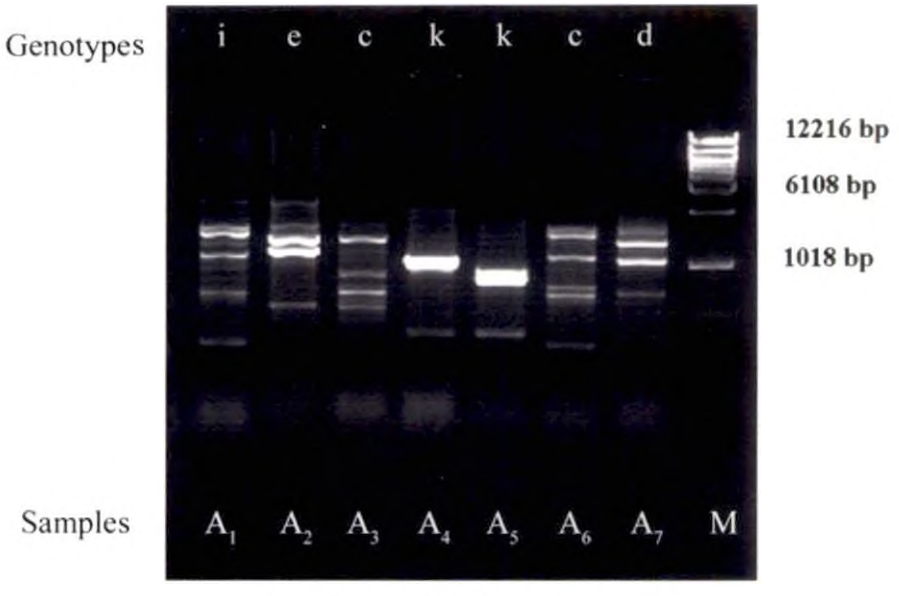

Plate 14. RAPD fingerprints of Staphylococcus *spp.* I

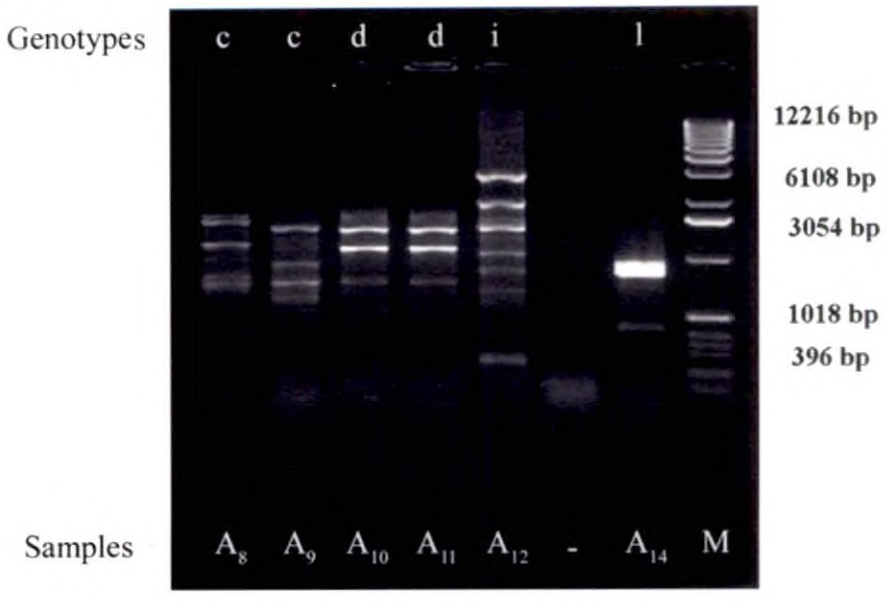

Plate 15. RAPD fingerprints of Staphylococcus *spp*. II

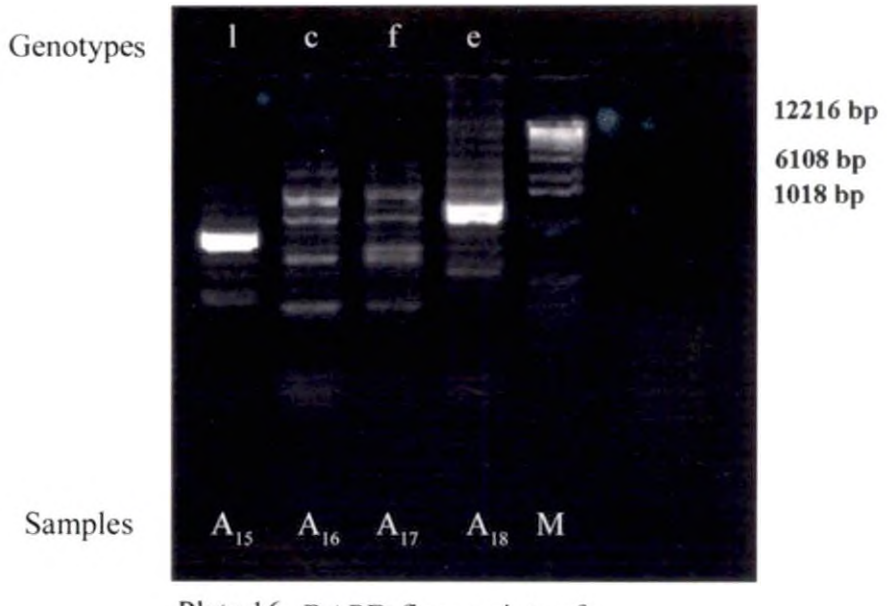

Plate 16. RAPD fingerprints of Staphylococcus *spp*. III

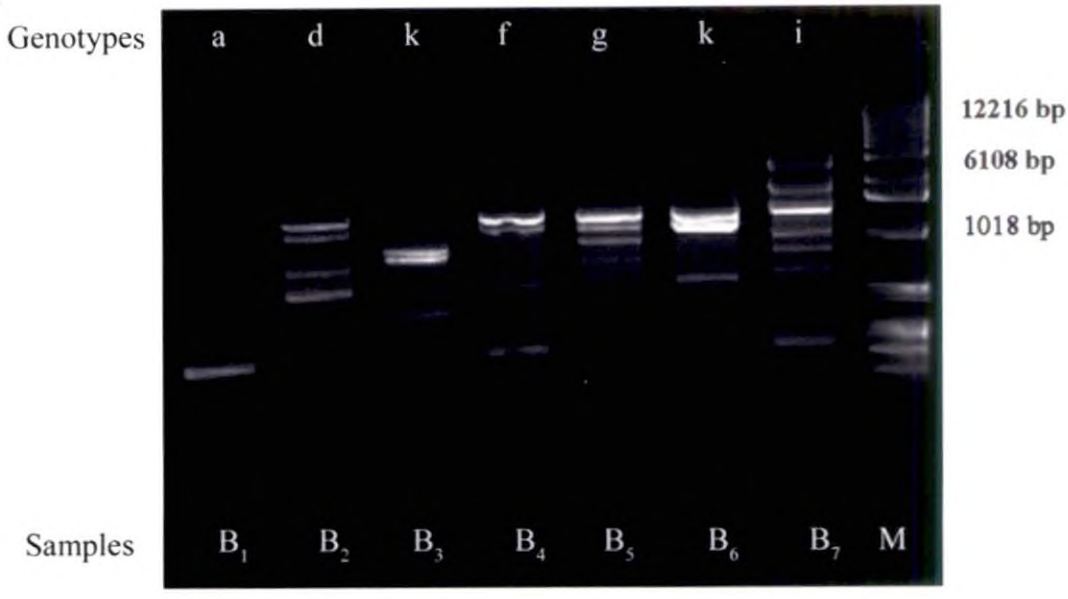

Plate 17. RAPD fingerprints of Staphylococcus *spp*. IV

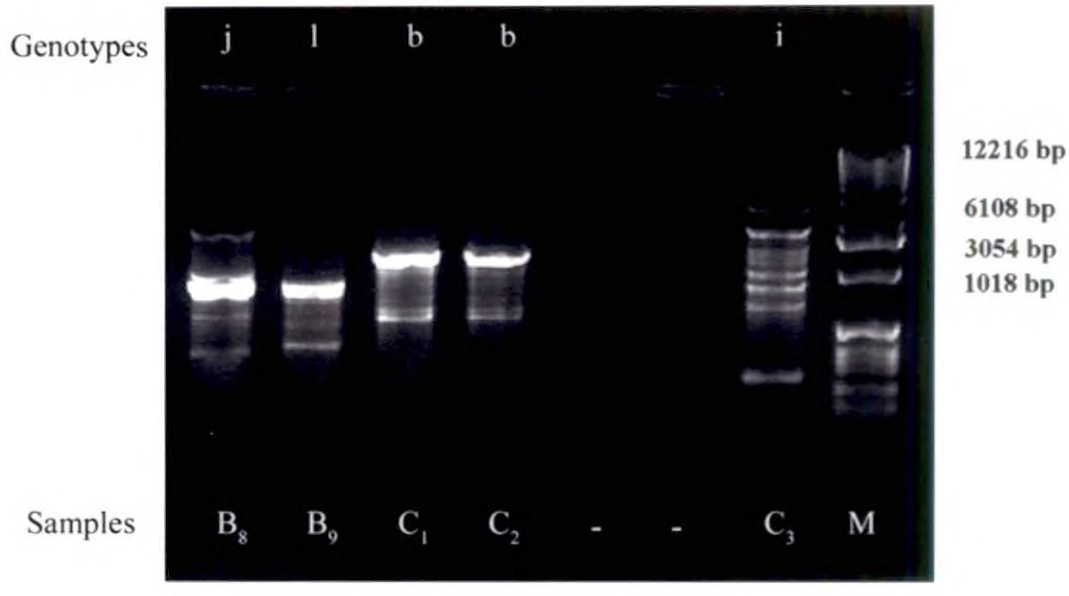

Plate 18. RAPD fingerprints of Staphylococcus *spp*. V

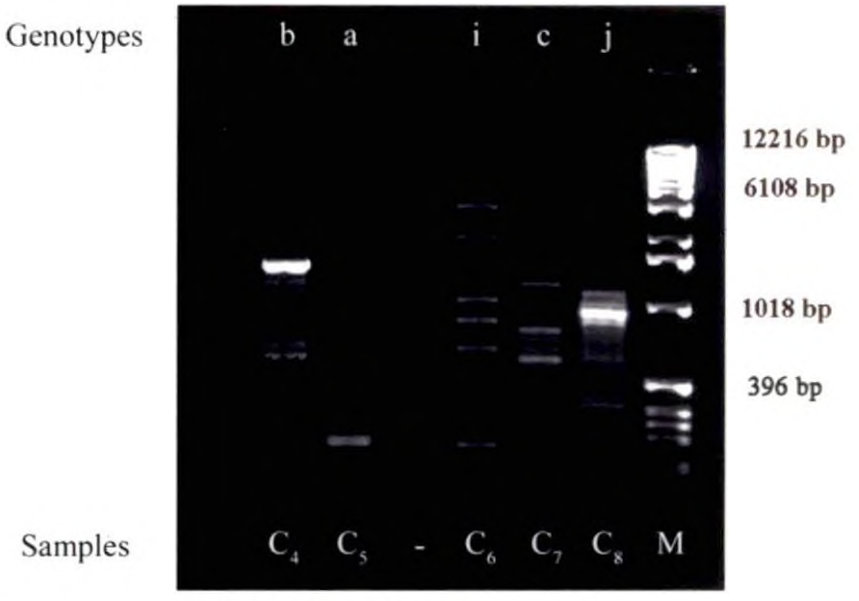

Plate 19. RAPD fingerprints of Staphylococcus *spp*. VI

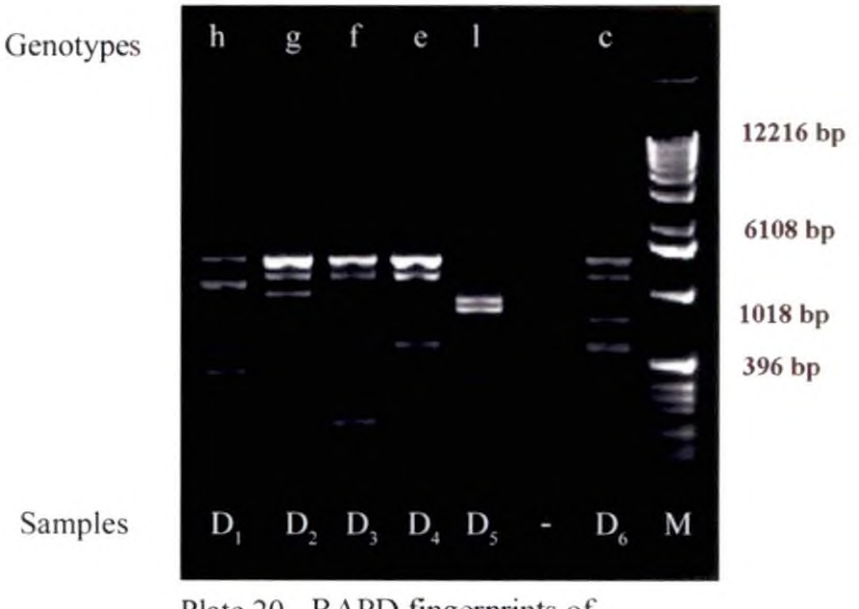

Plate 20. RAPD fingerprints of Staphylococcus *spp*. VII

Table 14. Cure rate of animals in 2 groups based on RAPD genotypes

Bacterial isolate	Group I treated with Ceftriaxone			
	RAPD type	No: of animals	Clinical cure	Bacteriological cure
S. aureus	c	3	2 (66.67)	2(66.67)
S. aureus	d	2	0 (nil)	0 (nil)
S. aureus	e	1	1(100.0)	0 (nil)
S. aureus	k	1	0 (nil)	0 (nil)
S. aureus	l	2	2 (100.0)	2(100.0)
Group II treated with Ciprofloxacin				
S. aureus	c	2	2 (100.0)	2 (100.0)
S. aureus	d	1	1 (100.0)	0 (nil)
S. aureus	e	1	0 (nil)	0(nil)
S. aureus	f	1	0 (nil)	0(nil)
Coagulase –ve Staphylococci	i	2	2 (100.0)	2 (100.0)
S. aureus	k	1	1(100.0)	1 (100.0)

Figures in parentheses indicate percentage

In Group I treated with ceftriaxone the highest cure rate (clinical and bacteriological) was for RAPD type 1 (100 per cent). The lowest clinical and bacteriological cure rate was for *S. aureus* RAPD type d and k (zero). Among eight animals in Group II, clinical and bacteriological cure rate was 100 per cent for RAPD types c and i. There was no clinical and bacteriological cure for *S. aureus* RAPD types e and f. The results are presented in Table 14.

Genetic fingerprinting and phylogenetic diversity between the different Staphylococcus isolates were determined by analyzing the RAPD data using RAPD distance software to generate a phylogenetic tree (Fig 6). It was observed that the RAPD genotypes a and b had less relationship when compared to RAPD types c, d, *f* and g.

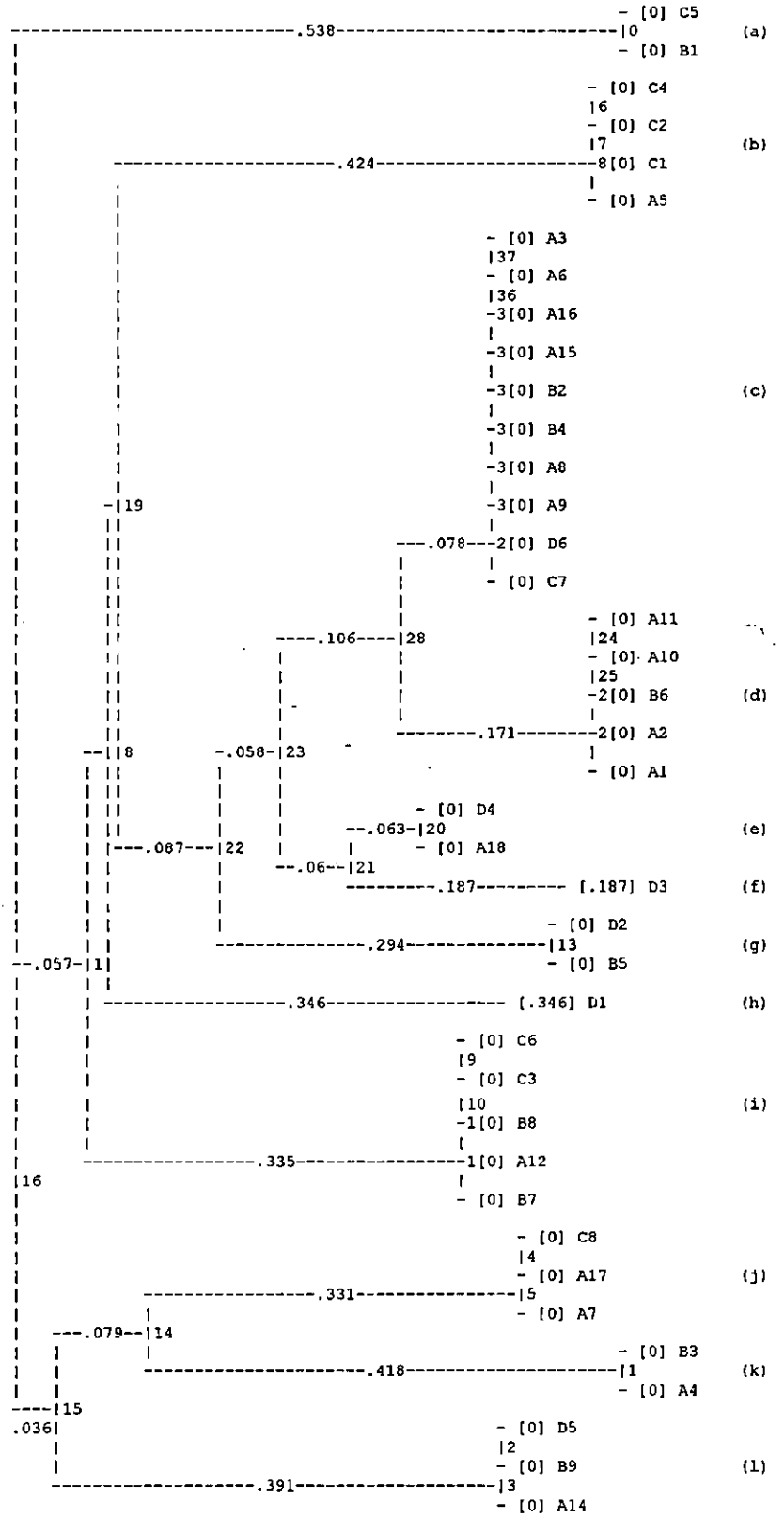

Fig.6 Phylogenetic tree of Caprine Staphylococcus isolates

Discussion

5. DISCUSSION

Mastitis is a disease complex having different causes, different degrees of intensity with variation in the duration and residual effects. Caprine mastitis is seen to be a more difficult problem to tackle when compared to bovine mastitis. Depending on intensity, the condition can be classified as subclinical and clinical mastitis. Since mastitis in goats is more of subclinical nature, it becomes imperative to evolve suitable methods for the diagnosis of the condition, therapy and control to prevent loss to the farmers due to mortality and lowered production.

Since goat milk is consumed raw especially in the rural areas, the condition of the udder is particularly important from the public health point of view. Due to indiscriminate use of antibiotics for mastitis and other diseases both in humans and animals, many antibiotic resistant strains have emerged. This necessitates the need to choose the most effective antibiotic for clinical mastitis cases of goats.

Even though mastitis in goats is an important problem, the research work done in our country is scanty. Keeping this in view, the present study on goat mastitis was taken up and the results have been discussed.

5.1. SCREENING FOR SUBCLINICAL MASTITIS

Mastitis in goats is predominantly subclinical (Contreras *et al.*, 1999). Subclinical mastitis is defined as the inflammation of the mammary gland that is not appreciable clinically and requires diagnostic tests for detection mostly based on the somatic cell count in milk. The disease condition should be diagnosed during the early stage itself so that appropriate treatment and preventive measures can be adopted.

The results of the present study shows that the occurrence of subclinical mastitis based on CMT in cross bred does from University goat and sheep farm was 30.2 per cent (Table 1). This agrees with the observations made by Contreras *et al.* (1995) and Boscos *et al.* (1996). As per bacteriological findings the occurrence of subclinical mastitis was 7.32 per cent which agrees with the findings of Dep *et al.*

(1985) and Contreras *et al.* (1997). The occurrence of subclinical mastitis was least (23.97 per cent) in the first screening. It showed an increase in the second and third screenings which was 33.82 and 35.71 per cent respectively. This may be because of the change in management conditions adopted during the period. The goats were mostly stall fed during this period. High stocking density particularly in intensively managed herds or flocks or during the suckling period may have resulted in large air concentrations of total microorganisms in the area which may have then lead to increased intramammary infection. Stress factors have been well documented to affect udder condition in goats. Neither teat dipping nor dry therapy is being followed in the farm. This may have contributed to the overall increase in the intramammary infections.

5.1.1 Teat characteristics

Most commonly mastitis begins as a result of penetration of bacteria through the teat duct to the interior of the mammary gland (Schalm *et al.*, 1971). Physical characteristics of the udder and teat are related to economically important traits of dairy cows but there are no reports of such studies in goats.

In the present study an attempt was made to find out whether there was any association between occurrence of subclinical mastitis, teat length and distance from teat tip to ground. There was no association between teat length and occurrence of subclinical mastitis. This is in accordance with Ameh and Tari (2000). The percentage of reactors of subclinical mastitis was lowest in goats having teat length below 14cm (0.6 per cent) and highest (22.67 per cent) in animals with teat length between 8 and 11cm. Studies on the relationship between floor to teat distance and occurrence of subclinical mastitis revealed that as teat end to floor distance decreased occurrence of mastitis increased. This shows that low teat end to floor distance is an important risk factor for mastitis. In animals where teat end to floor distance was 21 to 28 cm occurrence of mastitis was 61.03 per cent while it was zero in animals when the distance decreased to 14 cm (Table 2). High placed udders are conducive to better hygiene and has decreased incidence of teat

lesions and so less occurrence of mastitis. A similar observation was made by Sebastian, (2001) in subclinical mastitis of cattle.

5.2. CALIFORNIA MASTITIS TEST (CMT)

The discovery that anionic detergents react with mastitic milk to produce a visible effect that can be scored numerically with reference to the content of somatic cells made possible the introduction of a new and practical test for mastitis which could be conducted at the side of the cow, in the laboratory on mixed milk from mammary quarters and on bulk tank milk. This test, popularly known as the California mastitis test (CMT) is based on the fact that leucocytes in milk are ruptured by the reagent releasing the DNA which is the active principle of the test. Bacterial multiplication commonly leads to acid production and increase in the SCC of milk. This can be easily assessed by the CMT reaction which gives a rough estimation of two parameters at a time, the cell count and the pH of the milk. Preservatives other than boric acid alter the DNA so that no reaction occurs even with milk of high cell counts. Freezing of milk samples should also be avoided (Schalm *et al.*, 1971).

Many workers differ in their opinion with regard to the application of CMT for diagnosis of subclinical mastitis in small ruminants. Goat milk naturally has high concentration of epithelial cells than cow milk and it is generally believed that scores of 'T' or '1' (upto 1,000,000 cells/ml) are usually of no reason for concern (Smith and Sherman, 1994). In this study out of 642 milk samples examined 194 (30.2 per cent) were found positive and 448 (69.78 per cent) negative. Based on the type of reaction these samples were scored as 'T', '1', '2' and '3' (Table 1). Comparison of CMT with culture results revealed that, out of the 97 left halves positive by CMT only 22 (38.6 per cent) were culture positive and out of the 97 right halves positive by CMT, 25 (43.9 per cent) were culture positive (Table 3). Menzies and Ramanoon (2001) states that the drawback of CMT in goats is that false positive rate is higher when used in does in late lactation or with low production. In the present study all lactating animals were sampled without classifying their stage of lactation. Maisi (1990) had increased the threshold of

CMT score for caprine milk and found that in the second and third month of lactation score was '1' in uninfected glands. Out of CMT scores 'T', '1', '2' and '3', zero, 7.35 per cent, 38.75 per cent and 64.7 per cent were positive bacteriologicaly (Table 7). Most samples above score '2' were culture positive. So it can be inferred that a score of '2' could be indicative of udder troubles while samples with score 'T' or '1' could be considered suspicious and further confirmatory tests should be applied. CMT scores of '0' and '1' can discriminate subclinical mastitis (Fthenakis, 1995). In this study also it was seen that CMT scores of 'T' and '1' could differentiate infected and uninfected animals as all of the 29 animals graded as 'T' by CMT were culture negative compared to 5 animals out of 68 in score '1' which were culture positive.

Statistical analysis showed that CMT had good correlation with SCC (Table 6). Out of a total of 194 samples scored as 'T','1', '2' and '3' statistical difference was noticed between each score of CMT and for SCC values both untransformed and logarithmically transformed ($P<0.01$)(Table 4). Although CMT is recognized as an indicator of abnormal milk due to inflammation of udder tissue, this does not necessarily mean infected milk. This corroborates with the findings of Pettersen (1981) who found that a CMT score of '1', '2' or '3' should be regarded as normal in mid lactation.

SCC corresponding to the different CMT scores were determined. The arithmetic mean cell counts for each score was - score 'T' had a cell count of $0.736 \pm 0.033 \times 10^6$; '1' had $1.199 \pm 0.031 \times 10^6$; '2' had $1.732 \pm 0.086 \times 10^6$ and '3' had a count of $20.417 \pm 0.851 \times 10^6$ cells/ml. According to Schalm *et al.* (1971) CMT scores of '1' to '3' did not correspond to the cell count in milk from cows. This was because the number of cells in milk from goats scoring '1', '2' or '3' was higher than those of cow's milk. Moreover it has been observed in studies of goats that SCC increased without infection in low milk yield goats or goats in late lactation (Siddique *et al.*, 1988). Does with high SCC had no mammary gland tissue changes or other mastitic conditions in udder. CMT score of '1' was dominant throughout lactation in noninfected animals (Maisi, 1990) and goats with

85 per cent of halves infected by major pathogens had a CMT score over '1' (Poutrel and Lerondelle, 1983).

El Sagheer (1988) held the view that CMT would not detect certainly every cell count nor pick out every infected quarter but the test could be a valuable aid to mastitis control, enabling the veterinarian to reduce infection in a flock without undertaking large scale bacteriological tests. CMT is more useful as a screening test than for diagnosing mastitis in goats. It can be concluded that CMT may be useful as a screening test in goats to identify animals for culture, keeping in mind that a high percentage of the animals selected would be culture negative.

5.3. SOMATIC CELL COUNT (SCC)

Somatic cell count has been accepted as the best quantitative index of inflammation of the bovine mammary gland and is used both to evaluate the quality of milk and to predict udder infections. Several types of somatic cells have been reported to be present in normal milk (Schalm *et al.*, 1971). The content of these cells increases in mastitic milk primarily due to an overwhelming number of leukocytes infiltrating from blood. Enumeration of somatic cells is possible in different ways. Microscopic cell counting is a direct visual method for estimating the number of cells in milk. Today electronic cell counting (Fossomatic or Coulter counter) is often used for somatic cell counting. This is a very rapid and fairly accurate method but still the microscopic method serves as the reference.

In the dairy cow the concentration of SCC in milk is considered indicative of mastitic condition of mammary glands. However this conclusion may not be absolutely true in dairy goats. Milk secretion in goats is apocrine while that in cow is merocrine. As a result cytoplasmic particles are shed in to milk from the apical portion of mammary secretory cells (Paape, 2000). These cytoplasmic particles are similar in size to milk leucocytes ranging from 5 to 30 μm (Dulin *et al.*, 1983). Although majority of these particles are generally anucleated, some have been observed to contain nuclear fragments and this can lead to erroneous SCC while using the microscopic counting method. Also the differential mean SCC for goats

is higher than in cows, the neutrophils constituting 45 to 74 per cent in goat milk as opposed to 5 to 20 per cent in cow milk (Paape, 2000).

In the present study the somatic cells were enumerated by microscopic counting of Broadhurst-Paley stained milk smears. Except for CMT score '3', in all other scores the SCC were higher than that reported by Schalm *et al,* (1971). Average SCC determined for each CMT category agrees with the work of Dulin (1983) on goats. However, in CMT score of '3' the differentiation between leukocytic cells and cytoplasmic particles was difficult since these were mostly seen together as small clumps.

SCC in goat milk is highly variable. Hinckley (1990) reported that 56 per cent of milking does with no signs of mastitis contained as many as 5x 10^6 cells /ml. Droke *et al.* (1993) reported an average of 1.3 x 10^6 cells/ml in bulk tank goat milk. SCC in goat milk is seen to be influenced by many factors like season, stage of lactation and parity (Dulin *et al.*, 1983; Hinckley, 1983; Manlongat *et al.*, 1998; Zeng and Escobar, 1995). In this study, stage of lactation and parity were not considered while taking the observations. This may be another probable reason for the high SCC counts. Dairy goat lactation is seasonal and hence SCC naturally exceeds the limit especially in late lactation stages (Dulin *et al.*, 1983; Hinckley, 1990). SCC will be higher in goats with lower milk yield, parasitic invasion and dietary deficiency in feed like insufficient iron, proteins and vitamins in addition to intramammary infection (Atroshi *et al.*, 1986).

Out of the 194 CMT positive samples with elevated SCC levels only 47 (24.23 per cent) yielded pathogens. Bacterial origin of intramammary infection cannot explain all elevated cell counts in goats. Ninety percent of the difference in goat SCC was not due to intramammary infection (Wilson *et al.*, 1995). So prediction of intramammary infection by assessing the SCC levels is difficult. The current legal limit for SCC in bulk tank milk of goats in the United States is 1x 10^6 cells/ml. In this study it was found that majority of the samples exceeded this limit yet only 24.23 per cent were bacteriologicaly positive. Studies have shown that a single day test measurement of SCC will not be adequate to efficiently estimate the

mastitic condition of individual goats (Rota *et al.*, 1993). The threshold of 1x 10^6 cells/ml reported by Poutrel and Lerondelle (1983) will identify most of the infected halves but type II error (uninfected half considered as infected) is high.

It can be inferred that the SCC accepted by IDF as the best quantitative index of inflammation of the bovine mammary gland seems to vary a lot for goat milk and rapid diagnosis of mastitis in goats using only cell count needs further re-evaluation.

5.4 MODIFIED WHITESIDE TEST (MWST)

W. H Whiteside, bacteriologist at the Kennedy bacteriological laboratories limited, Canada is credited with the use of sodium hydroxide in mastitic milk to detect mastitis in cows. Murphy and colleagues were responsible for modifying the test as used today and suggested the designation Modified Whiteside test (MWST). Fresh somatic cells are necessary for the reaction to take place. Heating milk or adding preservatives like formalin and potassium dichromate destroys the reactivity of mastitic milk to sodium hydroxide. The principle of the test is that nucleic acid in the somatic cells form a sodium salt in the presence of sodium hydroxide producing a gelatinous mass to which serum solids and fat globules become adsorbed to form the characteristic precipitate of the reaction (Schalm *et al.*, 1971).

MWST was performed on all the 194 CMT positive milk samples from the three screenings out of which 72 (37.11 per cent) were classified as negative, 53 (27.32 per cent) as '1+', 55 (28.35 per cent) as '2+', 10 (5.15 per cent) as '3+' and 4 (2.06 per cent) were graded as '4+' (Fig 3). MWST detected 62.89 per cent as positive for subclinical mastitis out of the 194 CMT positive samples. The MWST phenomenon is a two-phase reaction involving somatic cells and fat. After formation of gelatinous mass by sodium hydroxide the fat globules rush together forming strings and isolated clumps (Schalm *et al.*, 1971). In goats the fat globules are small compared to cows and remain dispersed. This may be the reason for more number of negatives in MWST compared to CMT. The arithmetic SCC corresponding to each grading were 1.131 ± 0.049 x 10^6, 1.432 ± 0.054 x 10^6, 3.124 ± 0.760 x10^6, 5.254 ± 2.458 x10^6, and 14.259 ± 4.285 x10^6 cells/ml respectively.

High SCC in goat milk is natural especially in later stages of lactation (Zeng and Escobar, 1995).

Comparison of the results of the MWST with bacteriology showed that score '3+' identified maximum number of infected udder halves. Fthenakis (1995) recommended score '1+' for MWST as threshold value for subclinical mastitis offering 93 per cent accuracy of diagnostic methods. In this study also score '1+' can be taken as the threshold since score '0' correctly detected 70 samples as culture negative out of a total of 72 milk samples while scores '1+', '2+' and '3+' detected 20, 47.17, 70 and 50 per cent as infected compared with culture results.

The present study shows that MWST can be used as a screening test on goat farms for diagnosis of subclinical mastitis.

5.5 *MODIFIED AULENDORFER MASTITIS PROBE TEST (MAMP TEST)*

MAMP test is an indirect test for diagnosing subclinical mastitis based on the increase in somatic cell count in milk. The test was found to be effective in the diagnosis of bovine subclinical mastitis (Buragohain and Dutta, 1991; Sebastian, 2001). The suitability of the test in diagnosing caprine subclinical mastitis has been attempted to in this study. The principle of the test is that sodium lauryl sulphate, an anionic surface active agent in the reagent disrupts the somatic cells and makes it into a gelatinous mass. The urea in the reagent helps the mass to rise up to the top. Reactions in the MAMP test is measured in centimeters of the gelatinous product formed and classified as healthy udder half, mild subclinical and severe subclinical mastitis. The errors based on subjectivity are less as the result is actually measured but the major disadvantage is that the result can be read only after 24 hours.

MAMP test detected 43.29 per cent of CMT positive samples as having subclinical mastitis. The mean SCC corresponding to the three classifications based on MAMP were $1.2 \pm 0.04 \times 10^6$ for healthy half, $4.055 \pm 0.895 \times 10^6$ for mild subclinical mastitis and $7.109 \pm 1.723 \times 10^6$ cells/ml for severe subclinical mastitis. MAMP reactions between 5 and 8 helped in identifying 42.86 per cent of the bacteriologicaly positive samples as true positive. However, there are no studies

regarding the application of MAMP test in the diagnosis of mastitis in goats. It can be concluded that MAMP test can be used as a screening test in the diagnosis of caprine mastitis along with other tests like CMT and MWST.

5.6. TRYPSIN INHIBITORY SPOT TEST (TIST)

Trypsin inhibitory spot test is based on the principle that proteolysis of the gelatin coat by exogenous trypsin is inhibited by the trypsin inhibitors present in mastitic milk (Samad and Awaz, 1997).

Proteolytic activity of milk is due to proteases like elastase, collagenase and cathepsin G in the lysosomal granules of neutrophils which are released during mastitis to the surrounding tissue. These proteases help in controlling the intramammary infection but also subject the mammary gland to self digestion. Some proteolytic activity may also originate from the pathogen or from soluble endogenous sources activated during inflammation. Under these circumstances the trypsin inhibitors protect the mammary tissue and milk proteins from proteolytic damage.

Trypsin inhibitors in milk constitute a group of antiproteases whose level increases in milk during mastitis (inflammatory conditions) because of the permeability at the blood milk barrier (Mattila *et al.*, 1986). Spectrophotometry is used for quantification of trypsin inhibitors. The TIST is a less sensitive method when compared to spectrophotometry and detects only whether the level has increased beyond a certain cut off level. Hence it can only be used as a qualitative screening test.

In the present study 194 CMT positive milk samples were subjected to TIST out of which only 45 (23.20 per cent) were positive indicating subclinical mastitis. Compared to all the three tests TIST positive milk samples were less. CMT, MWST and MAMP are indirect tests based on SCC. Since SCC is more in goats and majority of it is contributed by neutrophils (45 to 74 per cent), protease activity is expected to be more. This is one of the reason for the less number of clinical cases of goat mastitis (1 case per 100 animals) when compared to cattle (50 to 80 clinical

cases per 100 animals) (Paape, 2000). When the protease level rises, trypsin inhibitor level should also rise proportionately and more positives are expected. But in this study majority of the CMT positive milk samples were TIST negative. This can attributed to the fact that antitrypsin is based on a different facet of inflammation, mainly permeability which happens only during mastitis and not influenced by increased SCC. Samad and Awaz (1997) observed that TIST was superior to CMT that it could accurately diagnose 95 per cent cases in bovine subclinical mastitis whereas CMT could diagnose only 70 per cent. A probable reason for this discrepancy may be that trypsin inhibitors from different sources have varying trypsin binding capacity (Honkanen-Buzalski and Sandholm, 1981).

It was seen that out of the 45 TIST positive milk samples 20 (44.44 per cent) were culture positive while 25 (55.55 per cent) were culture negative. The test detected more number of uninfected samples than infected ones. This is not in accordance with Bhujbal *et al.* (1999a) who observed that the reliability of trypsin inhibition activity compared to bacteriological examination of subclinical mastitis in goats was 100 per cent. This variation may be due to factors like stage of lactation and breed which were not considered during the study. Agreement between TIST and culture result was excellent in mid lactation buffaloes while false positive results for TIST were seen in initial and final stages of lactation (Das *et al.*, 2001).

It can be inferred from the present study that TIST can only be used as a rough guide and could only be used along with other tests for the diagnosis of subclinical mastitis in goats.

5.7 COMPARISON OF SCREENING TESTS

Long before the knowledge developed relating the involvement of specific bacteria in the production of mastitis, detection of the disease was solely based on examination of milk for gross deviations from the normal and on physical examination of udder for swelling, scar tissue and atrophy. As mastitis manifests itself, the composition of milk is altered in direct proportion to the extent and intensity of the inflammatory process. Mastitis changes the primary function of

mammary tissue from production of milk to protection against the injury and preparation for repair. These changes can be easily detected by indirect tests and these are capable of detecting mastitis in its earliest as well as in its advanced stage. Culture of pathogens from mastitic milk is considered to be the most sensitive method for detection of mastitis but it is time consuming, requires skilled technicians and expensive facilities. So it is unsuitable for routine monitoring of udder health.

In the present investigation the health status of the caprine mammary gland was established by simultaneous bacteriological and cytological examination of milk samples. Indirect tests like CMT, MWST, MAMP and SCC were employed for assessing the cytological status of individual udder halves. Statistical analysis of the results showed that there was positive correlation between CMT, MWST, MAMP and SCC with the highest correlation between CMT and SCC ($r = 0.829$). CMT is an indirect test based on the somatic cell count in milk. Gonzalez-Rodriguez and Carmenes (1996) reported a similar correlation coefficient of 0.82 between CMT and SCC. SCC could be of value if carried out along with bacteriological examination (Schalm *et al.*, 1971). The correlation coefficient between CMT and MWST was 0.570, CMT and MAMP 0.566, MWST and MAMP 0.525, MWST and SCC 0.470 and between MAMP and SCC it was 0.443. The three indirect tests namely CMT, MWST and MAMP showed a positive correlation with SCC. The little variation observed may be due to the difference in the scores of the different tests. CMT has four classifications ('T', '1', '2', '3') and MWST five classifications ('-', '1+', '2+', '3+', '4+') whereas MAMP has only three classifications (0 to 3cm, 3 to 5cm and 5 to 8cm).

The indirect tests were also compared with culture results to assess the influence of infection status on these tests (Table 7). CMT score '3' detected the maximum infected halves (64.7 per cent) followed by score '2'. Similarly score '3+' of MWST and grade '3' of MAMP reaction detected the maximum positive cases with respect to bacteriology. Out of the bacteriologicaly positive samples 44.44 per cent were detected as positive by TIST. Regression analysis showed significant relationship between CMT and SCC in bacteriologicaly positive halves.

It can be inferred that CMT and MWST can be used as screening tests in the diagnosis of subclinical mastitis in goats, alone or in combination, as CMT and MWST were found to correlate better with SCC of samples and with subclinical mastitis status of the mammary gland or culture results of the samples. MAMP and TIST can be used as farm tests but should be combined with any other test for increasing their sensitivity in the diagnosis of the condition.

5.8. CULTURE AND SENSITIVITY TESTS

5.8.1 Bacteriological findings

In the present study bacteriologicaly positive and negative milk samples accounted for 62.07 and 37.93 per cent in clinical mastitis cases and 24.23 and 75.79 per cent in subclinical cases.

Staphylococcus aureus was the most prevalent pathogen in both clinical and subclinical mastitis cases. It was isolated from 39.7 and 10.3 per cent of udder halves respectively, contributing to 51.19 per cent of the total isolates. Similar observations were made by Contreras *et al.* (1997). *S. aureus* is a major pathogen responsible for caprine mastitis (Smith and Roguinsky, 1977; Hunter, 1984; Manser, 1986; Deinhofer and Pernthaner, 1995). In most studies on subclinical mastitis coagulase negative Staphylococci was found to be the most prevalent pathogen compared to *S. aureus* (Lerondelle and Poutrel, 1984; Poutrel, 1984; East *et al.,* 1987; Deinhofer and Pernthaner, 1995; Kirk *et al.*, 1996; White and Hinckley, 1999; da Silva *et al.,* 2004). In this study *S. aureus* was found in 10.3 per cent of the subclinical mastitis cases. The high prevalence of *S. aureus* is a factor to be taken seriously. This can be attributed to the persistence of infection by this pathogen in the flock as *S. aureus* is a pathogen reported to cause persistent intramammary infection in goats (Contreras *et al.*, 1997). Failure to apply dry therapy may have contributed to the maintenance of *S. aureus* in goats mammary gland (da Silva *et al.,* 2004). *S. aureus* was isolated from all the seven gangrenous mastitis cases encountered in this study. Out of this four animals died and the other three goats did not show bacteriological cure. In three cases mixed isolates of *S. aureus* and *E. coli* were obtained. It could be observed that a large percentage of

the *S. aureus* isolates in the study were hemolytic. Hemolytic ability is an indication of the virulence of the pathogens and thus it can be inferred that caprine *Staphylococcus aureus* isolates are pathogenic. The pathogenicity of the genus Staphylococcus particularly for *S. aureus* is related to the production of a wide variety of exoproteins including the α, β and δ hemolysins which contributes to its ability to cause diseases in many mammalian species (da Silva *et al.*, 2005). It was noted that infection with *S. aureus* was confined to one half of udder in most of the clinical and subclinical mastitis cases. Both halves were affected in four of the seven gangrenous mastitis cases.

Though coagulase negative Staphylococci are regarded as minor pathogens seen mostly in subclinical mastitis, two cases from clinical mastitis yielded coagulase negative Staphylococci which points to the increasing threat of this organism in goat intramammary infection. A re-evaluation of the consequences of mammary infections due to coagulase negative Staphylococci capable of lasting through out lactation and dry period is of utmost importance (Poutrel, 1984). Coagulase negative Staphylococci contributed to 17.86 per cent (Fig 5) of the total bacterial isolates. This corroborates well with the findings of Deinhofer and Perthaner (1995) and Contreras *et al.* (1997). There are many species of coagulase negative Staphylococci which affect goats, the most common being *S. epidermidis, S. caprae, S. lentus, S. simulans, S. capitis, S. lugdunensis, S. xylosus, S. chromogenes, S. hominis, S. arlettae, S. warneri, S. scuiri* and *S. saprophyticus* (Mallikeswaran and Padmanabhan, 1989; Poutrel *et al.*, 1997; Contreras *et al.*, 1997; Sanchez *et al.*, 1999) but specific identification of coagulase negative Staphylococci were not attempted in this study. The main coagulase negative Staphylococci species causing intramammary infection reside on the skin of udder and teat of goats. Among the coagulase negative Staphylococci affecting goats, *S. caprae* is a commonly isolated pathogen. *S. caprae* is an agent to be watched closely as it has been detected in human infections and especially in bone and joint infections related to hospital operations and immunosuppression (Vandenesch *et al.*, 1995). Intoxication by staphylococcal enterotoxin is one of the most frequent causes of food poisoning in European countries and these enterotoxins could be

produced not only by *Staphylococcus aureus* but also by coagulase negative Staphylococci species (Contreras *et al.*, 2000).

Bacteria of the genus Streptococci were isolated to a lesser extent comprising only 6.9 per cent of the cases in clinical mastitis and only 0.5 per cent in subclinical cases (Table 8). These findings are in general agreement with those of Hunter (1984) who reported that Streptococci mostly caused clinical mastitis in goats. A number of epidemiological studies on subclinical mastitis even reported the absence of this pathogen. Streptococcal mastitis in goats may be associated with problems of environmental contamination particularly from poor bedding conditions. Despite the pathological importance of Streptococci in mastitis they do not represent a group of prevalent pathogen in goat farms and are usually reported in some what less than 5 to 10 per cent of caprine mastitis cases (Contreras *et al.*, 2003). The difference lies in the semi intensive nature of goat rearing as they spend less time with the pathogens residing on the floor. So agents like Streptococci that survive on their hide and in the bedding material establish within the udder when conditions are favourable.

In this study coliforms were identified from five cases of clinical mastitis out which four were *E. coli* and one was Enterobacter *spp.* In subclinical cases only one isolate of *E. coli* and two each of Proteus *spp* were obtained. This agrees with the findings of Contreras *et al.* (2003). Mastitis due to gram negative bacilli are less common in goats than in cattle but when they appear they usually cause severe acute clinical mastitis. The infection can lead to symptoms similar to those caused by *Staphylococcus aureus* or even acute mastitis of gangrenous nature. Three of the four *E. coli* isolates were isolated from gangrenous mastitis cases along with *S. aureus*. *E. coli* are the most frequently isolated gram negative bacilli in goats (East et al, 1987; Contreras *et al.*, 1997; Contreras *et al.*, 2000). The high somatic cell count in healthy goat udder and the dominance of neutrophils may be the reason for the low prevalence of mastitis by gram negative bacilli (Paape, 2000; Contreras *et al.*, 1997; Contreras *et al.*, 2000; Contreras *et al.*, 2003). The general environmental conditions of goat husbandry are usually cleaner and drier than in cows. Goat manure is as pellets and drier than cow dung which is much higher in water content

and normally sloppy and easily soil the flank of cows. So the *E. coli* generally considered as environmental pathogens have fewer chances of establishing within the caprine udder.

Corynebacterium *spp* are regarded as minor pathogen in goats (Contreras *et al.*, 2003). In this study these pathogens were isolated from 3.4 per cent of milk samples and only from clinical mastitis cases. Some Corynebacterium *spp* are commensals on the udder and some are widespread in sores, tonsils, mucous membranes and genital tract (Kalogridou-Vassiliadou, 1991). Another study on goat mastitis reports that Corynebacterium is mainly involved in subclinical mastitis and does not produce long lasting persistant infection (Contreras *et al.*, 1997).

Other gram positive organisms isolated in the study were Micrococcus *spp* and Bacillus *spp*. Involvement of Bacillus *spp* in small ruminant mastitis is rare although they have been reported on occasions (Kalogridou-Vassiliadou, 1991; Sanchez *et al.*, 1999). The high incidence of Bacillus in subclinical mastitis samples may be attributed to poor hygienic practices in the farm. Bacillus *spp* could be considered as potential pathogens because bacterial species isolated from milk samples are a reflection of the skin flora. In the absence of hygienic measures particularly teat disinfection, bacteria multiply on the teat surface and in the teat duct leading to intramammary infections (Kalogridou-Vassiliadou, 1991).

In this study 3.6 per cent of the milk samples from the screenings yielded Micrococci. This is in agreement with the reports of Kalogridou-Vassiliadou, (1991); Deinhofer and Pernthaner, (1995) and Ndegwa *et al.*(2000) and contrary to the reports of Poutrel, (1984) who did not find any Micrococci in goat milk. Colonisation of Micrococci produces an antibiotic substance antagonistic to the more pathogenic Staphylococcus. So invasion of udder tissues by coagulase negative Staphylococci and Micrococci acts as a preventive factor which reduces the occurrence of mastitis by inciting a leucocyte reaction (Schalm *et al.*, 1971).

The results of the present study agree with those of other workers who have tackled the study of small ruminant mastitis without discriminating between major

and minor pathogens and who consider minor pathogens as normal microflora of the udder tissue.

5.8.2 Antibiogram

Mastitis is the single most common reason for antimicrobial use in lactating does and therefore the antibiotic sensitivity patterns and the resistance of the mastitis pathogens has received a lot of attention in the past few years. Antibiotic sensitivity studies in mastitis are useful in choosing the most effective antibiotic against the common pathogens prevalent in an area. It can also be employed as a predictor of therapy outcome in clinical mastitis cases.

In vitro studies on 84 bacterial isolates from clinical and subclinical caprine mastitis cases revealed that chloramphenicol was the most sensitive antibiotic followed by ceftriaxone, ciprofloxacin, gentamicin, oxytetracycline and sulpha. Venugopal, (1978) reported similar results of sensitivity to chloramphenicol in an *in vitro* study on caprine mastitis isolates. A large number of isolates were found to be resistant to the long established antibacterial agents like oxytetracycline and sulpha compared to high susceptibility to the more recently developed compounds (chloramphenicol, ceftriaxone, ciprofloxacin and gentamicin). The emergence and dissemination of antimicrobial resistance is the result of numerous complex interactions among antimicrobials, micro organisms and the surrounding environment. The two main factors involved in the development of antibiotic resistance in bacteria are the selective pressure by the use of antibiotics and the presence of resistance genes. The observed resistance pattern to oxytetracycline and sulpha may commensurate with the antibiotic usage pattern in this area. This could also explain the sensitivity to chloramphenicol, ceftriaxone, ciprofloxacin and gentamicin due to their limited use in the treatment of mastitis. Sulpha resistance is often carried along with the tetracycline resistance in plasmids (Rajala-Schultz *et al.*, 2004) which could be the reason for more resistance to sulpha and oxytetracycline in this study. More isolates from subclinical mastitis were found to be susceptible to most of the antibiotics than from clinical mastitis perhaps because the former were not exposed to antibacterial agents as frequently as the latter.

All isolates of *S. aureus*, the most prevalent pathogen in caprine gangrenous mastitis cases showed sensitivity to chloramphenicol. This is in agreement with the results of antibiotic sensitivity studies conducted by Venugopal, (1978); Bhujbal *et al.* (1999b); Kapur *et al.* (1984); Gupta *et al.* (2002). Bone marrow suppression and blood dyscrasia are the most important adverse effect associated with chloramphenicol administration. Bone marrow injury can take two forms and the first type which involves a dose related suppression of the bone marrow precursor erythroid series is more common. This toxicosis is reversible and usually occurs when blood chloramphenicol levels are greater than 25 μg/ml. The second type of toxicity, aplastic anemia has been described in humans but not in animals. Chloramphenicol induced aplastic anemia in humans is important from a food animal residue stand point. If chloramphenicol is used to treat infections in food animals it is possible that low concentrations of chloramphenicol in milk, meat and other edible tissues will be consumed by people and cause aplastic anemia in susceptible individuals. Chloramphenicol residues have been known to persist for prolonged periods in food animals. Even though the amount consumed may be small, the reaction is not concentration dependant. Thus there is a public health risk for individuals consuming the products. Hence the use of chloramphenicol is prohibited by the Food and Drug Administration (FDA) in food producing animals in United States (Adams, 2001c). This factor also has to be considered while initiating treatment in cases like gangrenous mastitis where the life of the animal is in danger.

The coagulase negative Staphylococci which are considered as emerging pathogens in caprine mastitis were sensitive to ceftriaxone, ciprofloxacin, chloramphenicol, gentamicin and oxytetracycline. Fthenakis (1998) also reported that newer antibiotics like cefoperoxone, cefuroxime, cloxacillin, methicillin, enrofloxacin and clindamycin were more effective in Staphylococcal isolates of caprine clinical and subclinical mastitis cases. The sensitivity studies showed that gentamicin was effective against most of the organisms isolated in caprine clinical mastitis cases.

Sulpha was the antibiotic to which the isolated pathogens showed maximum resistance. Sulphonamides have been used clinically for approximately 50 years and many once quite susceptible organisms are being resistant now. Resistance by bacterial organisms has become widespread due to the extensive use of sulphonamides over many years. Plasmid mediated resistance, the most commonly encountered form of sulphonamide resistance occurs quickly and manifests itself via the impaired drug penetration mechanism in addition to producing sulphonamide resistant dihydropteroate synthase enzyme. If an organism becomes resistant to one sulphonamide it is generally resistant to all other sulphonamides (Adams, 2001a). Resistance to sulphonamides develops slowly in a graded manner. In such bacteria, usually the tetracycline concentrating mechanism becomes less efficient or the bacteria acquire a capacity to pump it out. Another mechanism is the plasmid mediated synthesis of a protection protein which protects the ribosomal binding site from tetracyclines (Tripathi, 2003). This may be the probable reason that many of the bacterial isolates in the study were resistant to both oxytetracycline and sulpha.

5.9. TREATMENT TRIALS WITH CEFTRIAXONE AND CIPROFLOXACIN

The treatment of mastitis can be highly effective in removing the infection from the udder half and returning the milk to normal composition. However, the yield of milk, although it can be improved by removal of congestion in the gland and inflammatory debris from the duct system, is unlikely to return to normal, at least until the next lactation. Parenteral treatment is advisable in all cases of mastitis in which there is a marked systemic reaction, to control or prevent the development of a septicemia or bacteremia and to assist in the treatment of the infection in the gland. To produce therapeutic levels of antibiotic in the mammary gland by parenteral treatment it is necessary to use higher-than-normal dose rates and to give a course of five to seven days rather than the conventional three days. If treatment is to be done in lactating animals, it should begin as soon as the clinical signs are noticed. Otherwise, increased destruction of milk secreting tissues may occur or the mastitis may turn gangrenous (Smith and Sherman, 1994). For lactating animals the preferred treatment is the use of an antibiotic which maintains

a minimum inhibitory concentration for 72 hours without the need for multiple infusions and without prolongation of the withdrawal time. This depends on the release time from the transport agent in the formulation and the particle size and diffusion capabilities of the antibiotic (Radostitis *et al.*, 2000).

In the present study treatment of clinical cases of caprine mastitis using two antibiotics, ceftriaxone and ciprofloxacin for a period of five days was attempted. Out of the 29 does presented with clinical signs of mastitis, treatment could be completed only in 24 animals, 12 each being treated by ceftriaxone and ciprofloxacin respectively. The animals were allocated into two groups at random irrespective of age, parity and stage of lactation. Twelve animals in Group I were administered ceftriaxone at a dose rate of 10 mg/kg intravenously for five days and 12 animals in Group II were administered ciprofloxacin at the rate of 10 mg/kg for five days. Majority of the animals presented showed pale watery milk with flakes or clots. All animals showed febrile reaction with temperature above 105°C, inappetance and enlargement of supramammary lymph node. Induration of udder was a common finding in majority of animals which persisted even after the completion of antibiotic therapy.

Seven cases of gangrenous mastitis were encountered in this study out of which four were fatal. The most common symptoms of gangrenous mastitis noticed in this study were serosanguinous secretion from udder accompanied by bluish discolouration, foul odour and coolness of udder skin. Such a condition warrants intensive therapy and usually a poor prognosis for return to function. *Staphylococcus aureus* was isolated from all seven cases of gangrenous mastitis and in three cases *Escherichia coli* also were isolated along with *S. aureus*. Gangrenous mastitis in goats is most frequently due to *Staphylococcus aureus* and the resulting toxemia is due to bacterial toxins and tissue destruction. Secondary invasion by *E. coli* and Clostridium *spp* contributes to the severity of the lesion and production of gas. The condition usually is restricted to the period of lactation. However sometimes gangrenous mastitis occurs during the last week of pregnancy, where often it causes loss of fetuses and death of the doe from toxemia. In early cases, the skin of the teat or udder floor becomes cool and edematous, and the goat appears

lame. A reddish blue disclouration is noticed next. The secretion becomes watery red and gas bubbles may be present which produce a squeaking sound when the teat is stripped. Death may occur within 24 hours. If the animal survives the acute phase, a clear blue line of demarcation forms on the udder and the gangrenous portions are sloughed after several days or weeks.

It is proposed that the browsing nature of goats predisposes it to teat abrasions and wounds which then lead to gangrenous mastitis. In addition, the relatively poor vascularisation of caprine udder compared to cattle and the lack of valves in large veins are the other predisposing factors (Abu-Samra *et al.,* 1988). Histologically there is venous thrombosis and the initial inflammatory changes are replaced by necrosis and sloughing of epithelial cells. This thrombosis is mainly responsible for the edema of the mammary gland and ventral abdominal wall. Despite the commencement of the antibiotic immediately on being presented, four animals succumbed to death and the other three did not show bacteriological cure. The alpha-toxin produces ischemia and thrombosis of large vessels preventing the paranterally administered drug from reaching the site of action. Local intramammary infusion of any antibiotic will not give the desired effect because the organism multiplies deep inside the parenchyma where the drug has no accessibility (Venugopal, 1978). This may be the reason for failure of treatment despite the fact that ceftriaxone and ciprofloxacin, the most sensitive drugs as per the antibiogram were administered. Amputation of the udder can be a life saving procedure if the goat is very toxemic with gangrenous mastitis. Another option is that the gangrenous gland can be infused with acriflavin (1 in 500) which kills the pathogens and removes the necrotic tissues. The gland thus treated will not return to production but the life of the doe may be saved.

Ceftriaxone belongs to the cephalosporin class of beta-lactam antibiotics which are classified by generation as first, second, third and fourth generations. The β lactam antibiotics exert their bactericidal effects by preventing bacterial cell wall integrity. Beta lactams bind to a series of enzymes known as penicillin-binding proteins which are involved in the final stages of cell wall synthesis. Penicillin-binding proteins vary between bacterial species. The affinity of various β lactam

antibiotics for the different penicillin-binding proteins may explain the differences in the spectra of activity of these antibiotics that are not caused by the presence or absence of β lactamases. Ceftriaxone is a third generation cephalosporin. It is non-irritant and remains in therapeutic concentrations in treated quarters for three to four milkings making it very useful in the treatment of bovine mastitis (Adams, 2001b). Out of the 12 animals where treatment with ceftriaxone was completed for five days, *S. aureus* was the most common isolate (ten cases). This group had a clinical cure of 58.33 per cent and a bacteriological cure of only 50 per cent. Cure rate of clinical and subclinical mastitis due to *S. aureus* in the lactating cow is very low. Emergence of beta lactamase producing strains of *S. aureus* may be another reason for the low clinical cure in this group. *S. aureus* isolated from goat mastitis cases showed a marked tendancy to invade and persist as foci in the acinar tissue (Radostitis *et al.*, 2000). It has been well documented that *S. aureus* is a pathogen having unique abilities to persist in microabscesses and infections become very difficult to cure (Smith and Sherman, 1994). In two cases the does were in their dry periods and the owners failed to notice the condition until it became systemic. In the other five cases the animals were presented for treatment only two to three days after the onset of clinical signs and by the time the udder was indurated leading to inaccessibility of the drug to the pathogen, ineffective drug diffusion and thus inefficient killing of the pathogen. Some drugs that are administered parenterally do not achieve adequate concentration in the udder. Cephalosporins are seen to have less permeability from blood to milk (Radostitis *et al.*, 2000). This may be the reason for the low bacteriological cure observed in this group.

Ciprofloxacin was found to have better clinical and bacteriological cure in the 12 animals when compared to ceftriaxone. Ciprofloxacin belongs to the fluoroquinolone group of antimicrobial drugs. The use of this class of antibiotics in veterinary medicine has increased tremendously in the last 10 years. Quinolones are bactericidal by inhibiting DNA replication and transcription. The two stranded DNA is tightly coiled in the cell and must be separated for transcription and translation. To facilitate coiling, winding and unwinding the enzyme DNA gyrase allows the strands to be cut and reconnected. The most common target site for fluoroquinolones is the A subunit of DNA gyrase coded by the gene *gyr A*. There

are very few scientific publications based on randomized clinical trials in which the efficacy of intramammary antimicrobials for treatment of clinical mastitis are compared to untreated controls. However, most of the reasons for treatment failure, while biologically attractive are hypothetical and have not been substantiated scientifically. One animal in this group presented with gangrenous mastitis involving both udder halves succumbed to death though the antibiotic was changed to chloramphenicol as per the antibiogram. In gangrenous mastitis death of the tissue is precipitated by thrombosis of the veins and this leads to loss of the gland or death of the animal due to toxemia. Clinical cure was 66.67 and bacteriological cure 58.33 per cent in this group. The systemic reaction can usually be brought under control by standard doses of antibiotics but complete sterilization of the affected udder half is seldom achieved because of the relatively poor diffusion of the antibiotic from the blood stream into milk. In most cases the affected gland showed fibrosis by the time treatment was initiated.

Two animals showed typical symptoms of anaphylactic reactions like dyspnea, cough, bloat, paddling movements of legs and severe arching of body in an effort to breathe on administering ciprofloxacin intravenously. It was brought under control by administration of chlorpheniramine maleate and dexamethasone. There have been no reports of anaphylactic reactions after administration of fluoroquinolones and these are claimed to have a remarkably good safety record. Fluoroquinolones injected rapidly intravenously or administered at high doses can induce CNS excitement. Fluoroquinolones can precipitate convulsions in some animals and should not be administered to animals that are prone to seizures (Adams, 2001d). The anaphylactic reactions may be attributed to stress during handling for intravenous injections as goats are animals very much prone to this condition. So it becomes important that extra care be taken while administering drugs intravenously to goats.

In vivo results of the treatment trials with ceftriaxone and ciprofloxacin were different from the *in vitro* antibiotic sensitivity patterns. According to Radostitis *et al.* (2000) *in vitro* laboratory testing of bacterial isolates is not necessarily a justifiable basis for selecting the antibacterial agents to be used in individual cows

and the response to treatment in clinical cases may be often unrelated to these results. This may be because of the failure of the drug to reach its sufficient concentration in the milk or due to its rapid excretion from the body. Comparison of antimicrobial susceptibility tests with clinical efficacy may be possible for mastitis pathogens that are confined primarily to milk but the comparison might be difficult for pathogens that invade tissues. The agreement between results of antimicrobial susceptibility tests and clinical efficacy in short term *S. aureus* intramammary infection is about 70 per cent; at 28 days post treatment the cure rate for chronic *S. aureus* infection is only 35 per cent and this reaffirms the intractable nature of this pathogen. Thus *in vitro* testing can be considered as a predictor for therapy outcome for intramammary infection caused by Staphylococcus *spp*, but cannot be considered as a useful predictor of efficacy for chronic infection caused by *S. aureus.*

Mastitis is a collection of syndromes. So antimicrobial therapy alone cannot bring back the affected gland to normal condition. Frequent stripping of the affected quarters and provision of other supportive therapy such as fluids and electrolytes are also crucial for the survival of the doe and minimization of the severity of mastitis and extent of permanent injury to the udder. Non steroidal anti inflammatory drugs administered may enhance recovery, reduce fever and edema in severe cases.

5.10. RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) ANALYSIS

Amplification of the DNA by PCR and detection and identification of specific bacteria using arbitrary primers are commonly employed to elucidate the genetic make up of the pathogen. PCR using arbitrary primers (AP-PCR or RAPD PCR) requiring no prior sequence information has revealed DNA polymorphisms that may be useful for fingerprinting (Onasanya *et al.*, 2003). Random amplified polymorphic DNA typing is based on the amplification of discrete DNA fragments in the genome by the use of short oligonucleotide primers with random sequences. Because of its small size the primers can anneal to multiple sites throughout the target DNA and the amplification of the fragment takes place whenever these

primers anneal together. Bacteria with different sequences will allow primers to anneal at different sites and thus produce a set of bands unique to each organism. The DNA band pattern obtained is similar to a bar code and allows the identification of each organism (Onasanya *et al.*, 2003).

In the present study on caprine mastitis eighteen Staphylococcus isolates from clinical cases of mastitis in goats brought for treatment and 23 Staphylococcus isolates from subclinical cases were typed by RAPD fingerprinting. The Staphylococcus isolates were grouped into 12 genotypes arbitrarily designated as a, b, c, d, e, f, g, h, i, j, k, and l based on their banding patterns (Table 13). The RAPD pattern of *S. aureus* from gangrenous mastitis cases belonged to type c, d and f. Genotype d had a low frequency (4.35 per cent) in subclinical mastitis cases and this may account for the less number of gangrenous mastitis cases reported from the farm. There was considerable variation in the distribution of *S. aureus* RAPD genotypes in clinical and subclinical mastitis. The absence of genotypes a, b, g, h and i in clinical mastitis cases shows that these genotypes are less pathogenic when compared to other isolates. It can be inferred that the caprine udder may be inhabited by many non pathogenic Staphylococci which in turn may prevent the colonization by pathogenic strains of *S. aureus* (Schalm *et al.*, 1971).

Out of the 12 genotypes two predominated in subclinical infections. This shows that caprine mastitis is of an infectious nature with Staphylococcus *spp* being passed from animal to animal via milkers hands. Only hand milking is practiced in the farm and this strengthens the view that infection of udder by opportunistic environmental isolates of *S. aureus* is unusual. The highest cure rate (clinical and bacteriological) was for the Staphylococcal isolates having RAPD genotypes i and k. There was no clinical and bacteriological cure rate for *S. aureus* RAPD type f (Table 14). This may be attributed to the virulence properties of the pathogen and many other factors like increased antimicrobial resistance and development of L forms of bacteria (Radostitis *et al.*, 2000). Half of the treatment failures are accounted for by new infections becoming established in a cured quarter. The guidelines of the Committee for Veterinary Medicinal Products (CVMP) in the European Union and the Center for Veterinary Medicines of the Federal Drug

Administration (CVM-FDA) in the United States assess the clinical efficacy of products used in the treatment of mastitis in the non lactating cow based on bacteriological cure. Genetic fingerprinting technique can be used to assess the impact of treatment on the perceived efficacy of the antibiotic. The sensitivity patterns of the known genotypes can be compared which makes the choice of antibiotic easy and also predict the therapy outcome of mastitis in goats. This technique can distinguish between strains of organisms before and after antibiotic therapy. Bacteriological techniques underestimate cure rate because new infection with a different strain cannot be differentiated by bacteriology (Reeve-Johnson, 2003). However, in this study no attempt was made to differentiate the strain of pathogens isolated after treatment by RAPD analysis.

Mastitis research is now focused on the development of suitable vaccines for combating the disease. This had been an area of research since 30 years and many mastitis vaccines are available commercially now. RAPD fingerprinting gives an idea about the most prevalent strain in an area and if this is known it will be possible to incorporate that strain as the vaccine antigen while formulating vaccines. This will certainly be of immense help in the prevention and control of this disease condition in that particular area. The phylogenetic tree (Fig 6) helps to get a comprehensive idea about the relationship between the different genotypes and how far a particular genotype is different from the more virulent one based on clinical and bacteriological cure rates.

It can be concluded that the use of RAPD fingerprinting in the diagnosis of caprine mastitis is a fast and reliable technique to define individual mastitis pathogens, the possible relationship between strains of organisms and also mutation and genetic variation among them.

Summary

6. SUMMARY

The present study 'Clinico-therapeutic studies on bacterial mastitis in goats' was undertaken with the objective of detecting subclinical mastitis in lactating goats, efficacy of various indirect tests in diagnosing the condition, identification of the various bacteriological agents responsible for the condition, their antibiogram, to assess the effect of two treatment trials in curing clinical mastitis and to differentiate the strain of pathogens isolated by RAPD fingerprinting.

The lactating does in the University goat and sheep farm were screened for subclinical mastitis once in three months using the California mastitis test. A total of 642 milk samples were examined for the presence of subclinical mastitis using CMT out of which 194 samples were found to be positive. The occurrence of subclinical mastitis was found to be 30.2 per cent. Statistical analysis showed no significant association between occurrence of subclinical mastitis and teat length whereas there was significant association between distance from teat tip to floor.

Unequal distribution of somatic cells in goat milk required logarithmic transformation of cell count values for statistical analysis. Among the CMT positive milk samples, 29 (14.95 per cent), 68 (35.05 per cent), 80 (41.24 per cent) and 17 (8.76 per cent) halves were graded as 'T', '1', '2', and '3' respectively. The arithmetic mean cell counts corresponding to CMT scores 'T', '1', '2', and '3' ranged between $0.736 \pm 0.033 \times 10^6$ and $20.417 \pm 0.851 \times 10^6$ cells/ml.

Out of 194 milk samples subjected to Modified Whiteside test, 72 (37.11 per cent) were classified as negative, 53 (27.32 per cent) as '1+', 55 (28.35 per cent) as '2+', 10 (5.15 per cent) as '3+' and 4 (2.06 per cent) were graded as '4+'. MAMP test performed on 194 CMT positive milk samples detected 110 (57.73 per cent) as healthy udder half, 56 (35.05 per cent) as having mild subclinical mastitis, 28 (7.22 per cent) as having severe subclinical mastitis. Among CMT positive samples MWST and MAMP detected 62.89 per cent and 43.29 per cent as positive for subclinical mastitis. Out of the CMT positive milk samples 50 (25.77 per cent)

were positive for TIST. TIST detected the maximum positive cases for CMT score '2' and MWST score of '2+'.

Comparison of screening tests revealed that significant positive correlation existed among the four tests namely CMT, MWST, MAMP and SCC. The highest correlation was between CMT and SCC (r = 0.829). Comparison with culture results showed that score '3' of CMT and score '3+' of MWST and grade '3' of MAMP reaction detected the maximum positive cases. Among the TIST positive samples 20 (44.44 per cent) were bacteriologicaly positive. CMT scores and SCC were found to have good correlation in bacteriologicaly positive udder halves.

Staphylococcus aureus predominated in both clinical and subclinical caprine mastitis occurring at a frequency of 39.7 per cent and 10.3 per cent respectively. Two isolates of coagulase negative Staphylococci were obtained from clinical mastitis cases and 13 from subclinical cases. The other bacterial isolates were *Escherichia coli,* Streptococci, Corynebacterium *spp,* Enterobacter *spp,* Corynebacterium, Bacillus, Proteus and Micrococcus *spp.*

In vitro antibiotic sensitivity pattern revealed that chloramphenicol was the most sensitive antibiotic followed by ceftriaxone and ciprofloxacin. *Staphylococcus aureus* isolates showed a sensitivity of 100 per cent to chloramphenicol, 97.14 per cent to ceftriaxone, 88.57 per cent to ciprofloxacin 51.53 per cent to oxytetracycline and 25.71 per cent to sulpha.

Treatment using two drugs, ceftriaxone and ciprofloxacin was attempted in 24 goats in different stages of lactation. Six animals had both clinical and bacteriological cure when treated with ceftriaxone and five of the 12 treated animals showed both clinical and bacteriological cure with ciprofloxacin. Two animals showed hypersensitivity reactions to ciprofloxacin administered intravenously. Gangrenous mastitis cases were seen to have very less clinical and bacteriological cure when compared to other cases. Considering the clinical and bacteriological cure of individual animals, it was found that Group I had 58.33 per cent and 50 per cent cure rates whereas Group II had 66.67 per cent and 58.33 per

cent cure rates respectively. Hence clinical and bacteriological cure was found to be better in the case of ciprofloxacin. However the risk of having anaphylactic reactions has to be considered when an option is to be made between ceftriaxone and ciprofloxacin in the treatment of caprine mastitis.

RAPD fingerprinting technique was used to type 18 Staphylococcus isolates from clinical mastitis cases and 23 Staphylococcus isolates from subclinical cases. Twelve different genotypes were identified among which genotype c predominated. Out of the four isolates from the gangrenous mastitis cases encountered in the study, two belonged to genotype d and the others to genotypes c and f respectively.

In Group I treated with ceftriaxone the highest cure rate (clinical and bacteriological) was for RAPD type 1 (100 per cent) and the clinical and bacteriological cure rates were maximum for RAPD types c and i in the ciprofloxacin treated Group II. Genetic fingerprinting and phylogenetic diversity between the different Staphylococcus isolates were determined by analyzing the RAPD data using RAPD distance software to generate a phylogenetic tree. The relationship between the genetic make up of the different Staphylococcal isolates could be made out from this relationship tree.

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CLINICO-THERAPEUTIC STUDIES ON BACTERIAL MASTITIS IN GOATS

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ABSTRACT

The lactating does in the University goat and sheep farm were screened for subclinical mastitis once in three months using the California mastitis test. The occurrence of subclinical mastitis was found to be 30.2 per cent. Statistical analysis showed no significant association between occurrence of subclinical mastitis and teat length whereas there was significant association between distance from teat tip to floor. Among 642 samples screened 194 samples were found to be positive by CMT.

The arithmetic mean cell counts for each CMT score ranged between 0.736 ± 0.033 $x10^6$ and 20.417 ± 0.851 $x10^6$ cells/ml. Among CMT positive samples MWST and MAMP detected 62.89 per cent and 43.29 per cent as positive for subclinical mastitis. Comparison of screening tests revealed that significant positive correlation existed among the four tests namely CMT, MWST, MAMP and SCC. Comparison with culture results showed that score '3' of CMT score '3+' of MWST and grade 3 of MAMP reaction detected the maximum positive cases. CMT scores and SCC in bacteriologicaly positive samples showed significant association. Among the TIST positive milk samples 20 (44.44 per cent) were culture positive.

Staphylococcus aureus was the most predominant isolate in both clinical and subclinical caprine mastitis. *In vitro* antibiotic sensitivity pattern revealed that chloramphenicol was the most sensitive antibiotic followed by ceftriaxone and ciprofloxacin. The isolated pathogens showed maximum resistance to sulpha. Comparison of treatment trials in 24 clinical goat mastitis cases using ceftriaxone and ciprofloxacin with 12 animals in each group revealed that clinical and bacteriological cure was better in the case of ciprofloxacin. Clinical and bacteriological cure was comparatively less in gangrenous mastitis cases.

Eighteen Staphylococcus isolates from clinical mastitis cases and 23 Staphylococcus isolates from subclinical cases were typed by RAPD fingerprinting. Twelve different genotypes were obtained among which genotype c predominated in clinical mastitis whereas in subclinical cases b and i were the common

Staphylococcal genotypes. Clinical and bacteriological cure rates were 100 per cent for RAPD type 1 in the ceftriaxone treated group and genotypes c and i in the ciprofloxacin treated group of animals. A possible relationship regarding the genetic make up of the different Staphylococcal isolates was elucidated from the phylogenetic tree generated from the RAPD fingerprints.