PATHOLOGY OF BACTERIAL MASTITIS IN BOVINES

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THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
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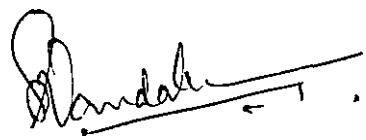
MANNUTHY, THRISSUR - 680651
KERALA

1998

DECLARATION

I hereby declare that this thesis entitled **"PATHOLOGY OF BACTERIAL MASTITIS IN BOVINES"** is a bonafide record of research work done by me during the course of research and that this 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 **"PATHOLOGY OF BACTERIAL MASTITIS IN BOVINES"** is a record of research work done independently by **Dr. Nandakumar, S.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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ACKNOWLEDGEMENT

I am deeply indebted to Dr. T. Sreekumaran, Chairman of Advisory Committee who has guided me throughout the course of my research work.

I greatly acknowledge the sincere help and cooperation from Dr. K.M. Ramachandran, Director and Head, Centre of Excellence in Pathology for the execution of this work.

The inerrant and astute suggestions rendered by Dr, Koshy Varghese, Assistant Professor, Centre of Excellence in Pathology is indeed worthy to be mentioned.

My words of praise to Dr. G. Krishnan Nair, Associate Professor, Department of Microbiology, who was very much associated with the ponderability of the text.

I am too appreciative of the technical support provided by Dr. A. Rajan, former Dean, College of Veterinary and Animal Sciences, Mannuthy, Dr. K.V. Valsala, Dr. C.R. Lalithakunjamma, Dr. N. Divakaran Nair, Dr. C.B. Manomohan and Dr. T.V. Anilkumar, Staff, Centre of Excellence in Pathology for the consummation of the thesis.

Had it not been for Dr. C.V. Sreeranjit Kumar, Research Associate, Department of Microbiology for his onerous efforts and admonition, the thesis would not have been licked into shape by now.

The timely help and countenance of Dr. J. Abraham, Dr. George T. Oommen and Dr. Sunil, M., Staff, Meat Technology Unit, Kerala Agricultural University has always patronised the furtherance of my research.

I am greatly obliged to Dr. Jacob Alexander, Dr. Biju, P., Dr. Silamban, Dr. Vigil Ambiah, Dr. Mohan and Dr. Suresh, who have always thrown in with me both in times of good and bad.

I take great pleasure in appreciating Dr. Sosamma Iype, Professor, University Livestock Farm, Mannuthy, Dr. Anil, K.S., Assistant Professor, Cattle Breeding Farm, Thumburmuzhi and Dr. Wahida, Assistant Professor, Livestock Research Station, Thiruvazhamkunnu for the facilities provided for this research work.

I would fail in my duty if I forget to mention Mr. A.P. Peter, Farm Assistant, Centre of Excellence in Pathology, who has always been with me through the thick and thin of my thesis work.

I am extremely grateful for the consistent support and encouragement lended by the teaching and non-teaching staff of Centre of Excellence in Pathology.

The assistance provided by Mrs. Beena, Ms. Sara, Ms.Seema and Ms. Jalaja, Research Associates, Centre of Excellence in Pathology is acknowledged in its full worth.

My colleagues Manoj, Sanjay, Sunil, Dinesh, Shihab, Madhu, Praveen, Jayakrishnan, Senthil and all other Post-graduate students have ushered me right from the inception to the eventuality of this work.

Mr. Noel, Ambika Computers and Mr. Ravindran, Peagles are those persons who have worked cheek by jowl in the typing of the manuscript and providing photostat facilities.

NANDAKUMAR, S.

Dedicated

To

My Family

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Introduction

INTRODUCTION

Mastitis is one of the most complex diseases of economic importance affecting the dairy industry. The widespread occurrence of mastitis in Indian dairy herds creates an estimated annual loss of Rs.1607.2 crores (Singh and Singh, 1994a). The economic losses incurred by the dairy industry are more due to the reduced milk production from affected quarters and culling of cows with non-functional quarters rather than by fatalities. As per Radostits *et al.* (1995), on an average an affected quarter suffers a 30 per cent reduction in productivity and an affected cow is estimated to lose 15 per cent of the production for the lactation. This excludes the additional untold losses from altered milk quality and composition and the effects on dairy products.

Mastitis, the inflammation of the mammary gland is characterised by physical, chemical and usually microbiological changes in the milk and by pathological changes in the glandular tissue of the udder. It is a disease complex having different causes, different degrees of intensity and variation in duration and residual effects. Many major and minor pathogens have been found to produce subclinical, clinical and chronic mastitis in cattle. Attempts have been made by several workers in

India and abroad to determine the causatives of the disease. Clinical mastitis is evidenced by the characteristic changes in the milk and mammary gland, whereas subclinical mastitis takes its toll in a rather stealthy manner in huge proportions among dairy cattle. Subclinical mastitis is universally present in almost all herds in one form or the other and a fairly high number of cows were reported to be suffering from this form of the disease (Ramachandraiah *et al.*, 1990).

California Mastitis Test (CMT) is usually employed for the detection of subclinical mastitis. The indirect screening tests for mastitis indicate the presence of products of inflammation in the milk and the results correlate directly with the concentration of somatic cells of blood or mammary origin. The *in vitro* culture and sensitivity tests of the milk samples to various antibiotics help to assess the prevalence of various microorganisms causing mastitis and opt suitable therapeutic measures. Moreover, knowledge of the type and epidemiology of the pathogen causing mastitis is highly essential for the selection of the most efficient mastitis control strategy.

Gross and histopathological studies of udder tissues and supramammary lymphnodes reveal the pathological changes taking place in the lactating and non-lactating glands.

Detailed studies on blood and milk samples of affected animals gives an idea of the progress or decline of the disease.

Immunocompetency of the host can be evaluated by assessing the cell mediated and humoral immune response, the deficiency of either of the two systems resulting in infections. Electrophoretic studies on whey samples gives an idea of the changes in the major whey proteins as a result of the disease process.

The present study will throw light on the pathological changes in the mastitis affected mammary gland and will help in the diagnosis and control of bacterial mastitis in bovines. The objectives of the study are the following:

1. To assess the prevalence of clinical and subclinical mastitis in bovines with special reference to Mycoplasmal mastitis.
2. To evaluate the clinico-pathological features.
3. To study the patho-anatomical features of mastitis affected udder.

Review of Literature

REVIEW OF LITERATURE

Mastitis is one of the most important diseases confronting the dairy industry producing huge economic losses every year.

Incidence

In India, mastitis was first reported by Joshi in 1928 (Krishnaswamy *et al.*, 1965). Schalm and Ziv-Zilberman (1968) reported a greater incidence of mastitis in residual milk than in fore milk. Rao and Naidu (1969) studied the quarterwise distribution of mastitis and found that left and hind quarters were affected more than right and fore quarters. Hunter and Jeffrey (1975) suggested the widespread occurrence of subclinical mastitis in many suckler herds. The incidence of clinical mastitis in cattle was highest in the third and fourth month of lactation and the rear quarters were more affected (Kapur and Singh, 1978). McDonald and Anderson (1981) reported that clinical mastitis occurred shortly after parturition, because these cows were frequently more dirtier providing better chance of exposure to *E. coli*.

A study of milk samples from cattle from different parts of Kerala during the period 1981-1984 revealed the incidence of mastitis as 50.42 per cent (Sudharma *et al.*, 1985). Badran (1988) revealed the factors affecting the

udder susceptibility to staphylococcal infection. He found that teat surfaces and teat apices were the major sources of udder infection with *Staphylococcus aureus,* by its transmission to the inside of the udder via the teat canal. Dutta *et al.* (1988) studied the lactational prevalence of mastitis in Jersey and crossbred cows and found that the risk ratio was 1.21 to 1.98 times greater in Jersey cows than crossbred cows.

Adkinson *et al.* (1993) studied the distribution of clinical mastitis among quarters in cows and found that front quarters had less clinical mastitis than rear quarters. Tuteja *et al.* (1993) conducted cultural examination of 697 quarter samples from 178 cows and showed that the incidence rate of subclinical mastitis was 78.1 per cent of the cows and 42.2 per cent of the quarters respectively. Milk samples collected from all the quarters of 123 crossbred cows and 241 buffaloe, when subjected to sodium lauryl sulfate test and further bacterial isolation, showed the incidence of subclinical mastitis among crossbred cows and buffaloe as 4.87 per cent and 2.59 per cent respectively. The lowest incidence of subclinical mastitis in cows and buffaloe was during the first month of lactation, which increased during subsequent lactations (Saini *et al.*, 1994). The incidence of subclinical mastitis in machine milked cows was found to be 49.29 per cent and 23.09 per cent on quarter basis (Singh *et al.*, 1994b).

Saharia *et al.* (1997) studied the effects of housing on the incidence of subclinical mastitis in cattle. 47.56 per cent and 55.74 per cent of the animals were found positive for subclinical mastitis under pucca and wooden floor housings respectively. The higher incidence of mastitis in wooden floors was due to the fact that the wooden floors remained comparatively damp and dirty. Staphylococcus mastitis was more during mid summer and the hind quarters were more affected (Umakanthan, 1997a).

Etiology

Parganoker (1956) reported high incidence of mastitis due to *Corynebacterium pyogenes*. Lee and Frost (1970) conducted bacteriological studies on the mammary gland of slaughtered cows and classified the major mammary pathogens as *Staphylococcus aureus*-42 per cent, *Streptococcus uberis* and *S. agalactiae*-25 per cent and other Streptococci-11 per cent. Out of the 304 milk samples examined, 54.4 per cent of the quarters were infected with pathogens, mainly Staphylococcus-68.6 per cent and Streptococcus-16.2 per cent (Chander and Baxi, 1975).

Jasper (1982) found that several species of Mycoplasma were common pathogens causing mastitis in California. Out of the ten species identified, *Mycoplasma bovis* and *Mycoplasma bovigenitalium* were the most common. *Mycoplasma bovigenitalium* was isolated from the milk of one cow and one buffalo out of the 28 animals screened (Pal *et al.*, 1982).

Kalorey *et al.* (1983) conducted cultural examination of milk samples from subclinical cases of bovine mastitis. Out of the 173 isolates obtained 45.6 per cent was *Staphylococcus aureus* and 31.7 per cent was *Staphylococcus epidermidis*. McDonald and Anderson (1983) reported that intramammary inoculations of *Staphylococcus aureus* and *Staphylococcus epidermidis* produced infections in 67.3 per cent and 20.8 per cent of the glands respectively. Staphylococcus was the predominant organism associated with clinical cases of mastitis, followed by Coliforms, Streptococci, Corynebacterium, Yeast, Gram positive bacilli and Pseudomonas species (Sudharma *et al.*, 1985). Bacteriological and histopathological studies on mammary glands of slaughtered cows revealed *Staphylococcus epidermidis* as the most predominant organism, followed by *Streptococcus uberis* and *Corynebacterium bovis* (Handique *et al.*, 1988).

Chanda *et al.* (1989) studied the incidence, etiology and diagnosis of bovine mastitis and *in vitro* sensitivity of the isolated pathogens. Staphylococci were the major pathogens (57.52%) followed by Streptococci (35.4%) Corynebacteria (5.3%) and *E. coli* (1.78%). Bacteriological examination of milk from clinically affected quarters showed the presence of Staphylococci (64.81%), Streptococcus (8.33%), *E. coli* (22.22%) and Pseudomonas (2.78%) (Prabhakar *et al.*, 1989). Char *et al.* (1993)

studied the bacterial flora and antimicrobial activity of various antibiotics in clinical cases of bovine mastitis. Out of the 145 strains of bacteria isolated from 74 subclinical cases of bovine mastitis, 40 isolates were *Staphylococcus aureus*, 34 coagulase negative Staphylococcus, 21 Bacillus species, 14 Streptococcus, 12 Pneumococcus and 8 Corynebacterium species (Saxena *et al.*, 1993b). Reddy *et al.* (1994) classified subclinical mastitis milk into different grades based on the intensity of the CMT reactions. He studied the bacteriological aspects of the different grades of milk based on standard plate count, coliform count, staphylococcal count and haemolytic bacterial counts. Saini *et al.* (1994) reported that Staphylococcus was the major pathogen in cases of subclinical mastitis among crossbred cows and buffaloe.

Bhattacharya and Rahman (1995) reported that Staphylococcus was the chief etiological agent of bovine mastitis, followed by Streptococcus, Corynebacterium, *E. Coli*, Pseudomonas and Bacillus species. Mitra *et al.* (1995) studied the etiological agents causing subclinical mastitis in buffaloe. Out of the 176 isolates obtained from 528 milk samples, 41.37 per cent was Staphylococcus species, 32 per cent Streptococcus, 28 per cent *E. Coli* and 6.89 per cent was Corynebacterium species. Bacteriological examination of 200 mastitic and 65 non-mastitic udder secretions from heifers revealed that 57.8 per cent of the

bacteria isolated were coagulase negative Staphylococci. This was followed by *Staphylococcus aureus* (20.1%), Streptococci (11.3%) and other pathogens (10.8%) (Myllys, 1995). Biju (1996) observed the predominance of Staphylcoccus species in 34 clinical and 193 subclinical cases of bovine mastitis. *Staphylococcus aureus* (75%) was the major bacterial agent in subclinical mastitis in cows, followed by *E. Coli* (19.75%), Micrococci and Proteus (14.58% each) (Ratnakumar *et al.*, 1996). Wadhwa *et al.* (1996) reported that mastitis due to Staphylococcus species was the highest (48.33%) followed by Streptococcus species (16.88%) *Escherichia Coli* (9.8%), Klebsiella (2.59%) and Diplococcus, Proteus and Corynebacterium (1.3%). Mallikarjunaswamy and Krishnamurthy (1997) isolated Staphylococcus species, Streptococcus, Bacillus, *E. Coli* and Pseudomonas in the decreasing order of prevalence from cases of bovine subclinical mastitis.

Somatic cells in milk

Various workers have estimated the somatic cells in normal and mastitis milk. A somatic cell count of 3 lakh to 10 lakh per millilitre of milk was found normal, depending on the age of the animal and stage of lactation (Little, 1938). Blackburn *et al.* (1955) compared the diagnostic value of total and differential cell counts of bovine mastitic milk. Babel (1958) revealed that the cell count in milk varied according to the stage of lactation.

Initially for the first two days of lactation, the counts were over 1,00,000 cells/ml. Later the average count of healthy cows fell to approximately 10,000 cells/ml.

Comparison of the variations of the estimated number of somatic cells in milk stained with Methyl green pyronin-Y with that of cells stained with Wright's stain was done by Paape *et al.* (1963). The number of cells determined in smears stained with Pyronin-Y methyl green was considerably more than that determined in smears stained with Wright's stain. Schalm and Lasmanis (1963) reported that neutrophils were the predominant cells involved in mastitic milk and they phagocytosed the organism causing the disease.

Cullen (1966) found out that the increase in number of cells in milk was due to the chemotactic stimuli as a result of the presence of microorganisms in the udder. Studies on cells in milk revealed that neutrophils were the major inflammatory cells seen (Lalithakunjamma, 1976). Syrstad and Ron (1979) reported variation in the somatic cell counts of milk samples from individual cows. Analysis of somatic cell volume by electronic somatic cell count method and relative cell volume distribution can be employed as an aid to the diagnosis of mastitis (Hoare *et al.*, 1980).

During the involutionary phase of mammary glands, there was an increase in the number of cells in milk due to the cessation of removal of milk and later a decrease in number was as a result of the afflux of neutrophils and macrophages from the gland. (Jensen and Eberhart, 1981). Emanuelson *et al.* (1987) compared some screening tests for the detection of mastitis. Wiggans and Shook (1987) developed a lactation measure of somatic cell count. Increase in somatic cell count with late lactation could either be due to infection or decreasing yield or physiological effects associated with lactation. Badran (1988) showed a marked increase in the somatic cell count during the first few hours of infusion of *Staphylococcus aureus*. Elevation of somatic cell counts was reported in response to conditions unrelated to infection like teat injury, excessive milking, stage of lactation and various forms of stress (Daley and Hayes, 1992).

Indirect screening tests based on the changes in chemical composition of milk were evaluated for their efficacy in detecting subclinical mastitis. Estimation of lactose, Aspartate transferase and somatic cell count were the most effective indirect tests to monitor subclinical infections (Pednekar *et al.*, 1992). Deluyker *et al.* (1993) evaluated the inter-relationship between somatic cell count and milk yield in a low somatic cell count herd. The somatic cell count was highest at lactation onset and cows

with clinical mastitis had significantly higher somatic cell count. Harmon (1994) reported that somatic cells in milk were primarily leucocytes, which includes lymphocytes, polymorphonuclears and occasionally macrophages.

Haematological evaluation

Cole and Easterbrooks (1958) reported leucopenia, neutropenia and relative lymphocytosis in experimental acute staphylococcal mastitis. Observations on clinical cases of acute mastitis indicated that there was reduction in number of neutrophils and lymphocytes leading to relative or absolute leucopenia and later there was leucocytosis (Theilen *et al.*, 1959). Schalm *et al.* (1971) evaluated the total and differential leucocyte count in cases of coliform mastitis. There was leucopenia with reduced number of mature neutrophils.

Yang *et al.* (1980) reported that the percentage and absolute number of peripheral blood B lymphocytes were significantly lower in cows with mastitis. Reddy *et al.* (1980) demonstrated acid alpha-naphthylacetate esterase activity in the peripheral blood leucocytes of bovines. Identification and enumeration of T and B cells in the peripheral blood of goats using erythrocyte antibody rosette technique and ANAE activity was carried out by Sulochana *et al.* (1982). Kaura *et al.* (1989) evaluated the status of T and B lymphocytes in the peripheral blood of

mastitic and healthy buffaloe. There was a reduction in B lymphocytes and an increase in T lymphocytes in mastitic animals.

Whey protein profile of mastitic milk

The paper electrophoretic pattern of whey proteins in cows with acute mastitis revealed the appearance of a fraction migrating at the rate of blood serum albumin (Leece and Legates, 1959). Fish *et al.* (1969) compared Coomassie blue R 250 and Amido black 10 B for the detection of whey protein bands after disc electrophoresis. A staining time of 1.5 to 2 hours for Comassie brilliant blue gave results comparable to staining by Amidoblack 10 B method. Green and Pastewka (1975) characterised milk proteins from BALB/C and C3H mice. The two major whey proteins were ß-lactoglobulin and α-lactalbumin, as characterised by their migration in sodium dodecyl sulfate gels. Darling and Butcher (1976) employed densitometric scanning of polyacrylamide gels for the quantification of whey proteins, mainly α-lactalbumin and ß-lactoglobulin. By comparing the peak areas with the standard peak areas, individual protein concentrations of the whey samples were determined. Whitney *et al.* (1976) had reported that the major whey proteins of cow milk were bovine serum albumin, ß-lactoglobulin and α-lactalbumin.

Benedet and Park (1982) subjected enzyme-treated casein fractions to sodium dodecyl sulfate polyacrylamide gel electrophoresis and the electrophoretic patterns were compared with those of the untreated casein fractions. The treated casein fractions showed a marked decrease in the intensity of the major α-S casein band. Gel filtration chromatography could be used for the rapid separation of whey proteins and other substances of molecular weight in the range of 10^2 to 10^6 daltons (Shimazaki and Sukegawa, 1982). Ng-Kwai-Hang and Kroeker (1984) evolved a rapid polyacrylamide gel electrophoretic method for the separation and quantification of major proteins in casein and whey fractions of bovine milk. Deshmukh *et al.* (1989) compared cellulose acetate electrophoresis (CAE) and polyacrylamide gel electrophoresis (PAGE) for the fractionation and quantification of milk protein fractions and confirmed that PAGE was generally precise for all protein fractions. Electrophoretic screening of milk samples on major casein and whey protein moieties revealed the impact of milk protein polymorphism on the incidence of mastitis in cattle (Jairam *et al.*, 1994).

Antibiogram of the various pathogens from mastitic milk

In a study of the antibiogram of Streptococcus isolated from bovine intramammary infection, it was found that the most sensitive antibiotics were carbencillin, cephalothrin, chloramphenicol, erythromycin and penicillin

(McDonald *et al.*, 1976). Gupta *et al.* (1979) conducted *in vitro* sensitivity studies of Staphylococcus species isolated from mastitic milk and milk products and found that nitrofurantoin inhibited 99.36 per cent and chloramphenicol, tetracyclines and streptomycin inhibited 97.35 per cent, 96.81 per cent and 94.9 per cent respectively. Sulphathiazole was found to be least effective. Kapur *et al.* (1979) found out that 98 per cent of the *Staphylococcus aureus* isolated from bovine mammary gland were sensitive to gentamycin, spiramycin, erythromycin, cephaloridine, cloxacillin, chloramphenicol, furadantoin and neomycin, whereas 95 to 80 per cent were sensitive to penicillin and tetracyclines. 74.3 per cent of the *Staphylococcus aureus* were sensitive to chloramphenicol. Tetracyclines, streptomycin, erythromycin and penicillin were effective to 52.9 per cent, 55.7 per cent, 54.8 per cent and 48.6 per cent of the isolates respectively (Kalorey *et al.*, 1983). Rahman and Baxi (1983) reported that Staphylococci were highly sensitive to neomycin (98.91%), followed by nitrofurantoin (90.81%) and chloramphenicol (86.73%). Penicillin was found to be least effective. Studies conducted by Sudharma *et al.* (1985) showed that Staphylococci were highly sensitive to gentamycin, followed by chloramphenicol, neomycin and tetracyclines. Coliforms were found to be sensitive to neomycin, gentamycin, chloramphenicol and tetracyclines. Streptococcal isolates were sensitive to ampicillin,

chloramphenicol, penicillin, gentamycin and neomycin. Antibiogram pattern of the isolates for a ten year period from mastitic milk samples revealed that 90.08 per cent and 85.38 per cent of the 595 isolates tested were sensitive to gentamycin and chloramphenicol respectively (Char *et al.* 1993).

The most effective drugs for bovine mastitis were lincomycin and clindamycin (98.62%). Tetracyclines showed moderately higher efficacy (92.41%) and the least effective drugs were penicillin, ampicillin, streptomycin and furazolidone (Saxena *et al.*, 1993a). Antibiogram studies on subclinical mastitis cases from machine milked dairy farms revealed that gentamycin was the most effective (93.08%) and penicillin was the least effective (52.31%) (Singh *et al.*, 1994b). Bhattacharya and Rahman (1995) studied the antibiogram of various pathogens isolated from clinical cases of mastitis. Chloramphenicol and neomycin were found to be most effective, followed by erythromycin, co-trimoxazole, oxytetracycline and chlortetracycline penicillin and streptomycin were least effective.

Reddy *et al.* (1995) studied the *in vitro* sensitivity of *E. Coli* to quinolone antibiotics like naldixic acid, pefloxacin and flumequin and reported that pefloxacin was the most effective and economical drug. In a study on subclinical mastitis in an organised buffalo farm,

Mitra *et al.* (1995) reported that most of the isolates were sensitive to ciprofloxacin (86.2%), followed by co-trimoxazole (82.75%), lincomycin (68.96%), chloramphenicol (65.51%), norfloxacin (62.06%) and gentamycin (58.62%). None of the isolates were sensitive to tetracycline, neomycin, erythromycin and streptomycin. Pal *et al.* (1995) used tri-sodium citrate alone and in combination with a suitable antibiotic in the management of clinical mastitis in dairy cows and the latter was found to be more effective. Mastitis in lactating buffaloe could be treated effectively with ampicillin-cloxacillin combination (Uppal *et al.*, 1995). Watts *et al.* (1995) studied the minimum inhibitory concentrations of antibiotics against the organisms isolated from the mammary glands of dairy heifers.

Biju (1996) found out that pefloxacin was the most effective and penicillin was the least effective in clinical and subclinical cases of bovine mastitis. Maiti *et al.* (1996) reported that enrofloxacin was highly effective in the treatment of bovine mastitis. Ratnakumar *et al.* (1996) reported that 95.83 per cent of the isolates were sensitive to chloramphenicol (66.66%) and gentamycin (50%) respectively in the decreasing order of merit. Umakanthan *et al.* (1996) conducted field trials for assessing the efficacy of certain drugs in bovine mastitis. When streptopenicillin-metronidazole combination, tobramycin

sulphate and cefotoxime were used, the recovery rates after two days treatment were 93 per cent, 80 per cent and 94 per cent respectively. Wadhwa *et al.* (1996) reported that the overall effective drugs against the organisms causing mastitis were cephaloridine (91%), gentamycin (88%), chloramphenicol (87%), nitrofurantoin (77.92%), tetracyclines (75.32%), ampicillin (64.94%), penicillin (50.65%) and streptomycin (33.76%). Mallikarjunaswamy and Krishnamurthy (1997) studied the antibiogram of bacterial pathogens isolated from cases of bovine subclinical mastitis. Staphylococci were highly sensitive to chloramphenicol, gentamycin, tetracycline, co-trimoxazole, ceftrioxime and cloxacillin. Streptococci were susceptible to chloramphenicol. Majority of the other isolates were sensitive to chloramphenicol and resistant to streptomycin, neomycin and penicillin group of antibiotics. Antibiotic sensitivity tests revealed that *Staphylococcus aureus*, non-haemolytic Streptococci and Coliform bacilli were sensitive to ceftriaxone (Umakanthan, 1997a). Umakanthan (1997b) reported that peracute mastitis in cows caused by *Staphylococcus aureus* could be treated with intramuscular injections of cefaperazone sodium.

Pathology of mastitis

The pathological changes in milk and mammary gland affected with mastitis have been studied in detail.

Histological changes in mastitic udders included interstitial oedema, vacuolation and desquamation of acinar epithelium and accumulation of fibroblasts and macrophages in the interstitium (Plastridge, 1958). Yamagiwa *et al.* (1963) classified the histopathological lesions of mastitis in slaughtered cows as tubular, diffuse and alveolar changes. In *E. coli* mastitis, the preliminary change was an increase in the vascular permeability. Later the alveoli were filled with inflammatory products and there was necrosis of the interstitial tissue (Heidrich and Renk 1967).

In Staphylococcal mastitis, signs of acute catarrh and aggregation of Staphylococci in the lactiferous ducts and secretory end pieces were noticed (Heidrich and Renk, 1967). Histopathological studies on mammary glands revealed regressive changes in the alveoli and milk ducts and infiltration of inflammatory cells in the inter alveolar areas (Lee and Frost, 1970). In mastitis caused by *Streptococcus agalactiae*, clots and flakes consisting of fibrin, leucocytes and bacteria formed plugs in the smaller ducts leading to involution of the secretory tissue. Increase in the connective tissue stroma resulted in increased firmness of the gland. Studies revealed that the alveolar and ductal epithelium showed vacuolation and accumulation of neutrophils in the interstitial tissue, milk ducts and alveoli. Pathologic effects of

Staphylococcus aureus strains varied from mild parenchymatous inflammation to acute gangrenous mastitis (Schalm *et al.*, 1971).

Schalm (1977) revealed that stromal tissue proliferation leading to fibrosis was the characteristic feature of Streptococcal mastitis. The Coliform sub-committee of the National Mastitis Council, USA (1979) reported that the inflammatory reaction in coliform mastitis was predominantly serous with oedema or haemorrhage. Alveoli were filled with serous fluid and desquamated epithelial cells. Hill *et al.* (1979) reported that in *E. coli* mastitis in newly calved cows, the impaired chemotaxis of neutrophils was associated with parturition or the stress of early lactation. Intramammary inoculation of *E. coli* into the teat cistern revealed that early stimulation of a sterile inflammation by the diffusion of the endotoxin through the streak canal rendered many mammary quarters refractory to subsequent microbial invasion (Schultze and Thompson, 1980). Nickerson and Heald (1982) studied the cells in local reaction to experimental *Staphylococcus aureus* infection in bovine mammary gland. Quantitative cytology demonstrated more number of lymphocytes, plasma cells, monocytes, macrophages and neutrophils in the infected quarters than in normal quarters. Dodd (1983) reported that the primary site of *Streptococcus agalactiae* was the infected udder quarters,

but teat cisterns and teat ducts could also be colonised. Corynebacterium mastitis was distinctly suppurative and occasionally there was extensive necrosis and sloughing of udder tissue. In long standing cases of mastitis, the firmness was the result of diffuse fibrous tissue proliferation that accompanied the chronic atrophying process (Jones and Hunt, 1983). Gudding *et al.* (1984) studied the histopathological changes of mammary glands taken at different post inoculation hours (PIH) of *Staphylococcus aureus*. At PIH-18, there was hydropic and vacuolar degeneration of the ductal epithelium with erosions and ulcers throughout the ductal system. At PIH 72 and 96, capillary and venuolar endothelium were surrounded by neutrophils and monocytes. Random foci of epithelial ulceration and extravasated erythrocytes and fibrin were present in the teat sinus and lactiferous ducts. Haraldson and Johnson (1984) reported that non-reactive necrosis and lesions induced by vascular reactions were the main features of experimentally induced *Staphylococcus aureus* mastitis in rats.

Nickerson and Pankey (1984) studied the neutrophil migration in bovine mammary quarters challenged with *Staphylococcus aureus*. The modes of migration of neutrophils across the luminal cells into the milk were by projection into degenerated luminal cells, penetration between intact epithelia and passage into milk along with

the desquamated luminal cells. The physiological and pathological factors influencing immunoglobulin G_2 concentration in milk was studied by Caffin and Poutrel (1988). They found that quarter infection with *Corynebacterium bovis* and *Staphylococcus aureus* increased immunoglobulin G_2 concentration in milk. Bacteriological and histopathological studies on mammary glands of slaughtered cows revealed fibrosis with corpora amylaceae, cystic dilatation of lactiferous ducts, serous exudation and desquamation of epithelial cells. Some glands revealed interstitial thickening and infiltration of mononuclear cells (Handique *et al.*, 1988).

Secondary challenges after a previous intramammary infection with *Streptococcus uberis* showed a significant reduction in infection, because the immunoglobulin G_2 antibodies specific to the bacterial surface components increased and exerted a protective effect against subsequent infection (Hill, 1988). Hurley (1989) reported that lactoferrin, hydrolytic enzymes, immunoglobulins and serum derived components increased in concentration during mammary gland involution. The increment of these factors reflect alterations in the function of alveolar epithelial cells resulting in increased disease resistance of the gland during involution. Histological studies of uninfected and infected mammary glands of cows revealed macrophages and lymphocytes as the predominant cells, followed by plasma cells, neutrophils and mastcells

(Nickerson, 1989). The most prevalent cell types within the epithelial lining and subepithelial stroma were macrophages and lymphocytes, whereas macrophages and neutrophils were the most prevalent cells in the alveolar lumen in cases of *Staphylococcus aureus* mastitis during lactogenesis (Sordillo *et al.*, 1989). Similar observations were made by Trinidad *et al.* (1990). In *E. coli* mastitis, the acini contained vacuolated desquamated epithelial cells, duct linings were destroyed and the cistern contained fibrin and coagulated casein. Haemorrhage and necrosis of the parenchyma were found in severe cases (Jones, 1990). Kennedy and Miller (1993) reported that in acute staphylococcal mastitis, the udder was tense, firm, hot and swollen with small quantity of blood stained serous fluid.

Nickerson (1993) reported that cells of the alveoli and ducts degenerated and sloughed from the cistern lining which along with the somatic cells occluded the milk ducts in Staphylococcal mastitis. Thomas *et al.* (1994) studied the pathological changes in experimentally induced *Streptococcus uberis* infection in the mammary gland of cows. The reaction in the glandular tissue and intralobular milk ducts was characterised by an acute inflammatory response. Secretory acini were filled with cellular exudate and cytoplasmic vacuolation of the secretory cells were noticed.

Histological changes in the regional lymphnodes after stimulation with conventional antigens were studied by Leduc *et al.* (1955). The lymphnodes were enlarged, containing many lymphoid follicles with active germinal centres. In Coliform mastitis, the supramammary lymphnodes draining the affected quarters were enlarged, oedematous and necrotic foci could be observed in the lymphnodes (Coliform Subcommittee of the National Mastitis Council, 1979). Nickerson (1989) reported that plant lectins, concanavalin-A and phytohemagglutinins could be used to induce proliferation of the protective tissue lymphoid cell population, thereby increasing the disease resistance of the gland. Thomas *et al.* (1994) observed the pathological changes of experimentally induced *Streptococcus uberis* infection in cows. The supramammary lymphnode of the affected side had intense follicular activity with accumulation of large number of leucocytes in the sinuses.

Materials and Methods

MATERIALS AND METHODS

3.1 Evaluation of milk samples

3.1.1 Collection of milk samples

Milk samples were collected from cows belonging to the University Livestock Farm, Mannuthy, Cattle Breeding Farm, Thumburmuzhi, Livestock Research Station, Thiruvazhamkunnu, District Livestock Farm, Kodapanakunnu and also from clinical cases in veterinary hospitals, Mannuthy and Kokkalai, Artificial insemination centres, Mannuthy and Kokkalai, veterinary dispensaries, Tarur, Vandazhi and Valoor and District Veterinary Centres, Ernakulam and Palakkad. Samples from distant places were transported in ice.

A total of 1031 samples were collected aseptically in sterile plastic vials for *in vitro* culture and sensitivity tests. Out of the 1031 milk samples, 489 samples were clinically positive and 542 milk samples were subjected to California mastitis test for the detection of subclinical mastitis. Somatic cell counts of a representative portion of the milk samples (108 samples which includes both healthy and mastitic) were carried out.

3.1.2 Detection of subclinical mastitis

A total of 542 milk samples were subjected to California mastitis test. The reagent was prepared and the test was conducted as per the procedure described by Schalm *et al.* (1971).

The results were interpreted as:

Negative	(-)	No precipitate
Doubtful	(±)	Distinct precipitate with a tendency to disappear
Slightly positive	(+)	Distinct precipitate that does not disappear
Positive	(++)	Thick mixture with precipitation and gelatinisation
Strongly positive	(+++)	Distinct gel formation which tend to adhere to the bottom with a central peak on mixing

3.1.3 Somatic cell count of milk

Total cell counts of 108 milk samples were studied. The milk smear was prepared as per the method of Prescott and Breed (1910).

The stain used was modified Broadhurst-Paley stain. The staining procedure was as per the method of Schalm *et al.* (1971).

The stained smears were then subjected to microscopic examination under a calibrated microscope.

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

3.1.4 Cultural examination of milk samples

The aseptically collected milk samples were streaked in Tryptone soy agar (TSA)-Himedia for the primary isolation of bacterial organisms. The TSA plates were incubated at 37°C for 24 hours and the colonies were identified by their morphology and biochemical characteristics (Cruickshank *et al.*, 1965). The plates which failed to show growth were again incubated upto 48 hrs to detect the presence of slow growing organisms.

3.1.4.1 Identification of the organisms

Smears were made from the colony, air dried, fixed in heat and stained with Gram's stain. Gram positive organisms were stained dark purple (Cocci and bacilli) and gram negative organisms were stained light pink (coccobacilli) (Cruickshank *et al.*, 1965).

Staphylococcus species appeared as gram positive cocci in pairs or clusters. Growth in mannitol salt agar (Hi-media), positive Voges-Proskauer test (VP), positive nitrate test and positive urease test were the confirmatory characters (Cowan, 1974).

Streptococcus was seen as gram positive cocci in pairs or chains and growth on streptococcus selection agar (Hi-media) was confirmatory (Cowan, 1974).

E. coli was confirmed by the growth of characteristic pink colonies in MacConkey agar (Hi-media) and a metallic sheen in Eosin-Methylene blue agar (Hi-media) (Cowan, 1974).

3.1.4.2 Culture for Mycoplasma

The culture of Mycoplasma was done as per the method of Chanock *et al.* (1962). The milk samples from clinical cases were streaked in PPLO agar plates and incubated at 37°C in an anaerobic jar. Examined the plates daily for one week under an inverted tissue culture microscope.

3.1.4.3 Antimicrobial sensitivity tests

The bacterial colonies after identification with Gram's reaction and other biochemical tests were subjected to antibiotic sensitivity tests (Bauer *et al.*, 1966). The colony was inoculated in peptone water and then swabbed on Mueller-Hinton agar plates.

The following antibiotic discs were used:

1. Pefloxacin - 5 mcg
2. Streptomycin - 10 mcg
3. Oxytetracycline - 30 mcg
4. Co-trimoxazole - 25 mcg
5. Gentamycin - 10 mcg
6. Chloramphenicol - 30 mcg
7. Penicillin - 10 units
8. Ampicillin - 10 mcg

After 24 hrs of incubation at 37°C, the results were read by noting the zone of inhibition around each disc and comparing it with the antibiotic disc sensitivity chart (Hi-media)

3.2 Haematological evaluation

Whole blood was collected from 24 animals (both healthy and mastitic) in glass vials with EDTA (1 mg/10ml) as the anti-coagulant. Five millilitre of blood was collected from each animal.

3.2.1 Total and differential leucocyte count

The total leucocyte count and differential leucocyte counts were determined according to the method described by Schalm (1965).

3.2.2 Determination of total protein in plasma

The Biuret assay method of Inchiosa (1964) was adopted for the estimation of total protein content in blood plasma.

3.2.3 Enumeration of acid alpha-naphthylacetate esterase positive cells in peripheral blood

Blood smears were prepared from the peripheral blood of healthy and mastitic (clinical and subclinical) animals. A total of 18 blood smears were examined for the enumeration of ANAE positive cells. The smears were fixed in fixative prepared as per the procedure of Reddy *et al.* (1980). Fixed smears were labelled and stored at room temperature to facilitate batch staining. The reaction mixture was prepared as per the procedure of Knowles *et al.* (1979). The stained slides were examined under the oil immersion of a microscope.

One or two localised nodular pink to red coloured reaction product could be noticed in the cytoplasm adjacent to the cell membrane. Some cells had more than two scattered punctate nodular reaction product of small size. Both type of cells were identified as T-lymphocytes (Knowles *et al.* 1978).

3.3 Sodium dodecyl sulfate polyacrylamide gel electro-phoresis (SDS-PAGE) on whey proteins

Milk samples from three mastitic animals and one healthy animal were used. The whey samples were prepared as per the procedure described by Ng-Kwai-Hang and Kroeker (1984). The supernatant whey, after the precipitation of casein was removed filtered and stored at -4°C.

3.3.1 Stock reagent preparation for SDS-PAGE

1. Acrylamide/Bis (30 per cent T, 2.67 per cent C)

Acrylamide	-	29.2 g
N-N' methylene-bis-acrylamide	-	0.8 g
Distilled water	-	100 ml

The solution was filtered through Whatman No. 1 filter paper and stored at 4°C in a dark glass bottle.

2. Tris-Hcl 1.5 M pH 8.8

Tris base	-	18.15 g
Distilled water	-	50 ml

Adjusted to pH 8.8 with 1 N Hcl, made upto 100 ml.

3. Tris Hcl 0.5 M pH 6.8

Trisbase	-	6 g
Distilled water	-	6.0 ml

Adjusted pH to 6.8 with 1 N Hcl, made up to 100 ml.

4. SDS - 10 per cent solution

5. Ammonium per sulphate (APS) - 10 per cent solution - prepared fresh just before use.

6. Sample buffer

Distilled water	-	20 ml
Tris Hcl pH 6.8	-	5 ml
Glycerol	-	4 ml
SDS (10 per cent)	-	8 ml
2-mercaptoethanol	-	2 ml
Bromophenol blue (0.05 per cent)	-	1 ml
		40 ml

7. Electrode buffer (stock 5 x)

Trisbase	-	45 g
Glycine	-	216 g
SDS	-	15 ml

Dilute 450 ml of stock with 1.8 litres of distilled water.

3.3.2 Formation of SDS resolving gel (12 per cent)

Acrylamide/Bis	-	16 ml
Distilled water	-	13.4 ml
Tris Hcl pH 8.8	-	10 ml
SDS (10 per cent)	-	400 µl
APS (10 per cent)	-	200 µl
TEMED	-	20 µl
		40 ml

3.3.3 Formation of SDS stacking gel (4 per cent)

Acrylamide/Bis	-	1.3 ml
Distilled water	-	6.1 ml
Tris Hcl pH 6.8	-	2.5 ml
SDS (10 per cent)	-	100 µl
APS (10 per cent)	-	50 µl
TEMED	-	10 µl
		10 ml

Fixing solution

Methanol	-	400 ml
Distilled water	-	500 ml
Glacial acetic acid	-	100 ml

Staining solution

Coomassie Brilliant Blue R250	-	1g
Fixing solution	-	1 litre

Destaining solution

Methanol	-	100 ml
Distilled water	-	825 ml
Glacial acetic acid	-	75 ml

The SDS - polyacrylamide homogenous vertical slab gels were prepared as per Laemmli (1970).

3.3.4 Preparation of sample

The total protein content of the whey sample from normal and mastitic milk was estimated by Biuret method. The whey samples were treated with urea at the rate of 0.4 g/ml. Since the whey protein content of mastitic and normal milk was 4.3 g/dl and 2.07 g/dl respectively, 100 μl of the former was diluted with 500 μl of the sample buffer and 100 μl of the latter diluted with 200 μl of the sample buffer, so as to give a protein content of 120-150 mg/20μl in both samples. 10 μl of the protein molecular weight marker-medium range (Hi-media) was mixed with the same volume of sample buffer.

3.3.5 Sample loading and electrophoresis

The comb was removed from the sandwich assembly and 20 μl of the sample mixed with the buffer was loaded into each well in duplicate leaving blanks in between the wells. Molecular weight marker (M-range) was also charged into one of the wells.

The electrophoretic tank and buffer dam was filled with the diluted electrode buffer. The power pack (Bio-rad, USA) was set to deliver a constant current of 25 mA, voltage 250 V, power 250 W and time 4 hrs. After 10 min. the current was increased to 35 mA without any change in the other parameters. Electrophoresis was stopped when the tracking dye reached the bottom of the gel.

The gel was then taken out and transferred to the fixing solution for 30 minutes, then to staining solution for 2 hrs. Excess stain was removed by placing the gel in destaining solution overnight and finally 2-3 changes of this solution at half hour intervals. The stained gel was then preserved in 7 per cent acetic acid in self locking polythene bags.

The gel was kept on a transilluminator and the position and thickness of the bands were traced in a transparency sheet. The molecular weights of the protein marker was plotted against the distance migrated in centimetres. From this the molecular weights of whey proteins were calculated as per the method of Shapiro *et al.* (1967).

3.4 Histopathology of mammary gland and supramammary lymph nodes

The udder tissues were collected from the municipal slaughter house, Thrissur and from animals brought for post mortem to the Department of Pathology. A total of 205 udder tissues from 56 animals and 20 supramammary lymph nodes were examined.

The udder tissues and the lymphnodes were examined in detail by dissection and the gross changes were noted.

Appropriate samples of tissues were collected in 10 per cent neutral buffered formalin for histopathological examination. Tissues were processed by routine paraffin embedding techniques (Armed Forces Institute of Pathology, 1968). Paraffin sections cut at five to six microns thickness were stained routinely with Haematoxylin and Eosin (H&E) method of Haris as described by Disbrey and Rack (1970).

Results

RESULTS

Subclinical mastitis

A total of 542 samples of milk from various quarters of 145 animals were examined for the detection of subclinical mastitis using California mastitis test. Two hundred and eighty samples (51.6 per cent) were found to be positive and 262 samples (48.3%) were negative. Depending on the intensity of the reaction, the samples were grouped as doubtful (±) - 62(22.14%), slightly positive (+) - 102 (36.42%), positive (++) - 97 (34.64%) and strongly positive (+++) - 19 (6.78%) which is diagrammatically represented in Fig.1.

The quarterwise distribution of subclinical mastitis in the 280 CMT positive samples are charted in Fig.2.

Somatic cell count in milk

One hundred and eight milk samples were collected for the assessment of total cell count in milk, which included 36 samples each from healthy animals, subclinical cases and clinical cases. The results are tabulated in Table 1. The appearance of somatic cells in milk smears stained with Broadhurst-Paley stain is shown in Plate 1.

Cultural examination of milk samples

A total of 1031 quarter milk samples which included 489 clinical cases and 542 subclinical cases were subjected to cultural examination to detect the various bacterial organisms. Out of the 489 clinical cases, 254 samples (51.9%) and 235 samples (43.35%) of the 542 subclinical cases were positive. The bacterial organisms isolated from mastitic milk are shown in Fig.3. Staphylococcus could be isolated from 158 samples (32.31%), *E. coli* from 92 (18.81%), gram negative bacilli from 87 (17.79%), mixed infections from 76 (15.54%), Streptococcus from 48 (9.87%), Yeast from 15 (3.07%) and grampositive bacilli from 13 samples (2.65%). Colonies of Staphylococcus grown on Mannitol salt agar (MSA) is shown in Plate 2. Streptococcus was confirmed by the growth in Streptococcus selection agar (SSA) as shown in Plate 3. *E. coli* appeared as characteristic pink colonies on MacConkey agar (Plate 4) and in Eosin-Methyleneblue agar (EMB agar) they could be seen as colonies with a metallic sheen (Plate 5). Culture for Mycoplasma under anaerobic conditions did not reveal any growth.

Antibiogram of the bacteria isolated from mastitic milk

The *in vitro* sensitivity results of the isolates to various antibiotics are represented in Table 2.

Staphylococcus, the predominant organism was sensitive to pefloxacin (97.4%), gentamycin (97.47%), chloramphenicol (87.34%), oxytetracycline (86.08%), co-trimoxazole (77.92%), streptomycin (76.58%), penicillin (15.19%) and ampicillin (13.29%).

Streptococci were found sensitive to pefloxacin (100%), chloramphenicol (100%), co-trimoxazole (100%), gentamycin (89.5%), oxytetracycline (70.83%), penicillin (64.58%), streptomycin (62.5%) and ampicillin (43.75%).

E. coli showed sensitivity to pefloxacin and gentamycin (97.83%), oxytetracycline (76.09%), chloramphenicol (65.21%), streptomycin (52.15%) and co-trimoxazole (50%).

All gram positive bacilli (GPB) were sensitive to pefloxacin, streptomycin, gentamycin, co-trimoxazole, chloramphenicol (100%), oxytetracycline (69.23%), penicillin and ampicillin (15.38%).

Gram negative bacilli (GNB) showed sensitivity to pefloxacin (100%), gentamycin (77.01%), chloramphenicol (66.67%), oxytetracycline (63.22%), cotrimoxazole (25.29%) and streptomycin (24.13%).

The sensitivity pattern in mixed infections showed varying results as represented in Table 3.

Haematological evaluation

Twenty four blood samples collected from healthy and mastitic (clinical and subclinical) animals were subjected to total and differential leucocyte counts. The leukogram is presented in Table 4.

Total leucocyte count

Mean leucocyte count of healthy animals was 9818±708 cells per ml of blood. In clinical cases of mastitis a slight decrease in leucocyte count was observed with a mean of 8331±652 cells per ml of blood. Pronounced leucopenia was noticed in subclinical cases with a mean leucocyte count of 4978±479 cells per ml of blood.

Differential count

Neutrophils were more in case of clinical and subclinical cases of mastitis when compared to healthy animals. Lymphocyte, monocyte, basophil and eosinophil count was normal in both clinical and subclinical cases (Table 4).

Total protein in plasma

In healthy animals, the total plasma protein showed a mean value of 8.15±0.69 gm%. Mean values in clinical cases were slightly higher 9.09±0.55 gm%. But subclinical cases showed a marked increase in plasma protein with a mean value of 10.56±0.97 gm%. The plasma protein values are shown in Table 4.

T-lymphocyte count

The number and percentage of ANAE positive cells in peripheral blood smear is charted in Table 5. Mean percentage of ANAE positive cells in normal healthy animals was 33.17 ± 3.27. But the percentage of ANAE positive cells in clinical and subclinical cases of mastitis were 38.08±2.39 and 30.42±1.49 respectively. ANAE positive cells appeared in the blood smear as shown in Plate 6.

SDS-PAGE on whey proteins

SDS-PAGE on whey samples of mastitis affected animals and healthy animals is shown in Plate 7. The electrophoretic pattern of the whey proteins in clinical (Group I) subclinical + (Group II) subclinical +++ (Group III) cases of mastitis and healthy animals (Group IV) is diagrammatically represented in Fig.4. The electrophoretic fractionation of whey proteins of clinical cases (Group I) revealed fifteen fractions comprising of five major bands of molecular weights 161 Kilo Dalton (Kd), 138 Kd, 84 Kd, 63 Kd and 54 Kd and ten minor bands of molecular weights 196 Kd, 193 Kd, 188.5 Kd, 184 Kd, 177 Kd, 171.5 Kd, 148 Kd, 126 Kd, 115 Kd and 71 Kd (Fig.5). Mild subclinical cases showing + reaction on California mastitis test (Group II) revealed three major bands of molecular weights 158 Kd, 82 Kd and 54 Kd and four minor bands of molecular weights 189 Kd, 171 Kd, 136 Kd and 62 Kd (Fig.6). Severe subclinical cases showing +++ reaction on CMT (Group III) showed four major bands of molecular weights 160 Kd, 84 Kd, 52 Kd and

38 Kd and seven minor bands of molecular weights 189 Kd, 175 Kd, 144 Kd, 140 Kd, 124 Kd, 95 Kd and 62 Kd (Fig.7). Whey samples of healthy animals (Group IV) contained five major bands of molecular weights 177 Kd, 168 Kd, 145 Kd, 51 Kd and 30 Kd and one minor band of 86 Kd (Fig.8).

Examination of mammary gland

A total of 205 quarters were examined for gross lesions. Thirty two quarters were visibly enlarged and revealed hyperaemic areas on dissection. On palpation, 14 quarters were found to be hard and on section, fibrosis was noticed. All the other glands were more or less normal in size and consistency.

Out of the 205 quarters, 114 quarters were lactating and 91 quarters were in varying stages of involution.

Histological changes in lactating glands

Fifty one quarters (44.7%) revealed inflammatory cells in the acini and interstitium. The inflammatory cells included polymorphs and mononuclears which were seen as masses in the dilated acini or scattered in the interstitium (Plate 8). Galactophoritis could be observed in seventeen (14.8%) quarters. There was desquamation of the epithelium lining the cistern and the lactiferous ducts and focal leucocytic infiltration could be seen in the sub epithelial areas of the milk ducts (Plate 9).

Five quarters revealed mild catarrhal inflammation characterised by mucus exudation, cellular infiltration and thickening of the interalveolar tissue (Plate 10). The alveolar and tubular secretory end pieces were of variable size with cuboidal epithelium. Acute mastitis characterised by intense infiltration of polymorphs and mononuclears in the dilated acini and widened interstitium could be observed in few cases. The other features were dilatation of the milk ducts with thickening of the interstitial connective tissue. Interstitial mastitis with diffuse interalveolar cell proliferation was observed in eight quarters (Plate 11). There was focal lymphoid cell accumulation in a few cases. Eleven quarters (9.65%) revealed irregularly dilated acini which contained lacteal secretions and widened milk ducts which contained a protein rich fluid with a few inflammatory cells (Plate 12).

Chronic changes were also observed in 35 quarters. The lesions were characterised by the presence of inflammatory cells, predominantly mononuclears in the acini and the interstitium. Cytoplasmic vacuolation of the secretory epithelium of the acini and focal leucocytic infiltration was observed in few cases (Plate 13). Degeneration and denudation of the secretory epithelium was found in severe cases. Polypoid thickening of the cisternal epithelium with desquamation of the epithelial cells and lymphocytic infiltration of the detached propria was observed in a few cases (Plate 14).

Histological changes in involuting glands

The glandular parenchyma was reduced and there was thickening of the interstitial connective tissue in healthy animals. Animals in late lactation revealed characteristic lesions. Widening of the interstitial septa and numerical reduction of the glandular lobules with non-secretory end pieces could be noticed in some quarters, Chronic galactophoritis and extensive involution of the glandular tissue with broadening of the interstitium and focal accumulation of inflammatory cells was observed in 9.89 per cent of the quarters in the involution stage (Plate 15). Cellular thickening of the interlobular supporting tissue between the non-secretory end pieces was observed in a few cases (Plate 16). Certain quarters revealed markedly dilated non-secretory end pieces and periductal fibrosis (Plate 17). In old animals, excessive fibrous tissue proliferation could be observed in the interacinar and interlobular areas with focal accumulation of polymorphs in these areas. Majority of the glands revealed the presence of mononuclears in the interstitium. Some glands revealed polymorphs in the acini and mononuclears in the interstitium.

Corpora amylacea could be observed in majority of the lactating and non-lactating glands. They were found in both the alveoli and interstitium, but mostly in the alveoli. They appeared as spherical lamellated bodies staining pink with haematoxylin and eosin (Plate 18). In

some cases, calcified corpora amylaceae appeared as round bodies with dark blue periphery and a light blue or pink centre.

Examination of supramammary lymphnodes

Lymphnodes of 20 animals were examined for gross abnormalities. The lymphnodes were slightly enlarged and soft in consistency. However, no lesions were seen on dissection.

Histological changes

The cortex revealed lymphoid follicles in various stages of activity. There was marked distension of the subcapsular sinuses with depletion of lymphocytes in the paracortex and proliferation of sinus histiocytes (Plate 19). Active secondary follicles which consisted of germinal centres having a dense zone packed with lymphoblasts and an apical zone with a few lymphocytes giving a starry-sky effect could be observed in majority of the lymphnodes (Plate 20).

In most cases, sinus catarrh characterised by the widening of the medullary sinuses with abundance of lymphocytes and lymphoblasts was noticed (Plate 21). Plasma cells could be seen in varying numbers in the medulla of the lymphnode. Medullary hyperplasia, as evidenced by sheets of lymphoblasts and congestion of the medullary blood vessels could be observed in a few lymphnodes (Plate 22).

Table 1 Results of somatic cell count

Group	No. of samples	Range	Mean
Clinical	36	12,00,000 - 21,28,000	17,12,000 ± 2,55,058
Subclinical	36	1,02,200 - 9,78,000	5,74,316 ± 2,04,224
Healthy	36	1,48,000 - 3,24,000	2,10,000 ± 44,931

Table 2 **Antibiogram of pathogens isolated from mastitic milk**

Organism	No. of isolates tested	Sensitive								Resistant							
		Pf	S	O	Co	G	C	P	A	Pf	S	O	Co	G	C	P	A
Staphylo cocci	158	154 97.47	121 76.58	136 86.08	120 77.92	154 97.47	138 87.34	24 15.19	21 13.29	4 2.53	37 23.41	22 13.92	38 24.05	4 2.53	20 12.66	134 84.81	137 86.71
Strepto cocci	48	48 100	30 62.5	34 70.83	48 100	43 89.5	48 100	31 64.58	21 43.75	- Nil	18 37.5	14 29.17	- Nil	5 10.43	- Nil	17 35.42	27 56.25
E. coli	92	90 97.83	48 52.15	70 76.09	46 50	90 97.83	60 65.21	- Nil	- Nil	2 2.17	44 47.83	22 23.91	46 58	2 2.17	32 34.78	92 100	92 100
GPB	13	13 100	13 100	9 69.23	13 100	13 100	13 100	2 15.38	2 15.38	- Nil	- Nil	4 30.77	- Nil	- Nil	- Nil	11 84.62	11 84.62
GNB	87	87 100	21 24.13	55 63.22	22 25.29	67 77.01	58 66.67	- Nil	- Nil	- Nil	66 95.86	32 36.78	65 74.71	20 22.99	29 33.33	87 100	87 100
Mixed infection	70	54 77.1	16 22.86	57 81.43	34 48.57	61 87.14	54 77.14	3 4.29	4 5.71	16 22.9	54 77.14	13 18.57	36 51.43	9 12.86	16 22.86	67 95.71	66 94.29

Pf - Pefloxacin
S - Streptomycin
O - Oxytetracycline
Co - Co-trimoxazole
G - Gentamycin
C - Chloramphenicol
P - Penicillin
A - Ampicillin

Table 3 Sensitivity results of mixed infections

Organism	No. of isolates tested	Sensitive								Resistant							
		Pf	S	O	Co	G	C	P	A	Pf	S	O	Co	G	C	P	A
GNB GNCB	16	16 100	8 50	13 81.25	9 56.25	10 62.5	12 75	- Nil	- Nil	- Nil	8 50	3 18.75	7 43.75	6 37.5	4 25	16 100	16 100
Staph Strept	2	2 100	- Nil	2 100	- Nil	2 100	2 100	- Nil	- Nil	- Nil	2 100	- Nil	2 100	- Nil	- Nil	2 100	2 100
Strept GNB	7	7 100	- Nil	7 100	3 42.8	7 100	4 57.1	- Nil	- Nil	- Nil	7 100	- Nil	4 57.1	- Nil	3 42.8	7 100	7 100
GNB,GPC, GPB	3	3 100	- Nil	3 100	- Nil	3 100	3 100	- Nil	- Nil	- Nil	3 100	- Nil	3 100	- Nil	- Nil	3 100	3 100
Staph GNCB	6	6 100	3 50	4 66.6	5 83.3	6 100	4 66.6	1 16.6	1 16.6	1 16.6	3 50	2 33.3	1 16.6	- Nil	2 33.3	5 83.3	5 85.3
Staph GPB	5	5 100	4 80	2 40	2 40	5 100	5 100	- Nil	- Nil	- Nil	1 20	3 60	3 60	- Nil	- Nil	5 100	5 100
GNB Staph	27	26	- Nil	24 88.8	13 48.1	26 96.2	20 74	- Nil	- Nil	- Nil	27 100	3 11.1	14 51.8	1 3.7	7 25.9	27 100	27 100
GPB GNB	3	3 100	- Nil	2 66.6	2 66.6	2 66.6	3 100	- Nil	- Nil	- Nil	3	1 33.3	1 33.3	1 33.3	- Nil	3 100	3 100
GPB Strept	1	1 100	- Nil	- Nil	- Nil	- Nil	- Nil	1 100	1 100	1 100	1 100	1 100	1 100	1 100	1 100	- Nil	- Nil

GNB - Gram negative bacilli GNCB - Gram negative coccobacilli GPC - Gram positive cocci GPB - Gram positive bacilli

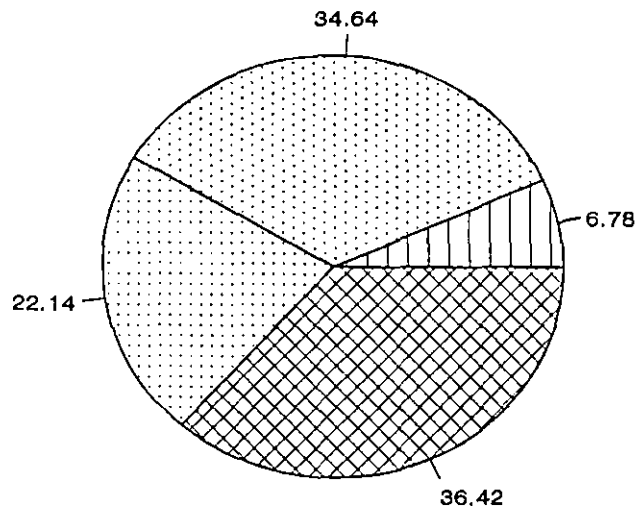
Table 4 Total and differential leucocyte count and total plasma protein in blood of mastitic and healthy animals

No.	Group	TLC Cells (% mm^3)	Differential count (%)					Total protein (g%)
			N	L	M	E	B	
1		8150	52	44	1	3	0	9.333
2		8800	62	35	0	3	0	8.882
3		7700	50	48	1	1	0	10.191
4	Clinical	8450	49	50	1	0	0	8.842
5		8250	53	45	0	2	0	8.827
6		7650	50	48	1	1	0	8.753
7		9650	54	46	0	0	0	8.430
8		8000	49	48	1	2	0	9.497
	Mean	8331 ± 652						9.09 ± 0.55
1		4950	43	55	1	1	0	9.608
2		5400	56	42	0	2	0	10.353
3		4880	49	48	1	2	0	11.392
4	Sub clinical	5400	52	47	1	0	0	9.333
5		4700	47	50	0	3	0	12.269
6		4200	64	36	0	0	0	9.962
7		5650	55	43	0	2	0	10.795
8		4650	49	50	1	0	0	10.753
	Mean	4978 ± 479						10.56 ± 0.97
1		9700	35	60	1	4	0	8.000
2		9600	40	58	0	2	0	8.971
3		11200	40	53	0	3	0	7.843
4		9100	38	62	0	0	0	8.63
5	Healthy	10500	34	63	2	1	0	7.412
6		9700	42	55	2	1	0	8.996
7		9100	31	65	2	2	0	7.804
8		9650	30	68	0	2	0	7.333
	Mean	9818 ± 708						8.15 ± 0.69

Table 5 Alpha napthyl acetate esterase positive cells in the peripheral blood of mastitic and healthy animals

No.	Group	ANAE +ve	Percentage	ANAE -ve	Percentage
1		61	40.5	139	69.5
2		74	37.0	126	63.0
3	Clinical	59	39.5	141	70.5
4		81	40.5	119	59.5
5		70	35.0	130	65.0
6		72	36.0	128	64.0
	Mean		38.08 ± 2.39		
1		64	32.0	136	68.0
2		72	30.0	128	64.0
3	Subclinical	75	31.5	125	62.5
4		53	28.0	144	72.0
5		60	30.0	140	70.0
6		62	31.0	138	69.0
	Mean		30.42 ± 1.49		
1		76	38	124	62
2		58	29	142	71
3	Healthy	63	31.5	137	68.5
4		65	32.5	135	67.5
5		72	36.0	128	64.0
6		64	32.0	136	68.0
	Mean		33.17 ± 3.27		

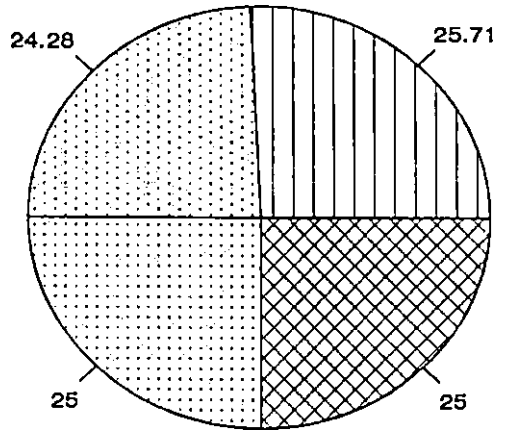
Fig.1 Results of California Mastitis Test



Total number of positive samples-280

Strongly positive | Positive | Doubtful | Slightly positive

Fig.2 Quarterwise distribution of subclinical mastitis



Total number of positive samples-280

Left hind Right hind Left fore Right fore

Fig.3 Organisms isolated from mastitic milk

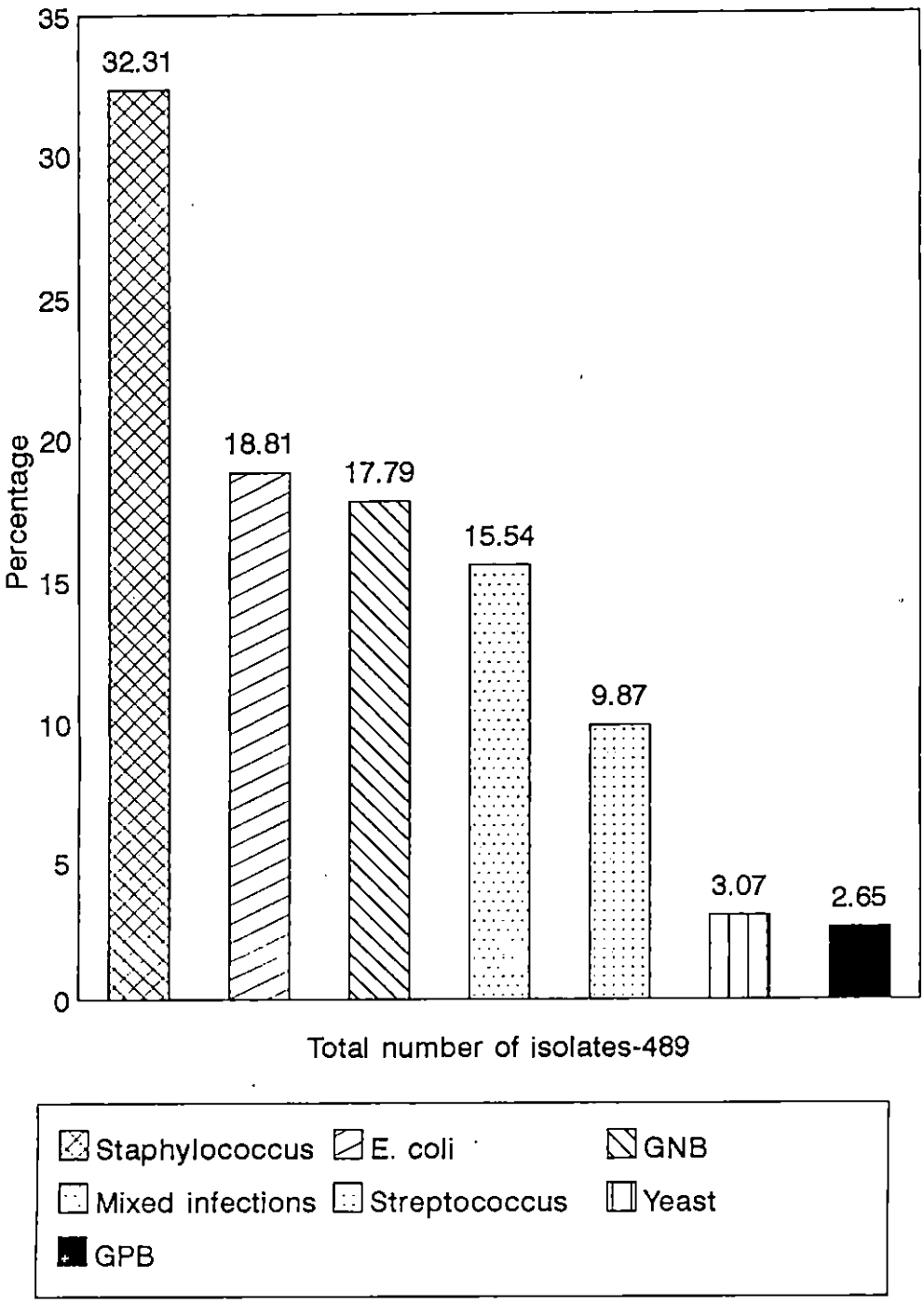
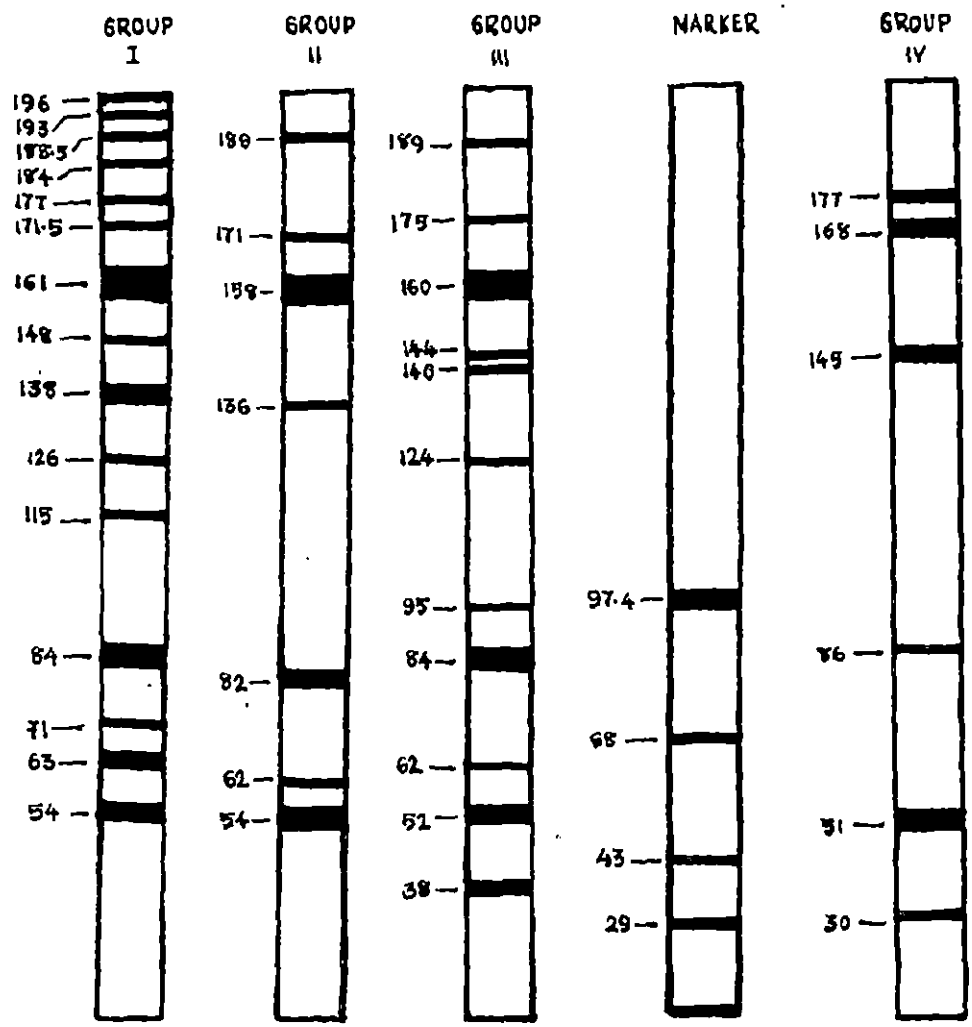


Fig. 4

ELECTROPHORETIC PATTERN OF WHEY PROTEINS



(Molecular weights in Kd)

GROUP-I - CLINICAL MASTITIS

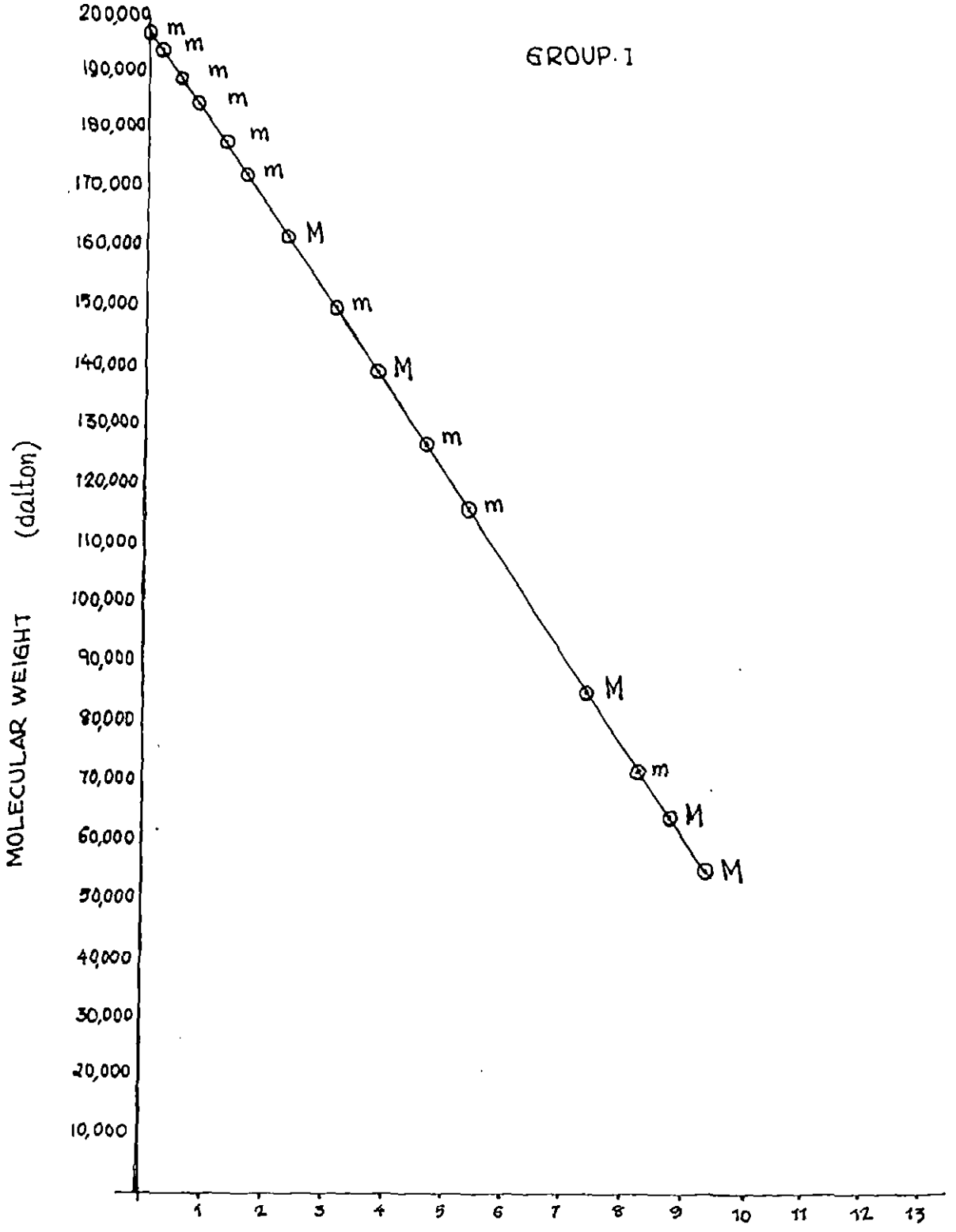
GROUP-II - MILD SUBCLINICAL MASTITIS

GROUP-III - SEVERE SUBCLINICAL MASTITIS

GROUP-IV - HEALTHY (NORMAL)

Fig. 5

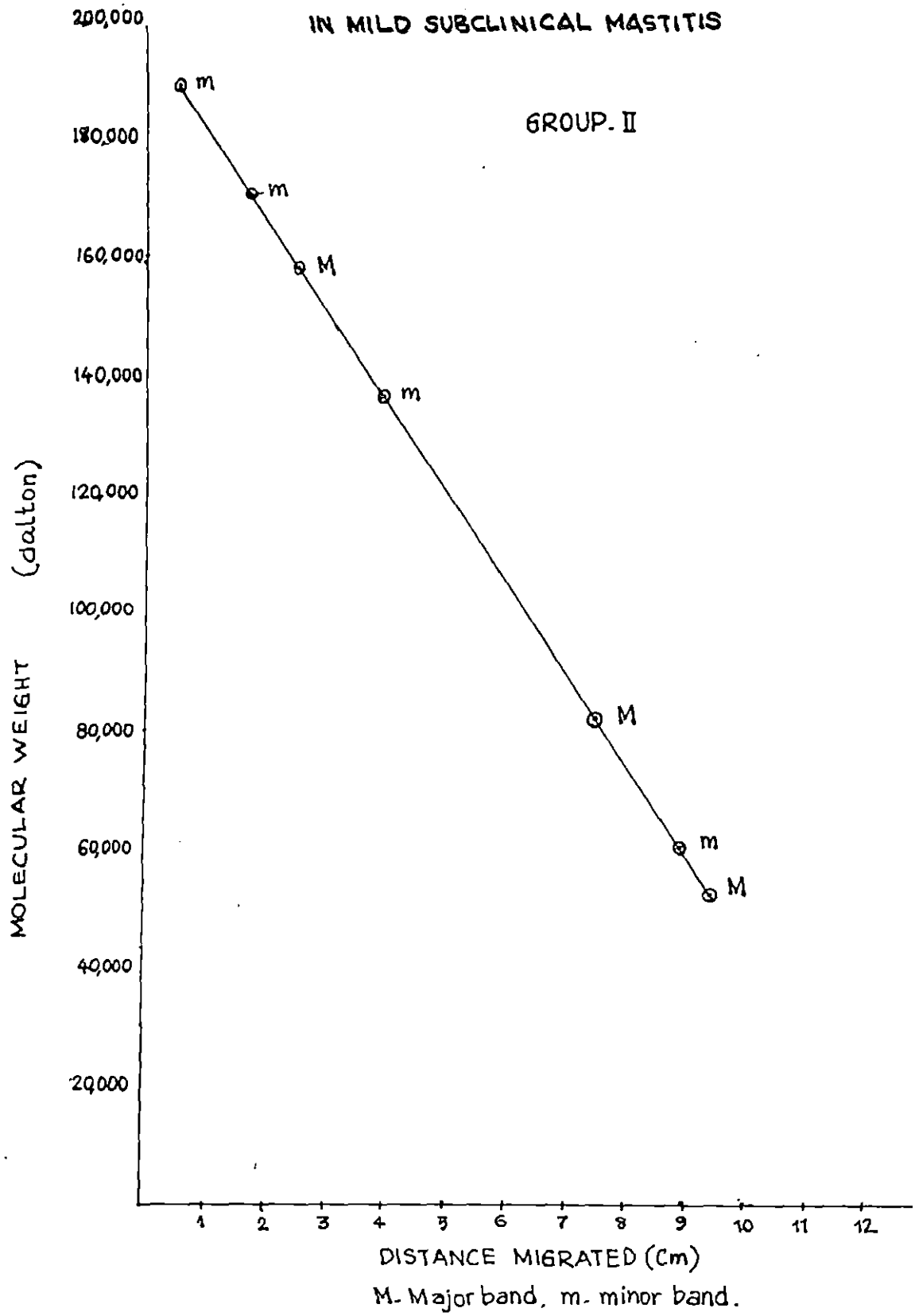
MOLECULAR WEIGHT DETERMINATION OF WHEY PROTEINS IN CLINICAL MASTITIS



M- Major band, m - minor band

Fig. 6

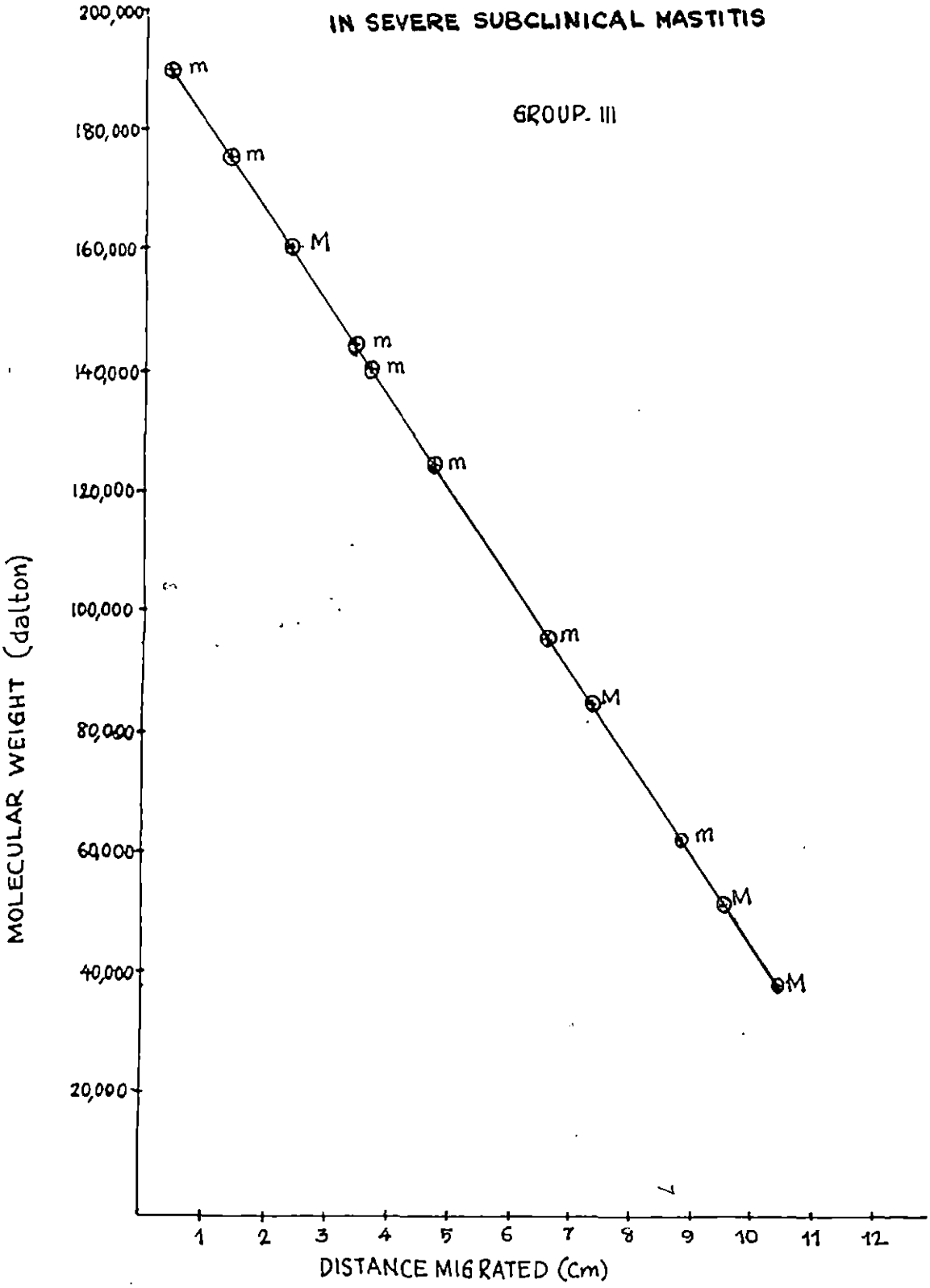
MOLECULAR WEIGHT DETERMINATION OF WHEY PROTEINS IN MILD SUBCLINICAL MASTITIS



M- Major band, m- minor band.

Fig-7

MOLECULAR WEIGHT DETERMINATION OF WHEY PROTEINS IN SEVERE SUBCLINICAL MASTITIS

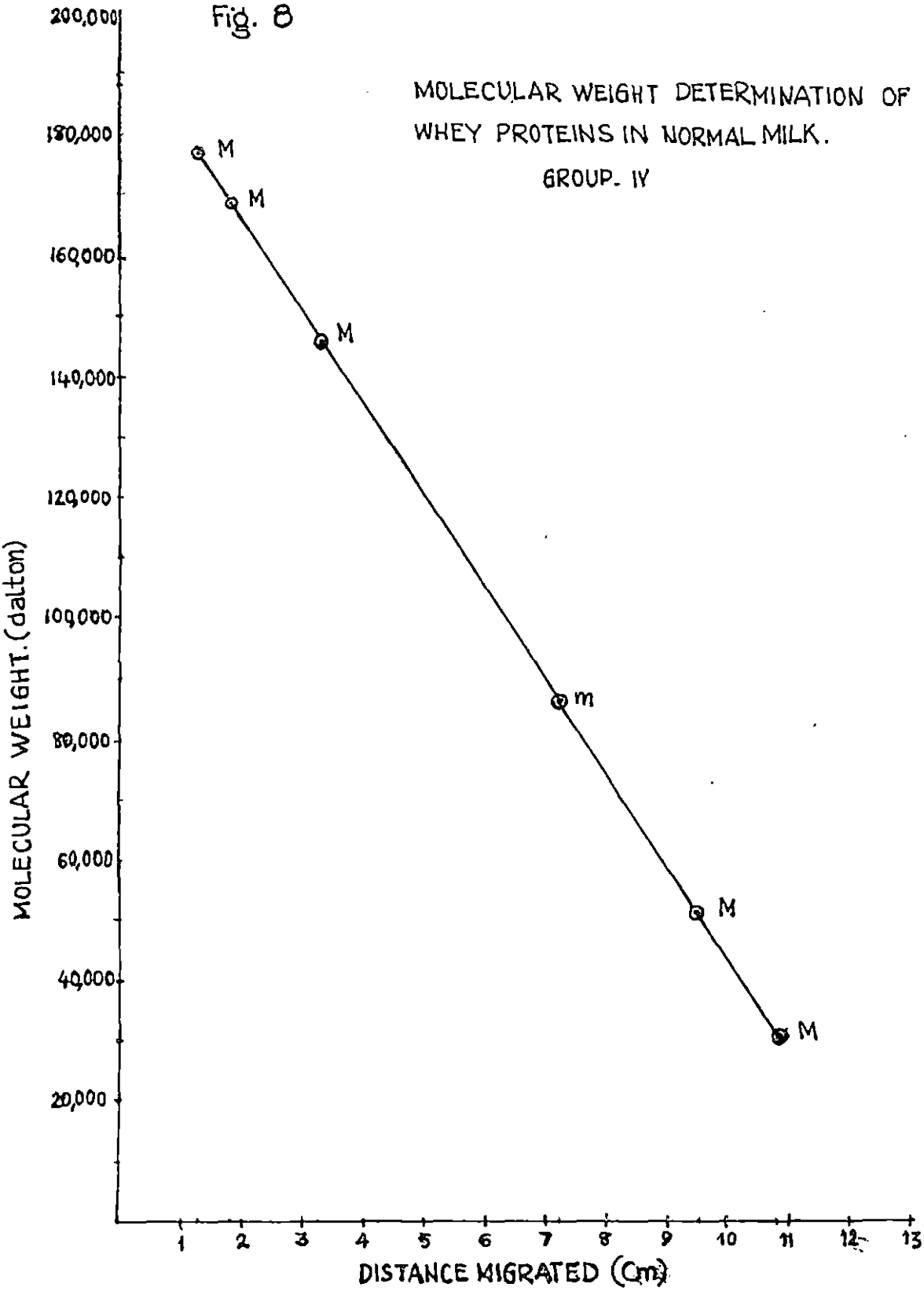


M - major band, m - minor band.

Fig. 8

MOLECULAR WEIGHT DETERMINATION OF WHEY PROTEINS IN NORMAL MILK.

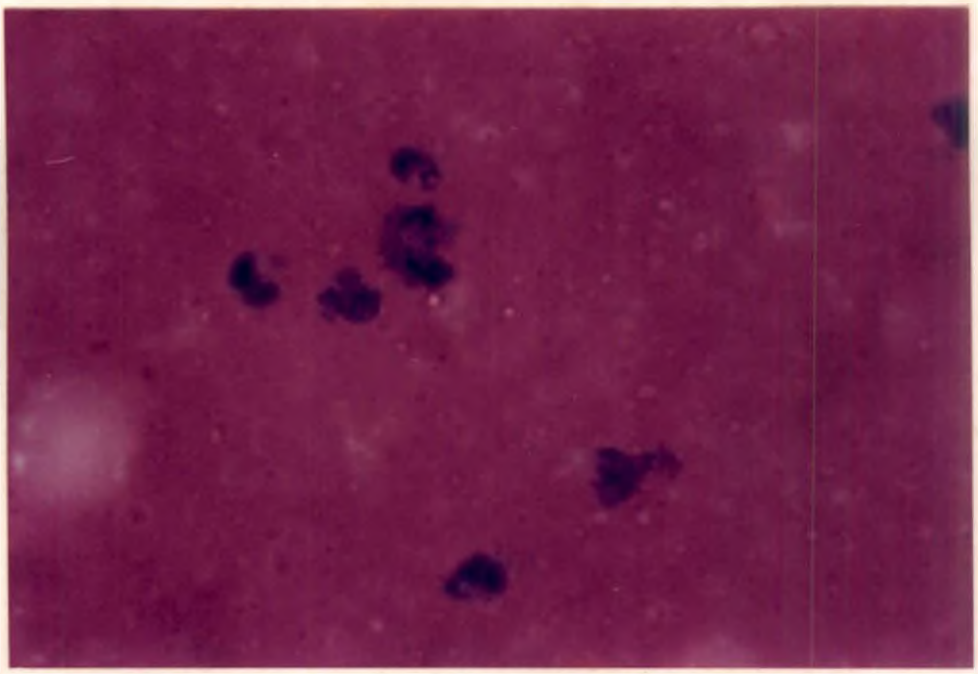
GROUP. IV



M. Major band , m- minor band

Plate 1 Milk smear stained with Broadhurst- Paley stain for the demonstration of somatic cells (1000x)

Plate 2 Growth of Staphylococci in Mannitol Salt Agar



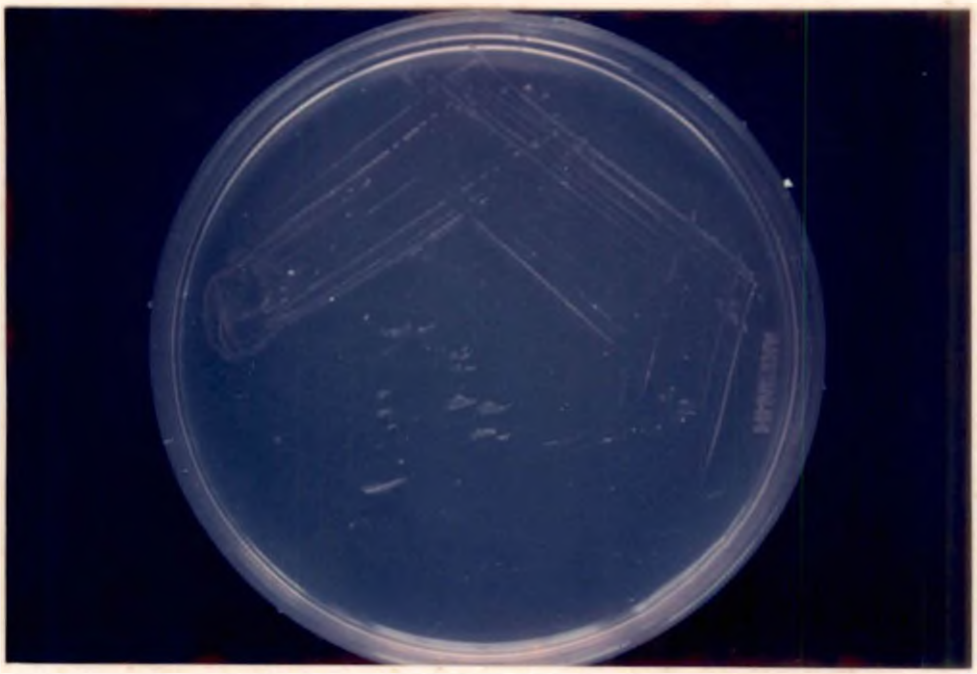
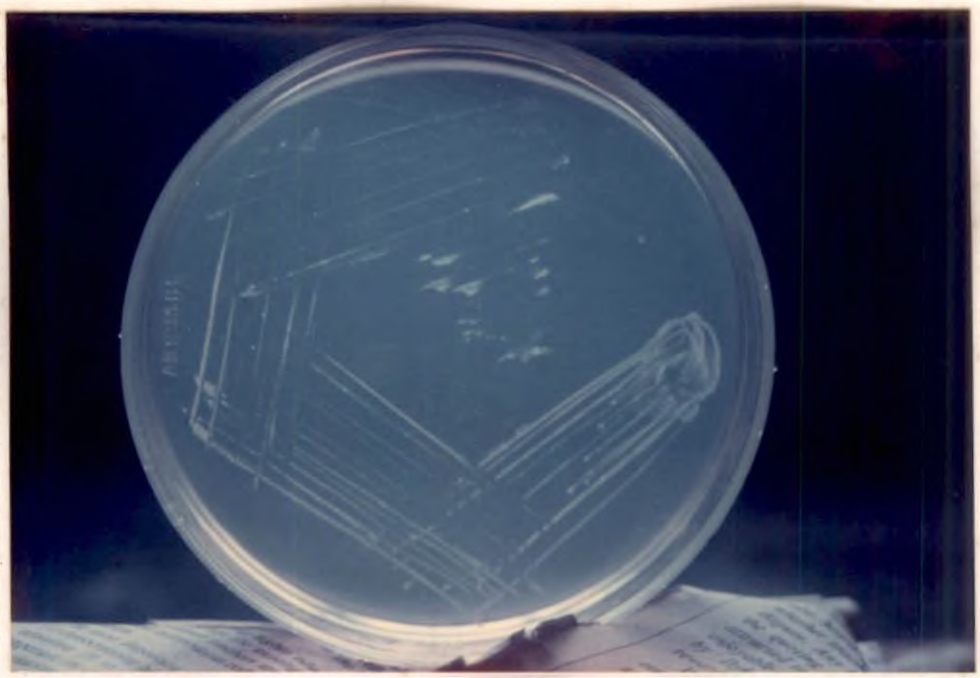


Plate 3 Growth of Streptococci in Streptococcus Selection Agar

Plate 4 Growth of *E. coli* in MacConkey agar



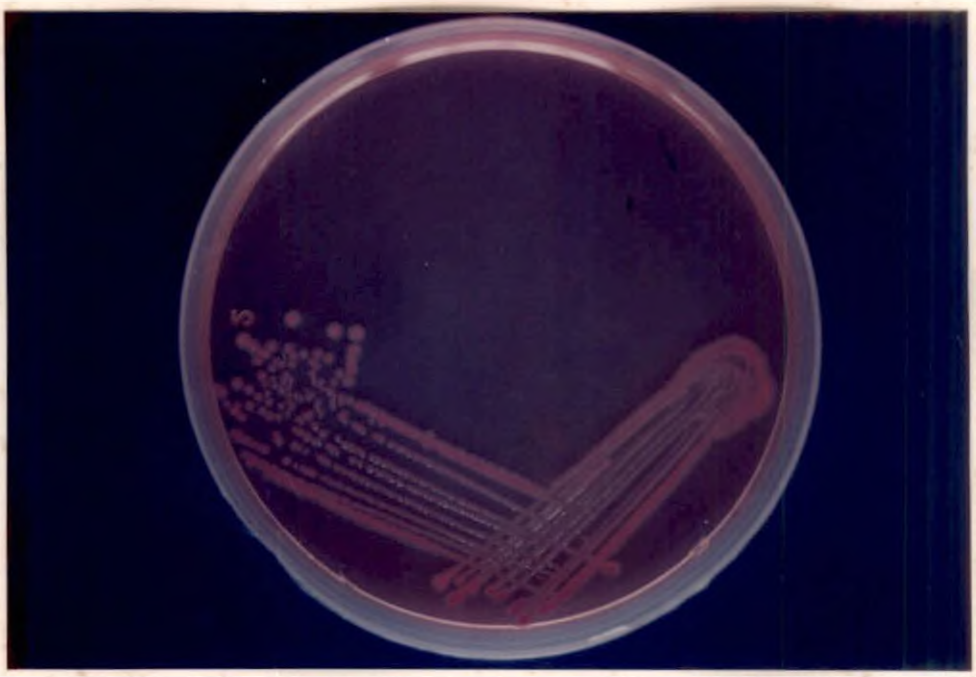
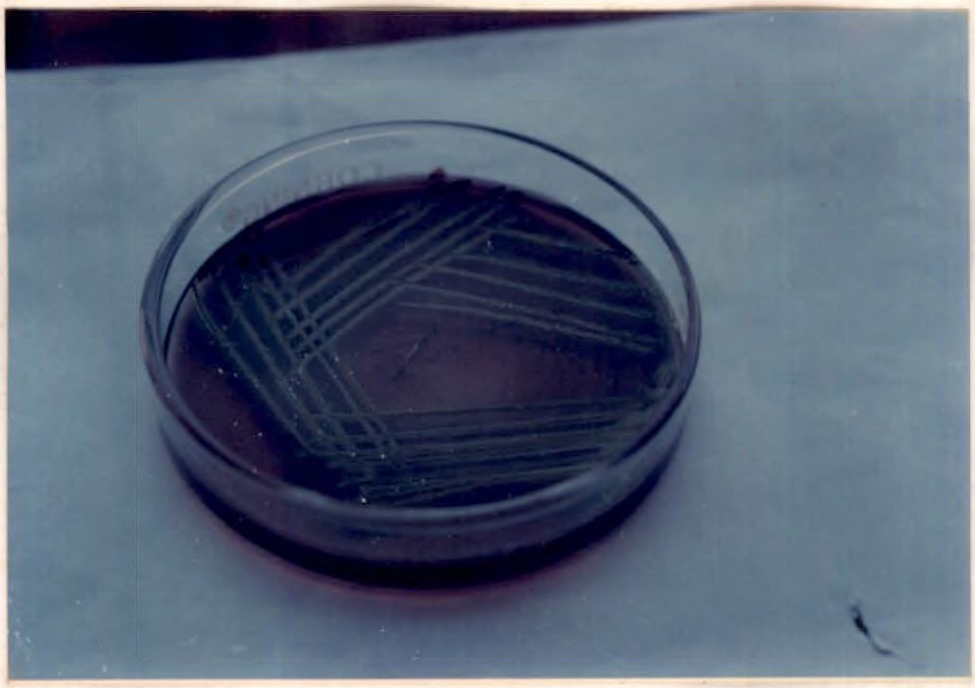


Plate 5 Growth of *E. coli* in Eosin-Methylene blue Agar

Plate 6 ANAE positive cells in the peripheral blood smear of bovines (1000x)



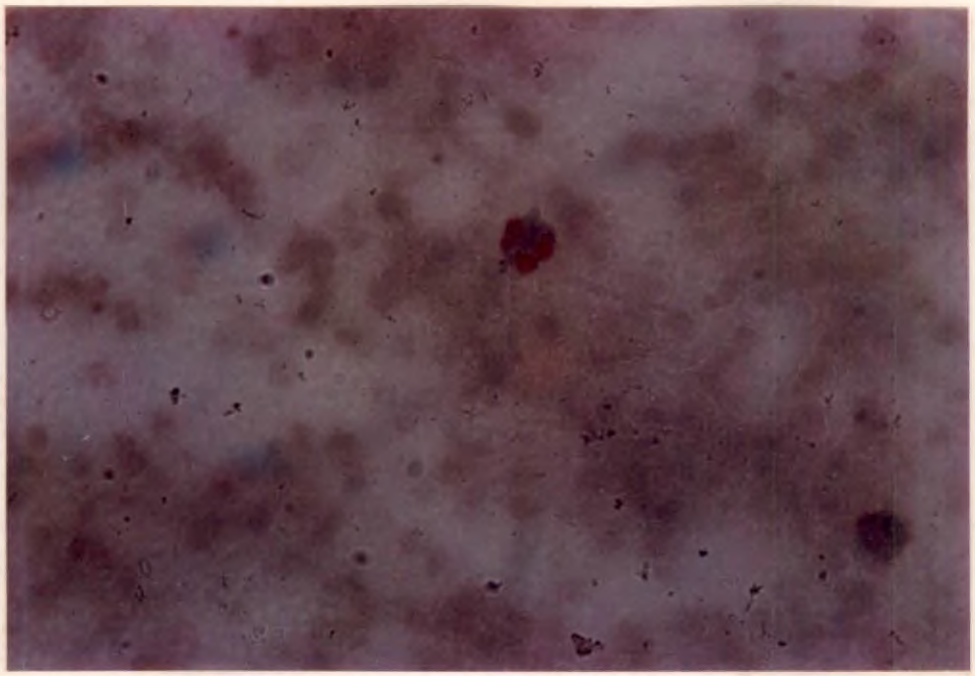
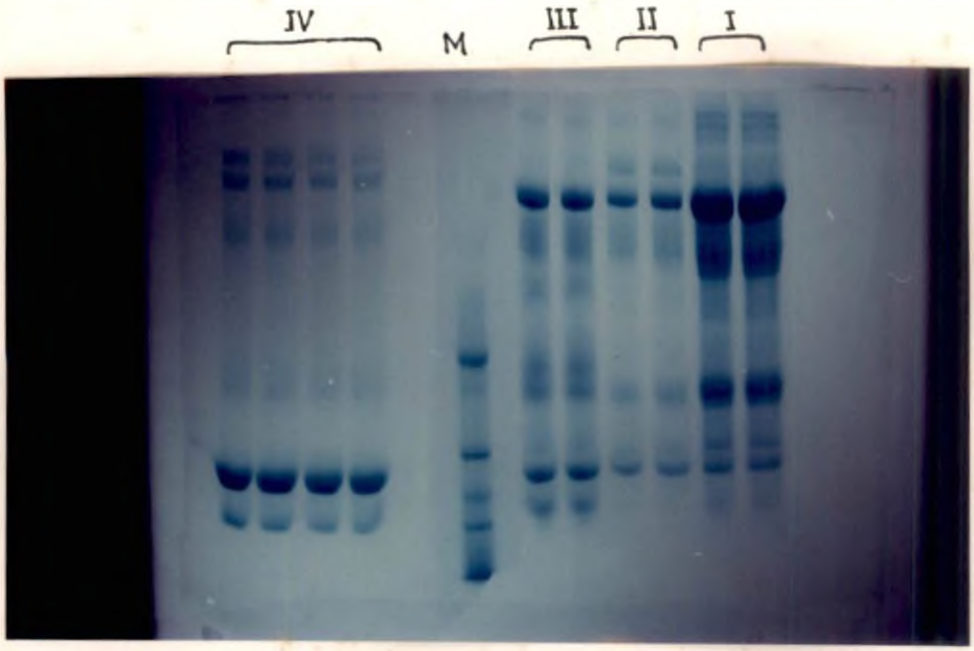


Plate 7 SDS-PAGE on whey proteins

Plate 8 Inflammatory cells in the dilated acini and interstitium of lactating glands (H&E, 100x)


IV
M
III
II
I

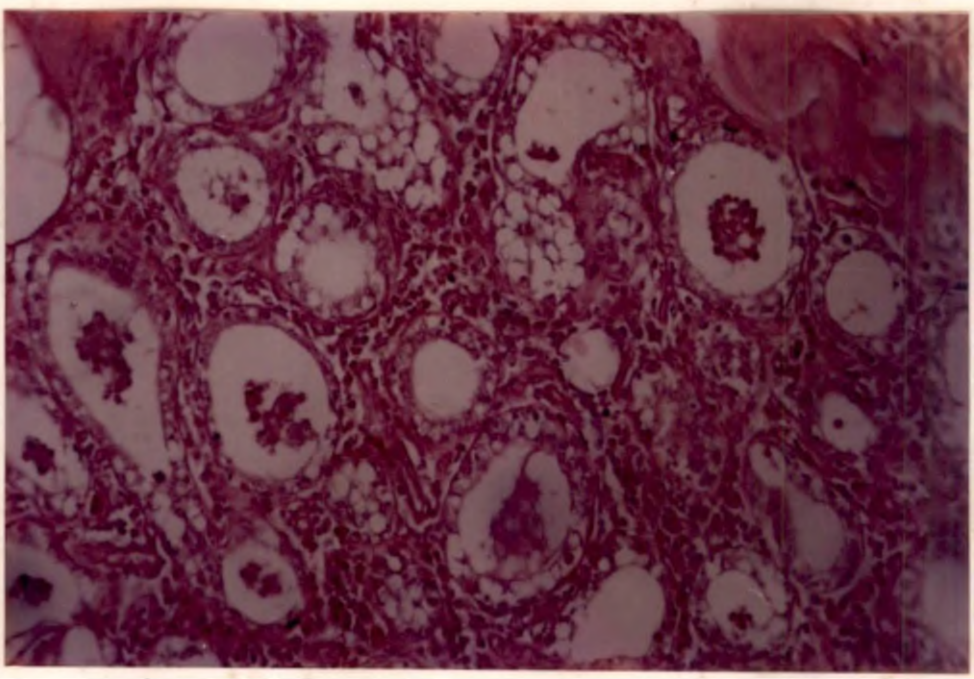
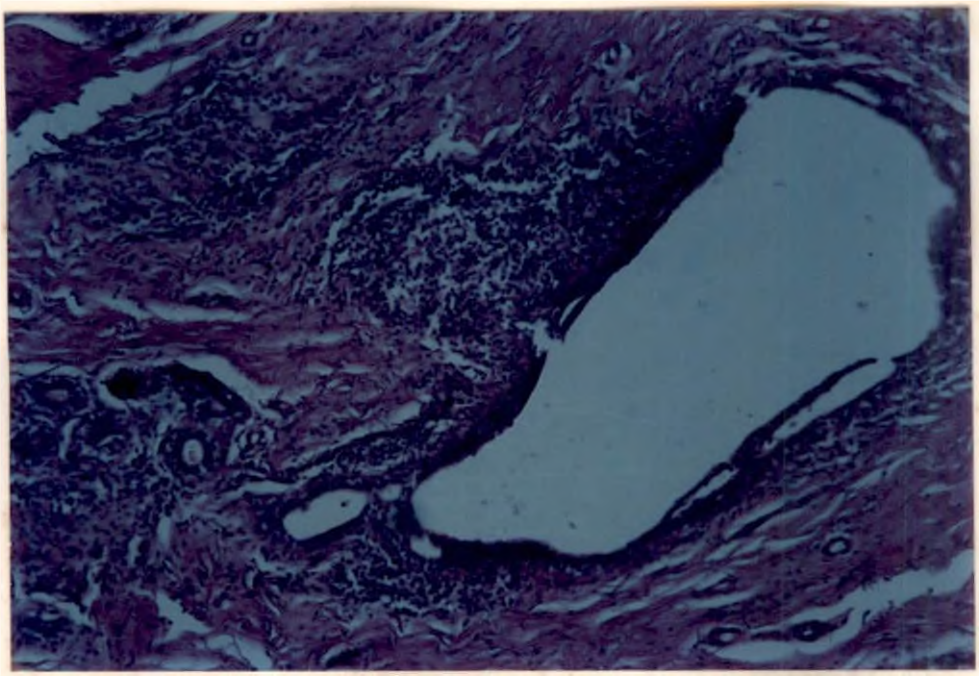


Plate 9 Galactophoritis with desquamation of the epithelium and focal leucocytic infiltration in the subepithelial areas in a lactating gland (H&E, 100x)

Plate 10 Catarrhal inflammation with cellular infiltration and thickening of the interalveolar tissue (H&E, 100x)



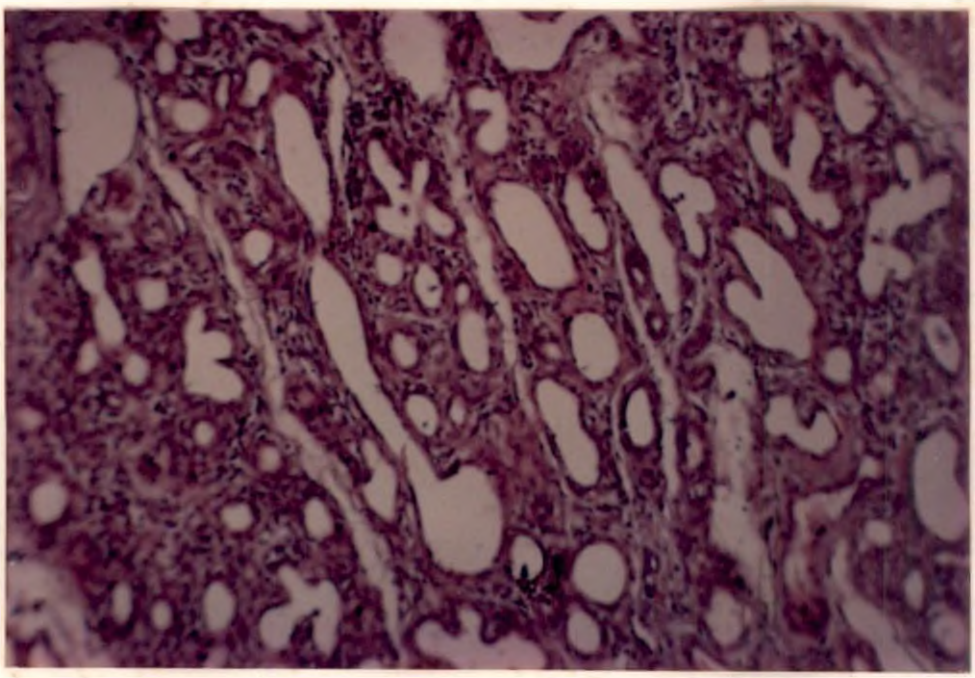
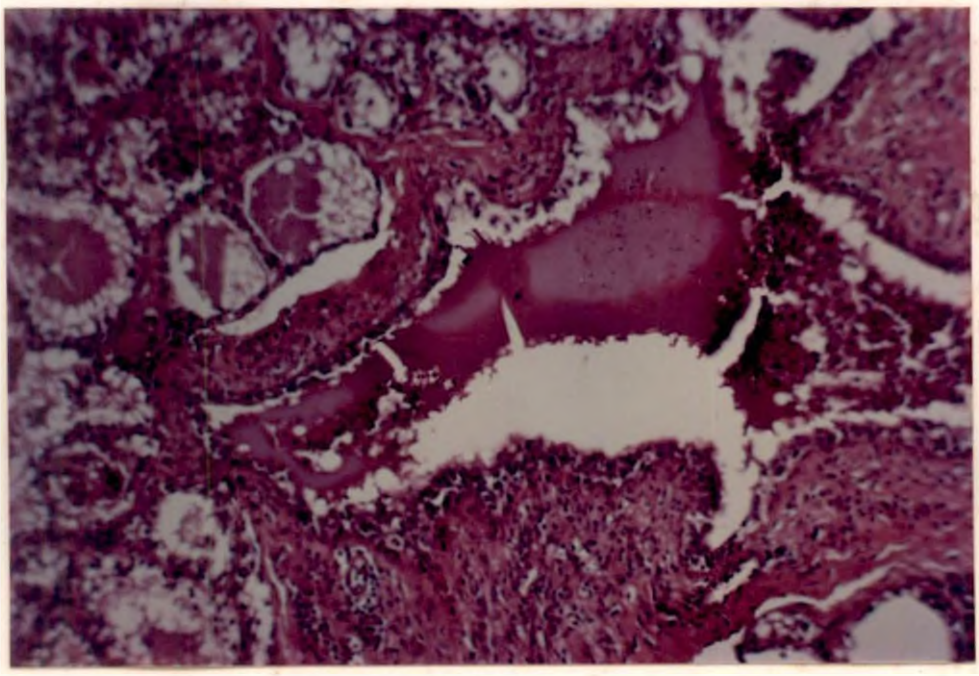


Plate 11 Interstitial mastitis with diffuse interalveolar cell proliferation (H&E, 100x)

Plate 12 Widened milk ducts with protein-rich exudate containing few inflammatory cells in a lactating gland (H&E, 100x)



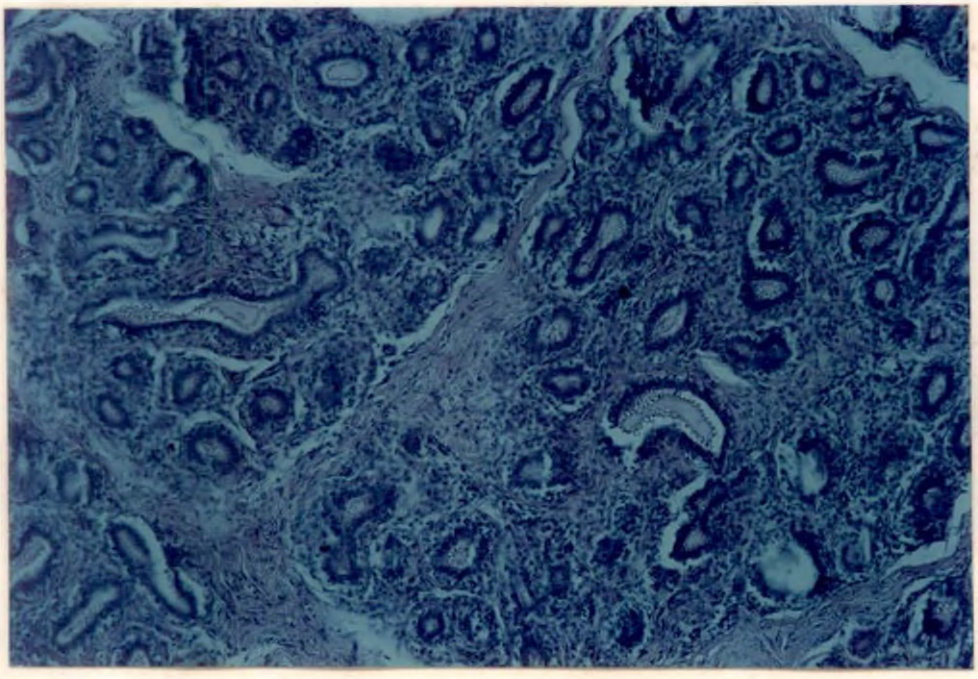
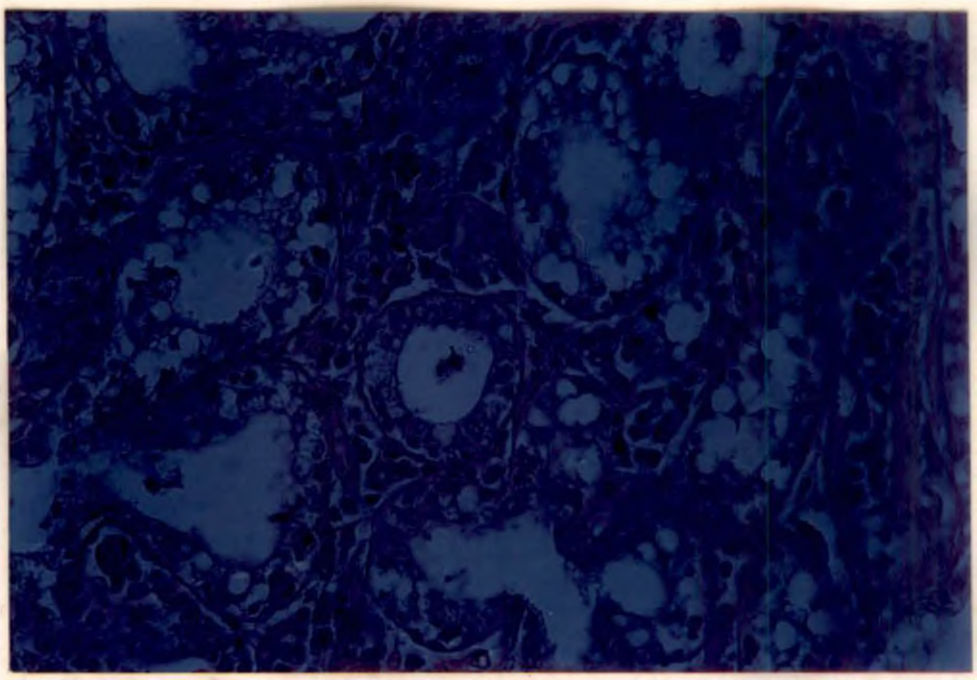


Plate 13 Cytoplasmic vacuolation of the secretory epithelium of the alveoli with focal leucocytic infiltration in a lactating gland (H&E, 400x)

Plate 14 Polypoid thickening of the cisternal epithelium with desquamation of the epithelial cells and lymphocytic infiltration of the detached propria in a lactating gland (H&E, 100x)



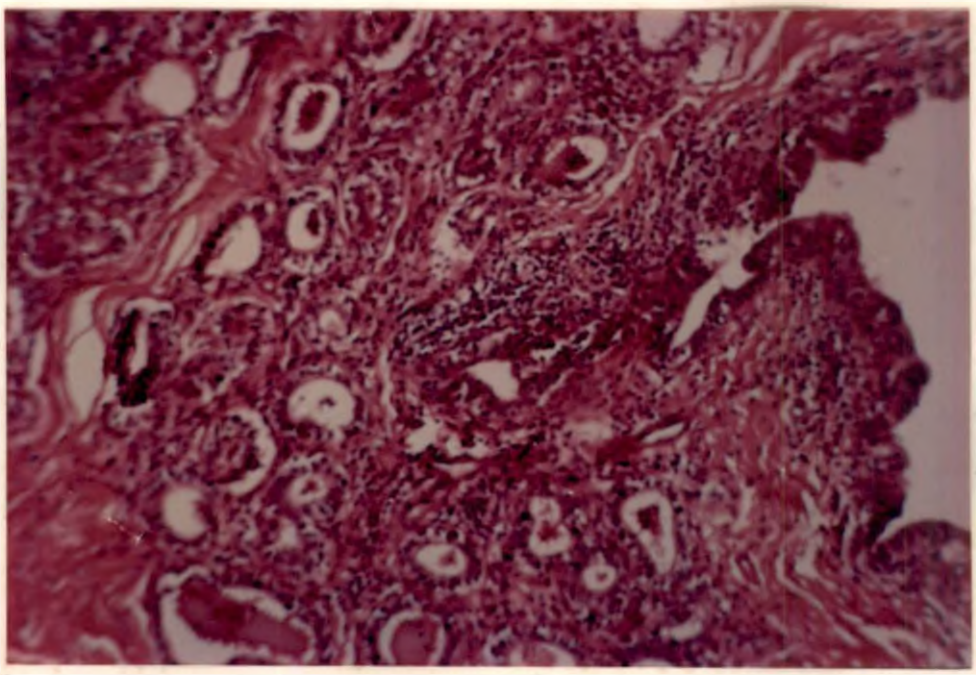
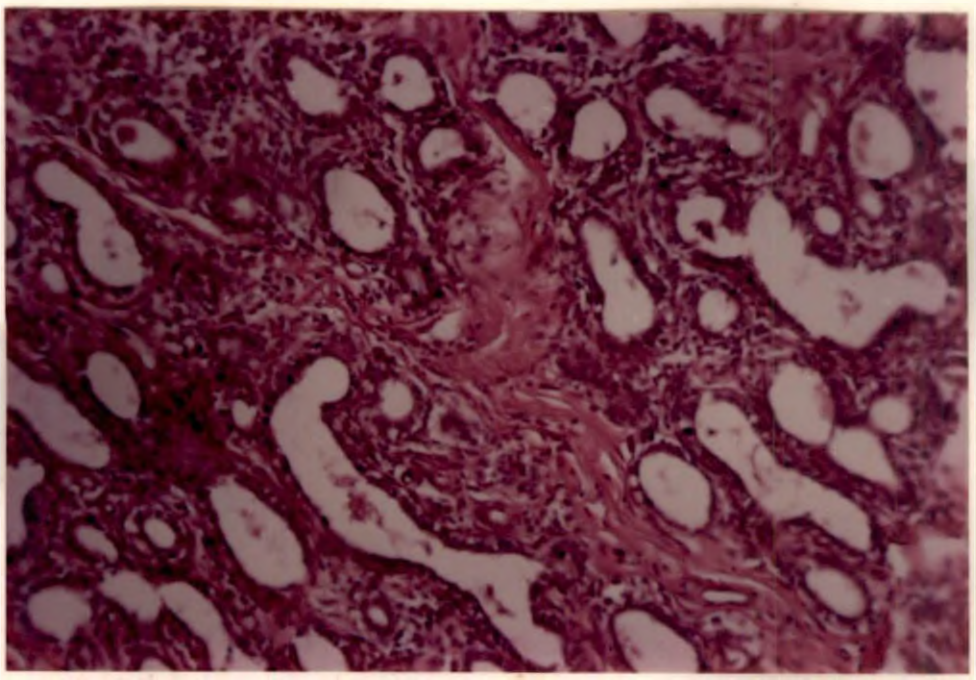


Plate 15 Chronic galactophoritis with extensive involution of the glandular tissue and broadening of the interstitium in an involuting gland (H&E, 100x)

Plate 16 Cellular thickening of the interlobular connective tissue between non-secretory end pieces in an involuting gland (H&E, 100x)



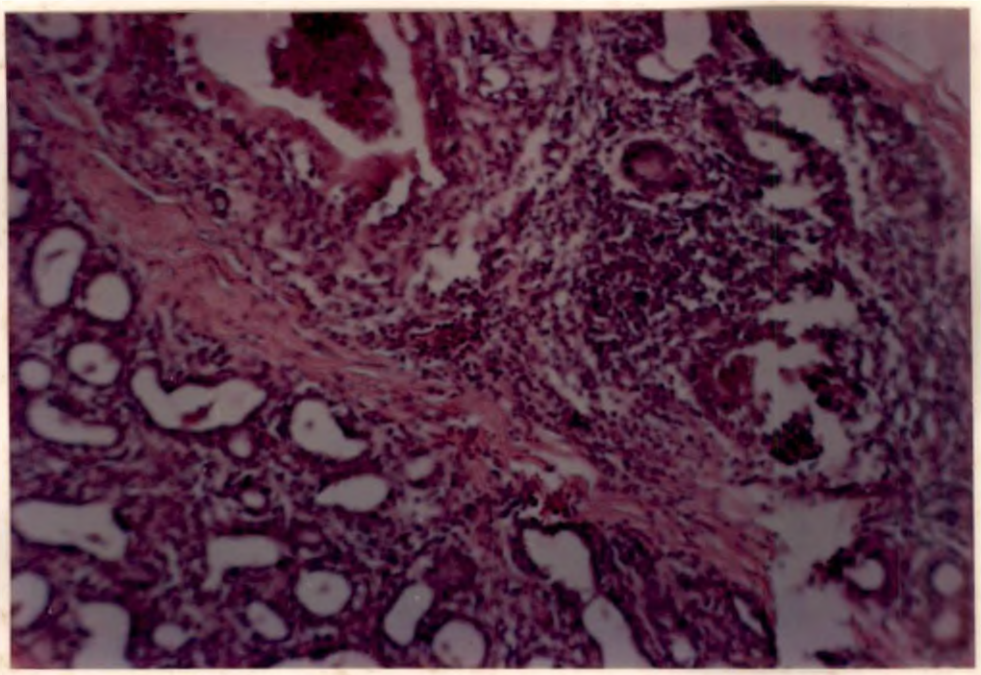
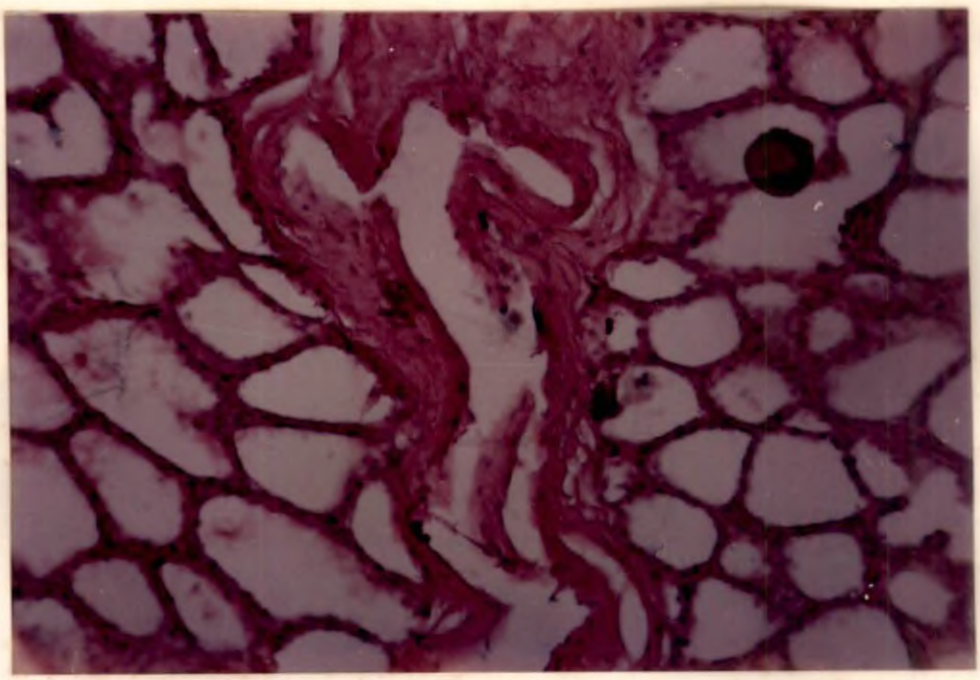


Plate 17 Markedly dilated non-secretory end pieces with periductal fibrosis in an involuting gland (H&E, 100x)

Plate 18 Corpora amylacea in the acini of a lactating gland (H&E, 100x)



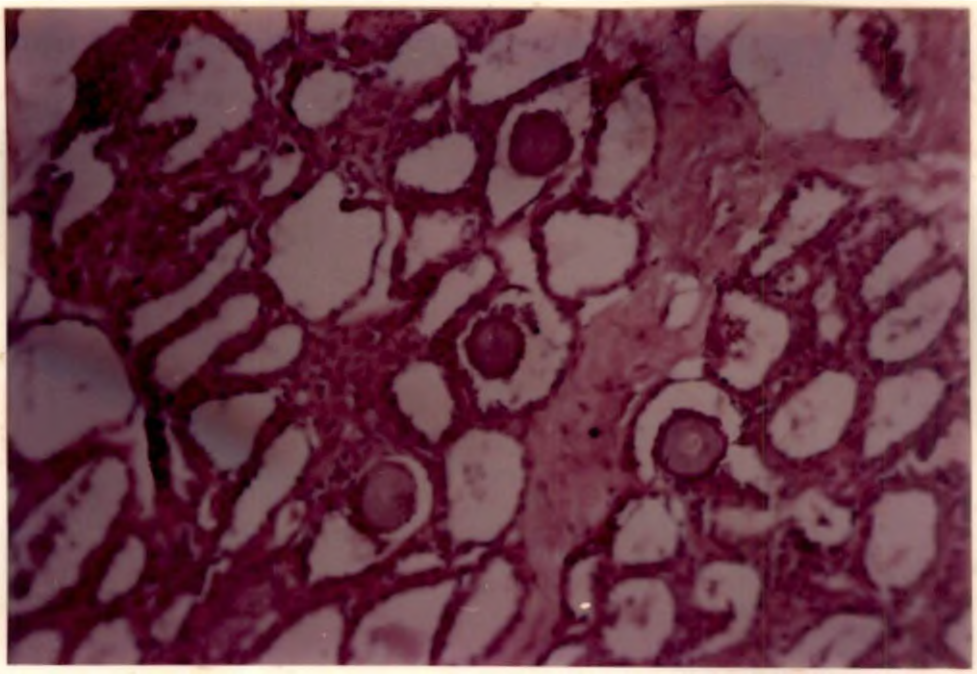
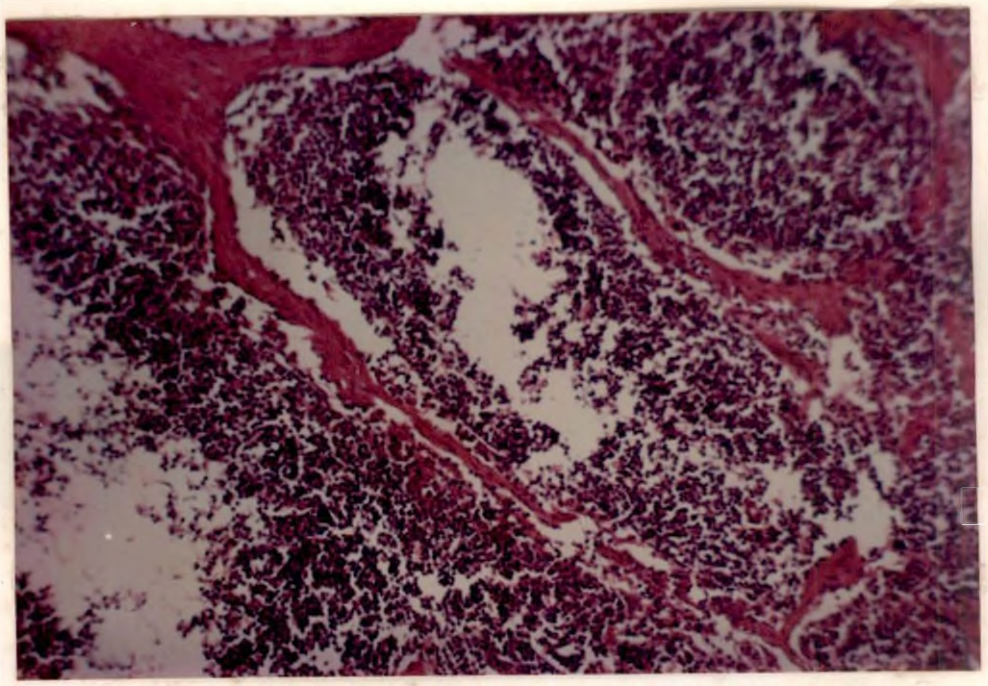


Plate 19 Distension of subcapsular sinuses containing lymphocytes in the supramammary lymphnode (H&E, 100x)

Plate 20 Active secondary follicles with germinal centres having a dense zone packed with lymphoblasts in the cortex of a lymphnode (H&E, 100x)



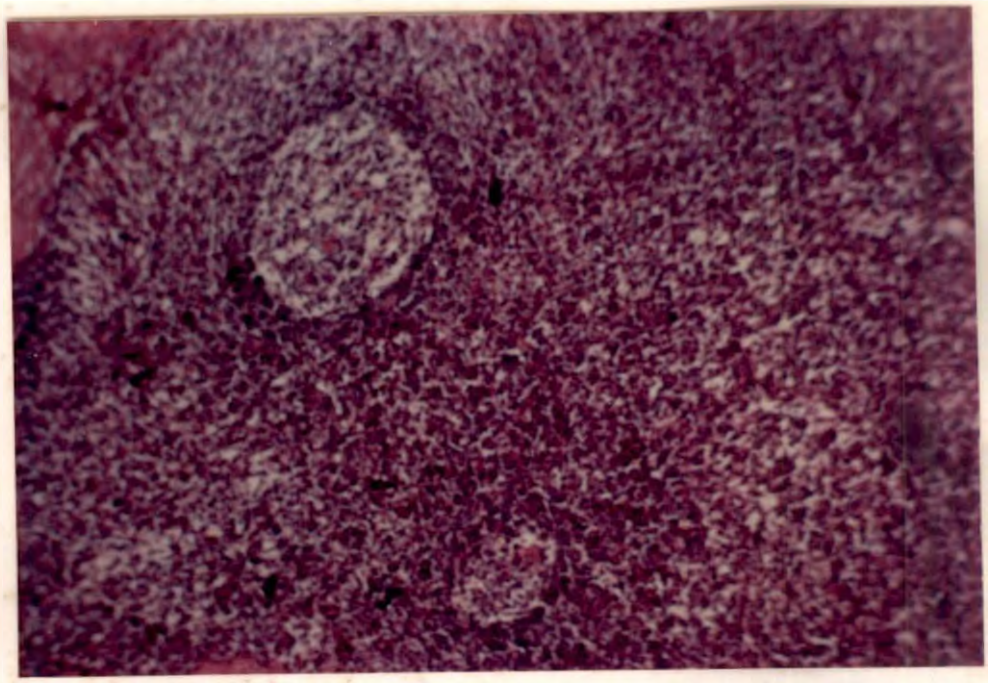
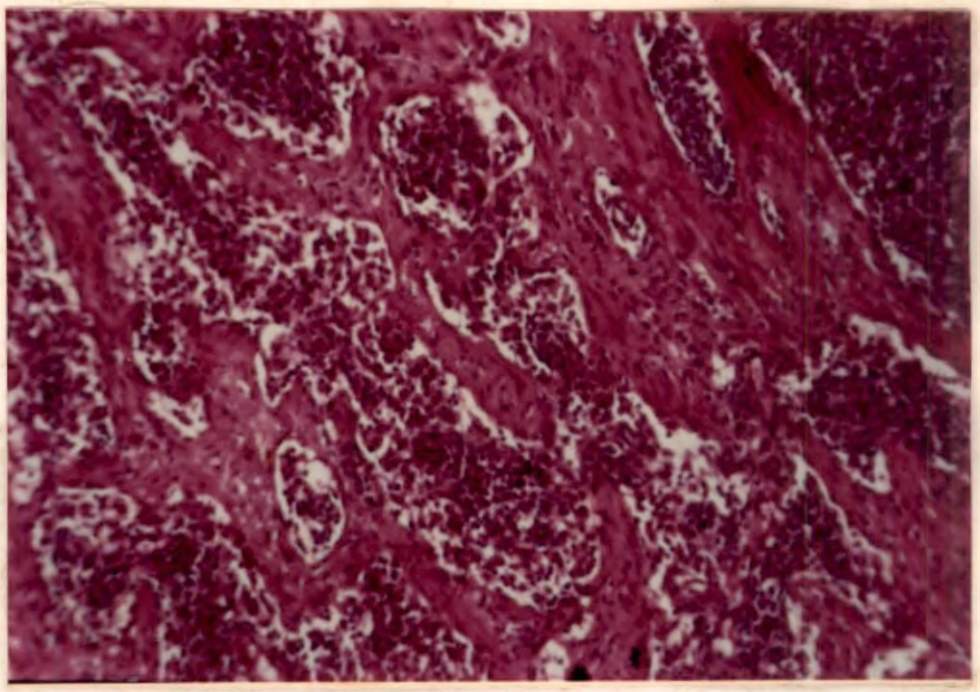
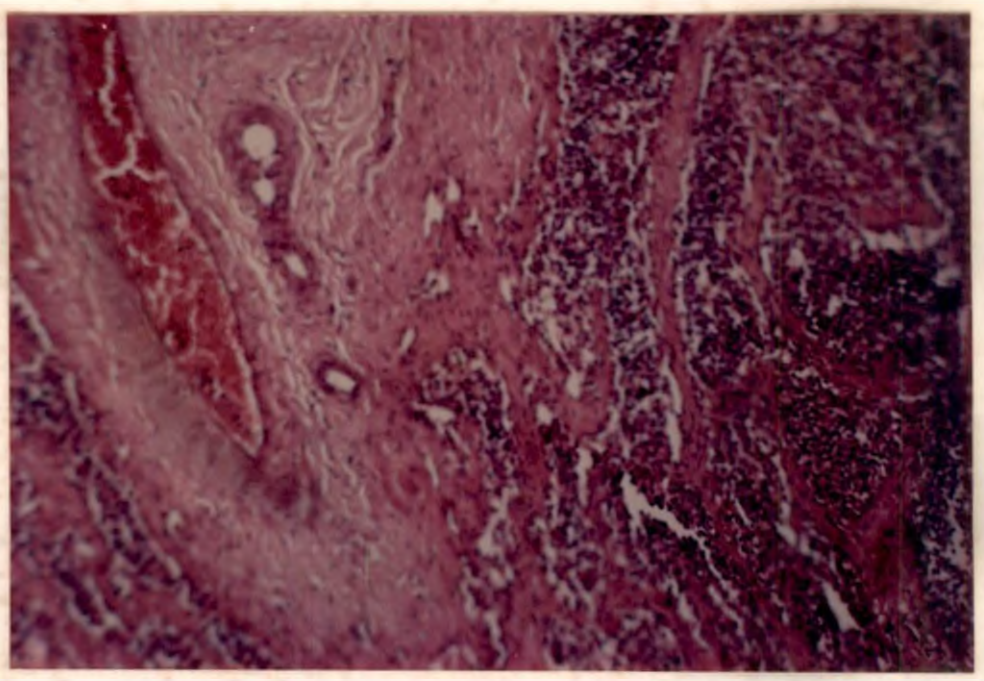


Plate 21 Sinus catarrh in the medulla of the lymph node (H&E, 100x)

Plate 22 Congested blood vessel in the medulla of a supramammary lymphnode (H&E, 100x)





Discussion

DISCUSSION

In the present investigation, the incidence of subclinical mastitis in all the four quarters was found to be the same. This may be possibly due to unhygienic barn practices and contaminated hands of milkers giving equal chances of exposure to all the four quarters for setting up an infection. Adkinson et al (1993) is of the view that all the four quarters of a cow have equal chances of clinical mastitis than individual quarters from different animals.

Kehrli and Schuster (1994) reported that normal milk contains upto 5,00,000 cells per ml of milk. However, somatic cell counts of 3 lakhs to 10 lakhs per ml of milk can be normal, depending on the age of the animal and stage of lactation (Little, 1938). The results show that total somatic cell counts of subclinical cases does not show a significant increase, hence it cannot be considered as a reliable aid for the diagnosis of subclinical mastitis. Many physiological and pathological conditions can affect the number and types of somatic cells in milk. High somatic cell counts can occur in conditions unrelated to infection like teat injury, excessive milking, stage of lactation and various forms of stress (Daley and Hayes, 1992). Exudation of leucocytes from blood and sloughing of epithelial cells from the mammary tissue into the milk of

mastitic glands is a continuous process, which varies in its degree with the intensity of action of the irritant (Schalm *et al.*, 1971). This explains the increased number of somatic cells in clinical cases of mastitis as observed in the study. Somatic cell count can be of value only if carried out along with bacteriological examination (Schalm *et al.*, 1971). As a result of the microbial invasion, the inflammatory reaction increases and reaches a stage, so as to result in a good correlation between infection and demonstration of the reaction by California mastitis test. A fairly high percentage of the milk samples showed positive results on cultural examination. Staphylococcal and Streptococcal infections revealed milk samples with high somatic cell counts. This increase in number of cells in milk is due to the inflammatory reaction initiated by these microorganisms in the udder (Cullen, 1966).

In the present study the bacterial organisms isolated from cases of bovine mastitis were Staphylococci followed by *E. coli*, gram positive bacilli and Streptococci. Similar findings have been reported by Krishnaswamy *et al.* (1965), Sudharma *et al.* (1985), Saxena *et al.* (1993b), Tuteja *et al.* (1993b), Singh *et al.* (1994b) and Wadhwa *et al.* (1996).

Staphylococci were most sensitive to pefloxacin, gentamycin, chloramphenicol, oxytetracycline, co-trimoxazole

and streptomycin. Similar observations has been made by Char *et al.* (1993), Jha *et al.* (1994), Mitra *et al.* (1995) and Biju (1996). Penicillin and ampicillin were least effective. This may be due to the indiscriminate use of these antibiotics in Kerala owing to the low cost and easy availability.

All the streptococcal isolates in the present study were sensitive to pefloxacin, chloramphenicol and co-trimoxazole. Moderate efficacy was shown by gentamycin, oxytetracycline, penicillin and streptomycin. Ampicillin was least effective. Pefloxacin (Reddy, 1995) and gentamycin (Chanda *et al.* 1989) were found to be the most effective and economical drug for *E. coli* mastitis. Pefloxacin and gentamycin were the most effective drugs for *E. coli* mastitis. The organism was moderately sensitive to oxytetracycline, chloramphenicol, streptomycin and co-trimoxazole.

The Gram postive bacilli were 100 per cent sensitive to pefloxacin, gentamycin, co-trimoxazole and chloramphenicol. Similar observation was made by Sudharma *et al.* (1985).

Observations of field veterinarians indicate that the parenteral administration of antibiotics do not give the same results obtained in the laboratory by sensitivity

test (by personal communication). This could be due to the failure of the drug to reach its effective concentrations in the milk or due to its rapid excretion (Sisodia *et al.*, 1973). Presence of degenerated tissue, pus, serum, divalent cations - Mg and Ca may also interfere within the *in vivo* effect of these antibiotics (Sudharma *et al.*, 1985).

Haematological evaluation in the present study revealed leucopenia with relative neutrophilia in clinical and subclinical cases of mastitis. These results are in agreement with those of Cole and Easterbrooks (1958). Clinical cases showed only a mild leucopenia. This may be due to the indefinite time of sampling in respect to onset of the disease. Within hours of onset, there is marked leucopenia with less number of neutrophils and lymphocytes. This change is attributed to the massive migration of the neutrophils into the affected gland (Coliform Sub Committee of The National Mastitis Council, 1979). This can also be due to the action of endotoxins released by bacteria causing destruction of the blood leukocytes (Schalm *et al.*, 1971). Differential leucocyte counts in blood reveal existence of a neutropenia and degenerative left shift (immature neutrophils) as the bone marrow reserve supply of mature neutrophils becomes exhausted. Granulopoiesis becomes intensified leading to restoration of neutrophil reserves in the bone marrow. This is followed by the

return of mature neutrophils into the circulation in normal or greater numbers (Schalm, 1977). Similar observations were made by Theilen *et al.* (1959). The total plasma protein in subclinical and clinical cases of mastitis showed an increase in the present study. Sordillo *et al.* (1989) observed that alpha lactalbumin, a whey protein escapes into the circulation through leaky gaps left by sloughed or damaged epithelial cells.

T-lymphocytes were identified by demonstration of alpha naphthyl acetate esterase (ANAE) activity. ANAE positive cells have been demonstrated by many workers (Knowles *et al.*, 1978 and Pinkus *et al.*, 1979). In the present study, T-lymphocyte count was slightly higher in clinical cases of mastitis. Similar observations were made by Ishikawa and Shimizu (1983). This is because the lymphocytes are the most important cells conferring protection to the host and an increase in T-lymphocyte population is a defence mechanism of the body to ward off the infection. Exposure of the sensitised lymphocytes to specific bacterial antigens may initiate a series of immunological reactions leading to production of immunoglobulin and lymphokines (Targowski, 1983). Interleukin-2 is a lymphokine elaborated by T helper cells with a wide range of immunologic effects including the proliferation and differentiation of B lymphocytes and stimulation of immunoglobulin secretion by plasma cells.

Interleukin-2 and interferon-γ produced by the T helper cells cause activation of the macrophages effecting the intracellular killing of the bacteria (Tizard, 1996). Kaura *et al.* (1989) also observed an increased number of T lymphocytes in the peripheral blood of mastitis affected buffaloe.

Electrophoretic studies of whey samples from mastitic animals revealed additional major and minor bands when compared to healthy animals. The primary response of the host to the infectious agent or the toxin produced by them is an increase in capillary permeability, resulting in an outpouring of plasma proteins from the circulation into the milk in a readily detectable quantity (Leece and Legates, 1959). So the major bands may be contributed by these plasma proteins. Nickerson and Heald (1981) suggested that the toxins produced by the multiplying bacteria spread to the secretory parenchyma, invoking cellular damage. Moreover, neutrophil migration into the mammary gland and release of hydrolytic enzymes also causes cytolysis of secretory cells (Harmon and Heald, 1982). The additional minor bands observed in the present investigation may be a result of these cellular proteins and bacterial proteins in mastitic milk, which are otherwise absent in normal milk. Major bands with molecular weight 161 kilodaltons represent immunoglobulins, which are markedly high in subclinical and

clinical cases when compared to healthy animals. Leece and Legates (1959) had also observed an increase in whey immunoglobulins in acute mastitis in bovines.

Histopathological examination revealed inflammatory cells like neutrophils and mononuclears in the interstitium and alveolar lumen of lactating glands. Research conducted by Sordillo *et al.* (1989) revealed similar findings. The monocytes in the alveolar epithelium mature into macrophages and phagocytose milk constituents, bacteria, neutrophils and degenerated macrophages. They also process the antigen in the subepithelial lymphoid cells in the interalveolar stroma. Presence of lymphocytes and neutrophils near the gland cistern indicates preferential infiltration and an attempt to contain the infection in the lower extremities of the gland. Lymphocytes also act as plasma cell precursors (Nickerson and Heald, 1982). Jones (1990) observed less number of neutrophils in the mammary tissue of affected animals. This was due to the rapid growth and degeneration of toxigenic bacteria producing large levels of endotoxin causing reversed neutrophil chemotaxis.

Many lactating quarters revealed degeneration of the secretory epithelium. This may be due to the fact that the toxins produced by the multiplying bacteria in the gland spreads to the secretory parenchyma invoking cellular

damage. Moreover, neutrophil migration and release of lysosomal enzymes from the degenerated neutrophils were implicated in the cytolysis of the secretory cells, Sordillo *et al.* (1989).

Excessive fibrous tissue proliferation could be noticed in involuting glands. Streptococcus is an obligate parasite of the bovine mammary gland, producing chronic mastitis with periodic exacerbations. Fibrin clots may plug smaller ducts leading to rapid involution of the leucocyte filled alveoli. Stromal tissue proliferates leading to fibrosis, which is characteristic of Streptococcal mastitis (Schalm, 1977).

The protein rich cellular exudate could be noticed in the alveoli which later fills up the lactiferous ducts and the gland cistern (Heidrich and Renk, 1967). The secretory epithelium undergoes cytoplasmic vacuolation with isolated focal areas of necrosis. Similar observations have been made by Thomas *et al.* (1994) on experimentally induced *Streptococcus uberis* infection in cows. A heat stable protease-nuclease and hyaluronidase resistant toxin derived from the capsular layer of *S. uberis* prevents phagocytosis of other bacteria by neutrophils. Furthermore, neutrophils possess tissue damaging and disease inducing properties like generation of reactive oxygen species, lysosomal enzymes and eicosanoids which promote the adherence of bacteria to the mammary tissue inducing mastitis, Thomas *et al.* (1994).

The degeneration and denudation of secretory epithelium observed in a few cases may be due to the toxic substances released by the microorganisms adhered to the epithelium. *Staphylococcus aureus* nuclease was demonstrated in secretions from all glands with epithelial lesions, Gudding *et al.* (1984).

Corpora amylaceae could be observed in majority of lactating and non-lactating glands. It is probable that these originated from inspissated milk proteins which later become hyalinised and very often calcified (Lalitha Kunjamma,1976). Presence of corpora amylacea is suggestive of a preceeding infection (Schalm *et al.*, 1971).

In the supramammary lymphnodes, there was a proliferative type of reaction in which the lymph sinuses were widened and filled with lymphocytes. When inflammation sets up in the mammary gland, the supramammary lymphnode, which drains the affected part also undergoes similar changes. The reason for this is obviously that the infectious agent is drained into the regional lymphnode producing the same inflammatory lesions as it does in the gland. The results are beneficial by virtue of the filtering capacity of the node to prevent further progress of the pathogenic organisms (Jones and Hunt, 1983). In the cortex of the lymphnode, the secondary follicles showed

germinal centres with darkly staining lymphocytes at the periphery and a pale centre, due to the proliferation of the pale staining lymphoblasts. Reactive hyperplasia could be noticed in the medulla. This is due to the fact that the defence mechanism of the body is enhanced either by action of the infectious agents or the toxins produced by them (Coliform Subcommittee of National Mastitis Council, 1979).

Summary

SUMMARY

A study was conducted on the pathology of bacterial mastitis in bovines. The prevalence of clinical and subclinical mastitis was studied. Clinico-pathological features and the patho-anatomical features in mastitis were investigated in detail.

A total of 542 quarter milk samples from 145 animals were subjected to California mastitis test to detect the presence of subclinical mastitis. Based on the intensity of the reaction, the milk samples were grouped into categories ranging from doubtful to strongly positive. 51.6 per cent of the milk samples tested were positive. The quarterwise distribution of subclinical mastitis was also studied. All the four quarters showed equal incidence of mastitis.

Somatic cell count was carried out in both clinical and subclinical cases of mastitis and compared with the healthy animals. The total cell count was increased in both clinical and subclinical mastitis.

Cultural examination of 1031 milk samples revealed positive results for 254 clinical cases and 235 subclinical cases. Staphylococcus was the most predominant organism,

followed by *E. coli*, Gram negative bacilli, mixed infections, Streptoccus, Yeast and Gram positive bacilli in the decreasing order of prevalence. Antibiogram of the various etiological agents revealed that all the major pathogens were sensitive to pefloxacin, gentamycin and chloramphenicol. Penicillin was found to be the least effective. Haemogram of mastitis affected animals revealed leucopenia in both subclinical and clinical cases, but more pronounced in the former, when compared to healthy animals. Relative neutrophilia with more number of immature neutrophils was observed in mastitis affected animals. Total plasma protein was markedly increased in subclinical cases of mastitis, whereas clinical cases showed only a slight increase. T-lymphocyte count as indicated by the number of ANAE positive cells was increased in clinical cases of mastitis.

Electrophoretic studies on whey proteins from mastitic milk revealed additional major and minor bands which resulted from the plasma proteins, cellular proteins and bacterial proteins. Immunoglobulins were increased in cases of acute mastitis.

Histopathological examination of the lactating mammary glands revealed the presence of polymorphs and mononuclears in the acini and interstitum. Galactophoritis, polypoid

thickening of cisternal epithelium, cytoplasmic vacuolation of secretory epithelium and interstitial mastitis were the other lesions. Involuting glands showed increased fibrous tissue proliferation with focal accumulation of polymorphs, mainly in aged animals. Animals in late lactation showed widening of the interstitial septa and numerical reduction of the glandular lobules. Corpora amylacae could be observed in majority of the lactating and non-lactating glands. Histopathological studies of supramammary lymphnodes revealed distension of subcapsular sinuses, active secondary follicles in the cortex, sinus catarrh and medullary hyperplasia.

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PATHOLOGY OF BACTERIAL MASTITIS IN BOVINES

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ABSTRACT OF A THESIS

Submitted in partial fulfilment of the
requirement for the degree of

Master of Veterinary Science

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1998

ABSTRACT

The pathology of bacterial mastitis in bovines was studied considering the following aspects such as bacterial isolation; changes in milk, antibiotic sensitivity pattern, haematology, whey protein pattern and histopathological examination of the mammary gland and supramammary lymphnodes.

Two hundred and sixty two samples were found to be subclinically positive based on California Mastitis Test. The organisms isolated from clinical and subclinical cases were Staphylococcus, *E. Coli*, Gram negative bacilli, mixed infections, Streptococcus, Yeast and Gram positive bacilli in the decreasing order of prevalence. All the major pathogens were sensitive to pefloxacin, gentamycin and chloramphenicol and resistant to penicillin.

Leucopenia could be noticed in clinical and subclinical cases of mastitis. Relative neutrophilia could be observed in mastitic animals. Total plasma protein was also increased. There was an increase in T-lymphocyte count in mastitis affected animals.

Somatic cell count was increased in clinical and subclinical cases, but was not indicative of infection, unless confirmed by bacteriological examination.

SDS-PAGE on whey proteins revealed an increase in protein content in mastitic milk as a result of the plasma proteins, cellular proteins, bacterial proteins. There was marked increase in immunoglobulins in mastitic milk, when compared to normal.

Histopathology of mammary glands revealed the presence of inflammatory cells in the acini and interstitium of mammary glands as the main lesion. Varying degrees of inflammatory reaction could be noticed in almost all the quarters. Supra mammary lymphnodes revealed distension of subcapsular sinuses, active secondary follicles in the cortex, sinus catarrh and medullary hyperplasia in mastitis affected animals.