STUDIES ON PATHOLOGICAL CONDITIONS IN THE MAMMARY GLANDS OF CATTLE AND GOATS

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled STUDIES ON PATHOLOGICAL CONDITIONS IN THE MAMMARY GLANDS OF CATTLE AND GOATS is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to of any degree, diploma, associateship, fellowship, or other similar title of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled STUDIES ON PATHOLOGICAL CONDITIONS IN THE MAMMARY GLANDS OF CATTLE AND GOATS is a record of research work done independently by Kumari. C.R.Lalithakunjamma under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to her.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

INTRODUCTION

INTRODUCTION

Since the intensification of the cross breeding programmes and adoption of scientific methods of management, the profile of the average Indian cow is gradually changing from one of a casual supplier of milk to that of vigourous milk producer. With the evolution of high yielding animals it becomes imperative to evolve suitable methods of disease diagnosis, therapeutics and control to prevent loss to the farmer either due to mortality of animals or lowered production.

The importance of diseases of the udder, especially in dairy industry needs no special mention. There is not only loss of milk yield, but the quality of milk is also affected. Dhanda and Sethi (1962) reported an annual loss of 105 million rupees due to discarding of milk and involution of affected quarters. In addition to the economic aspects, the public health significance of mastitis has also to be seriously taken into consideration.

During the past few decades an enormous amount of work has been done and results published on different aspects of mastitis in India and other countries. A thorough knowledge of the pathological processes in the mammary gland, in addition to the agents involved in the production of these lesions, is essential to understand

the disease process completely. In Kerala, the information available on these aspects of the diseases of mammary glands is scanty. It is now well established that the severity of pathological changes in the udder, especially mastitis is determined not only by the nature of the infecting agent but also by the natural mechanisms of resistance of the animals in which apart from the immunological processes, the animal's hormonal status has a significant role.

There is considerable disagreement on the number of somatic cells encountered in milk in physiologic and pathologic conditions but it is now well established that there is marked increase in the somatic cells in mastitis. Even though the microscopic counting method has its own limitations with regard to accuracy and reproducibility, in conjunction with other tests used for detection of mastitis, this could be very usefully employed to assess the status of the mammary gland during disease processes.

The aim of the present work was

i) To study the various pathological lesions encountered in the mammary gland of cattle and goats from specimens selected from slaughter house and obtained from autopsy cases.

ii) To study the somatic cell count in milk from clinical and sub-clinical cases of mastitis.

squamous metaplasia, dystrophic calcification, calcification of arterial wall, epitheliosis, haemorrhage, acute catarrhal, acute suppurative, necrotic, subacute and chronic mastitis and some neoplastic conditions.

The teats, orifices, the main duct, and the cisternae exhibit many alterations, some of which may be held responsible for difficult milking. Increased number of teats (Polythelia), obliteration of the main ducts, lack of external orifice, thelitis, teat and milk fistulas, parturient udder oedema, hyperemia, haemorrhage, haematoma and udder ulcers are occasionally encountered (Steere et al. 1960; Heidrich and Renk, 1967). Skin diseases of the udder and teats like urticaria, burns, frostbite, photosensitization, exogenous and endogenous eczemata, lesions of Foot and Mouth disease, Pox and Pseudo cow pox, furunculosis, traumatic wounds of skin and teats and Stephanofilariasis have been recorded (Dirksen, 1959). Skin gangrene of the udder and teat in milking cows was reported during the milking period (Gold, 1943; White et al. 1959).

Some other pathological conditions frequently encountered were non specific catarrh of the udder (Peterson, 1938), chronic oedema of the udder, heterophic presence of melanophores in the mammary gland, cystic

Friedrichs (1919) reported a case of fibrosarcomata under the skin of the teats of heifers. A cystadenoma papilliferum and a sarcoma were encountered in a goat by Cotchin (1956).

A case of mammary intraductal carcinoma in a buffalo was reported by Mandal and Iyer (1969).

Singh and Iyer (1973) could describe fibrocystic disease or cystic hyperplasia, fibroadenoma, lipofibroadenoma and intraductal carcinoma in goats.

Etiology of Mastitis

More than 50 species of bacteria and more than 20 species of yeast like fungi had been demonstrated as causes of mastitis (Jubb and Kennedy, 1970). According to Plastridge (1958) a multitude of organisms are incriminated. The principal organisms associated with the mastitis were Streptococcus agalactiae, Streptococcus uberis and Micrococcus pyogenes (Nanjiah, 1956).

In a survey pertaining to the etiology of mastitis in cows in India, it was found that 41.2% of the affected animals had Staphylococcus, 15% Streptococcus agalactiae, 14.5% Streptococcus dysagalactiae, 7% Streptococcus uberis, 0.5% Streptococcus pyogenes, 0.2% Streptococcus zooepidemicus and 1% Streptococcus equisimilis. The corresponding figures for buffaloes are 30.5%, 7.9%,

11.3%, 17.2%, 4%, 4.6% and 10.6% respectively (Dhanda and Sethi, 1962). The chief etiological agent of clinical mastitis in goats was staphylococcus (80%), but Streptococcus agalactiae, Streptococcus dysagalactiae and Streptococcus pyogenes as well as other organisms were also observed (Kalra, et al. 1962). Waite and Blackburn (1962) studied a case of subclinical mastitis with Micrococci and Staphylococci. VanKruingen (1963) isolated Pseudomonas from 12 quarters of a herd of 25 cows by cultural examination of mastitis milk. Kalra and Dhanda (1964) found that in both clinical and latent infections 50% was due to Staphylococcus. Mastitis by Corynebacterium pyogenes in cows was found to be very common by Pargaonker, (1956). Krishnaswamy et al. (1965) examined 91 quarter samples of milk from 35 cows and nine buffaloes for etiological agents. The results were Streptococci 31.4% and 55.6% and Staphylococcus 51.4% and 22.2% of the quarters of cows and buffaloes respectively. Mixed infections of Streptococci and Staphylococci were observed in 5.7% of cows and no such case was seen in buffaloes. Very few cases with Pseudomonas, Coliforms and diptheroids were observed.

The commonest pathogen causing mastitis in goats in U.A.R. was Corynebacterium pyogenes and the second largest incidence was due to Staphylococcus aureus (26%).

Streptococcus agalactiae, Corynebacterium ovis, unclassified Corynebacterium, Pseudomonas aeruginosa and Escherichiae coli were also isolated (Farrag, 1966).

Staphylococcus were present in 61% of the milk and 64% of mammary parenchyma samples and 19% of the supra mammary lymph nodes in a study of bacterial flora of the udder of goats (Rao and Seetharaman, 1967).

Tannour and Malik (1968) reported the occurrence of Corynebacterium pyogenes in freshly drawn bovine milk.

Rao and Naidu (1969) in a laboratory investigation of the bacterial flora of clinical mastitis found that there was a preponderance of gram negative organisms (68.11%) over gram positive organisms (31.89%). Escherichiae coli was found to be the chief etiological agent. The other common organisms in the order of frequency were Klebsiella aerogenes, Staphylococcus pyogenes, Beta haemolytic streptococci, Pseudomonas pyocyanes, Faecalis alkaligens, and Paracolon species.

Cullen (1969) could isolate Streptococcus uberis from lactating and non-lactating cows.

Etiology of sub-clinical mastitis in cattle in Rajasthan from a preliminary survey on three cattle farms was found to be 50.9% Staphylococci, 13.2% Streptococci and 11.3% Pseudomonas(Bhatnagar and Meharotra, 1969).

Lee and Frost (1970) classified mammary pathogens as Staphylococcus aureus 42%, Streptococcus agalactiae 15%, Streptococcus uberis 10% and other Streptococcus 11%. In an abattoir survey of udder pathogen from culled dairy cows, no udder pathogens was found in 44% of the udders (Ziv and Nachman, 1972). Streptococcus, other than agalactiae were isolated from 44.5%, Staphylococcus aureus from 20.6%, Pseudomonas aeuriginosa from 8.1% and Streptococcus agalactiae from 3.2% of the udders.

Chander and Baxi (1975) examined milk samples from 304 quarters from apparently healthy cows and found that 56.4% of the quarters were infected. The organisms included 68.6% Staphylococcus and 16.2% Streptococcus.

Pepper et al. (1968) reported a severe outbreak of mastitis due to Pasteurella septica in a herd of cows.

Lepper (1964) reported a case of Mycotic mastitis in a dairy goat. Mastitis caused by Candida parapsilosis was reported in cows by Prasad and Prasad (1966). Singh and Singh (1968) isolated fungi from clinical cases of bovine mastitis. In Kerala, Candida albicans was isolated in pure culture from one milk sample out of 68 which were subjected to cultural

analysis (Rajan and Sivadas, 1969).

The prevalence of mycotic mastitis in cows and buffaloes in Hariana was found to be 6.7% and 3.7% respectively. Apparently healthy udders were found to harbour fungal infection in cows and buffaloes to the extent of 0.9% and 5% respectively. In goats 1.8% of diseased halves of the udders had mycotic infection (Monga and Kalra, 1971). They found that the fungi frequently isolated were Cryptococcus neoformans, Candida albicans, Candida krusei, Candida parapsilosis and Secharomyces species. Experimentally the disease could be produced in healthy goats by inoculating Cryptococcus neoformans and candida isolated. Candida albicans was found to be the common agent causing mycotic mastits (Sinha et al. 1974). Farnsworth and Sorensen (1975) experimentally infected the cows udder with candida but did not cause clinical mastitis and the subsequent treatment with antibiotics produced an increased leucocyte count. Jand and Dhillon (1975) reported mastitis caused by fungi and found that the incidence of mycotic mastitis in buffaloes, cows and goats was 5.8%, 9% and 0% in clinical cases and 6%, 9% and 9% in subclinical cases. Candida parapsilosis was the most frequent isolate, and the others were Candida gullermondie, Candida tropicalis,

Candida stellaloiden, Cryptococcus ater, Rhodotorula glutinis and Penicillium species.

Mastitis was also reported to be caused by Nocardia asteroids (Johnston and Connole, 1962 and Bruhl, 1963). From chronic lesions in the mammary glands of goats Nocardia was identified by Sharma and Iyer (1974). Actinomycotic lesions of mammary glands was reported by Yamagiwa et al. (1963).

Hale et al. (1962) reported a severe outbreak of mastitis in a dairy herd caused by Mycoplasma agalactiae var ovis. A similar case caused by Mycoplasma bovigenitalium was recorded by Stuart et al. (1963). Carnole et al. (1967) also encountered Mycoplasma mastitis in cattle.

Wilkinson (1963) recovered insect larvae from a case of mastitis in a cow.

Tuberculous mastitis in cows and buffaloes was usually due to Mycobacterium bovis (Singh et al.1957; Prasad, et al. 1968; and Mandal and Iyer, 1971) but Mycobacterium tuberculosis and Mycobacterium avium and saprophytic Mycobacterium were also isolated isolated from bovine udder (Heidrich and Renk, 1967).

Mycobacterium Johnei has been isolated from milk, udders and associated lymph nodes from clinically

affected cows (Doyle, 1954).

Testi (1962) described an eosinophilic mastitis in two cows and he could not elucidate the etiology and considered that the condition might be a delayed allergic phenomenon after the topical use of antibiotics.

Bovine ulcerative mammilitis and subsequent mastitis was found to be caused by a virus of the Herpes group (Martin et al. 1966 and Pepper et al. 1966).

Teat injury was found to be predisposing to mastitis (Kalra et al. 1962). Adams and Rickard (1963) recorded the anti-streptococcal activity of bovine teat canal keratin. Factors affecting the bacterial invasion of the bovine udder via the teat duct were patency of teat, suction back into the udder during and after milking, growth of the invading organisms through teat canal, hypersensitization of udder duct to previous infection and intra-mammary factors such as a germicidal factor in normal milk and changes produced in the udder after infection has commended and the presence of inhibitory or stimulatory intraductal substances, the strain, number and state of growth of the invading organisms (Newbould, 1964). Hickman (1964) described that the teat shape and size in relation to mastitis in dairy cattle. He found that funnel shaped teats had signi-

ficantly lower frequency of mastitis than cylindrical shaped teats.

There are many reports regarding mastitis caused by machine milking (Schalm et al. 1971). There was a severe outbreak of mastitis as a result of machine milking and the machine parts were found to harbour large number of organisms (Anon, 1976).

Pathological Lesions

Histopathological examination of udder tissue infected with Streptococcus agalactiae producing either clinically normal or abnormal milk, showed that acute inflammatory foci occur in few alveoli in one or more lobules (Jubb and Kennedy, 1974). There was interstitial oedema vacuolation and desquamation of acinar epithelium and accumulation of fibroblasts and macrophages. These areas later became fibrotic and new areas of inflammation developed (Plastridge, 1958). Udder tissue infected with Staphylococci and Micrococci revealed small abscesses and damage to the duct system. The milk had abnormal number of cells and chemical composition (Waite and Blackburn, 1963). They found that these changes were better indications of tissue damage than the presence of organisms. The incidence of active lesions in the lobules of all the quarters was only 1 to 6% but half or more of all the lobules were

involved. Brown and Scherer (1958) reported that the histological construction of Staphylococcal mastitis with nodules consisting of purulent necrotic mass with bands of connective tissue simulates actinomycotic process. Gangrenous mastitis has been reported in experimental Staphylococcal mastitis in goats (Derbyshire, 1958) and natural occurrence in some dairy cows (Minett, 1938; Parshall, 1934 and Schalm, 1944). The mild chronic mastitis is characterised by slowly progressing pathologic changes. A patchy effect in the tissue is noted initially with normal lobules intermingled with affected lobules (Stabenfeldt and Spencer, 1965).

Lee and Frost (1970) found that the organisms were present in all parts of glandular tissue with the exception of Staphylococcus aureus infection, where the isolation deceased in the dorsal part of the gland. The histopathological picture revealed scattered foci of mild changee in the alveoli and milk ducts. The interalveolar areas were oedematous and infiltrated with neutrophils and lymphocytes. Yamagiwa et al. (1963) studied and classified the histopathology of mastitis in slaughtered cows as lobular, diffuse and alveolar.

Jain and Sharma (1964) in their experimental study of Corynebacterium mastitis in goats found that non-

lactating goats mostly showed a severe reaction while the lactating animals exhibited only moderate inflammation. The histopathological changes in the udder were typical of acute suppurative mastitis. Abscess formation and necrosis were followed by fibrous tissue proliferation leading to pressure atrophy of surviving lobules. Toxin of Corynebacterium pyogenes inoculated via the teat canal in non-lactating goats produced transient reaction. The change, were almost similar to those with the live organisms.

Granulomatous mastitis caused by fungus produced granulomata varying from 0.25 to 2 cm. in diameter (Lepper, 1964). He described the lesions which were hard to cut in some cases but were not calcified while larger ones were filled with greyish-yellow fluid.

Mastitis due to Nocardia has been described by Sharma and Iyer (1974). The lesions of both gross and microscopic was those of typical granulomatous mastitis.

Actinomycotic mastitis showed numerous foci of granulomas (Yamagiwa et al. 1963). Ewes with mastitis due to Actinobacillus ligneresi showed acute necrotising mastitis, which later became purulent and fibrosed (Laws and Elder, 1969).

Hale et al. (1962) studied the pathology of Mycoplasma mastitis. There was acute purulent reaction

which subsequently became granulomatous. In the lumen of the ducts and alveoli there was coagulated secretion, fibrin deposit on the interalveolar connective tissue and lobular atrophy.

There are many reports of tuberculous mastits in India (Singh et al. 1955; Singh and Sen Gupta, 1957; Prasad et al. 1968 and Mandal and Iyer, 1971). Heidrich and Renk (1967) classified tuberculous mastitis on histological basis into productive lobular infiltrating tuberculosis, miliary tuberculosis, and acute exudative and caseating type.

Testi (1962) described eosinophilic mastitis in cows. It appeared as small green nodules in the parenchyma with strands of connective tissue, particularly around the cistern. Histologically the lesions were eosinophilic granulomas.

It has been reported that infection with Brucella abortus can hardly be recognised clinically or grossly but can be recognized histologically as a non-purulent interstial mastits (Renk, 1962). The quarters show cellular accumulations in disseminated lobules and in the vicinity of lactiferous ducts. These accumulations infiltrate and increase between the alveoli or the walls of the lactiferous ducts or they form round foci.

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The aim of the present work was

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REVIEW OF LITERATURE

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Published literature on the pathology of the udder is relatively few when compared with the reports on the clinical, bacteriological and economic aspects of mastitis. Various aspects of diseases of the mammary glands of domestic animals have been described in many text books (Little and Plastridge, 1946; Neiberle and Cohrs, 1966; Heidrich and Renk, 1967; Runnells et al. 1967; Jubb and Kennedy, 1970; Schalm et al. 1971 and Smith et al. 1972).

Incidence

Of the various pathological conditions of the udder the most important one considering the loss to dairy industry is mastitis. In India, mastitis was first reported by Joshi in 1926 (Krishnaswamy et al. 1965). Dhanda and Sethi (1962) reported that the incidence of clinical mastitis in cows was 3.9% while 48.8% had latent infections. In buffaloes it was 2.3% and 20.7% respectively. The corresponding figures in goats were 9.4% and 45.5% respectively (Kalra et al. 1962). The average incidence of mastitis in Punjab, both in rural and urban dairy establishments, was found to be 9.62% in cows and 10.55% in buffaloes. In these cases the hind quarters were found more often involved.

52.14% of the cows and 61.74% of the buffaloes were having occult infection; in this no special involvement of the hind quarters was noticed (Kalra and Dhanda, 1964). The incidence of sub-clinical mastitis in cattle from three dairy farms in Rajasthan was 11.7%, 21.5% and 24% respectively (Bhatnagar and Meharotra, 1969).

Krishnaswamy et al. (1965) found that there was increased incidence of mastitis with increasing lactation and more incidence in the first month of lactation. Farrag (1966) reported that the incidence of mastitis in goats in U.A.R. to be 22.8%. Rao and Naidu (1969) from a laboratory investigation found that the left half and hind quarters were affected with mastitis at greater frequency than right half and fore quarters. They could observe that the highest incidence of mastitis was seen during fourth lactation and fourth to sixth month after calving.

Another pathological condition of the udder is bovine ulcerative mammilitis (Martin et al. 1966). According to them the average incidence in milking stock was 50% and out of these 22% developed mastitis. A similar case was reported by Pepper et al. (1966).

Singh and Iyer (1972) studied the pathological conditions of goat udder and reported fatty changes,

squamous metaplasia, dystrophic calcification, calcification of arterial wall, epitheliosis, haemorrhage, acute catarrhal, acute suppurative, necrotic, subacute and chronic mastitis and some neoplastic conditions.

The teats, orifices, the main duct, and the cisternae exhibit many alterations, some of which may be held responsible for difficult milking. Increased number of teats (Polythelia), obliteration of the main ducts, lack of external orifice, thelitis, teat and milk fistulas, parturient udder oedema, hyperemia, haemorrhage, haematoma and udder ulcers are occasionally encountered (Steere et al. 1960; Heidrich and Renk, 1967). Skin diseases of the udder and teats like urticaria, burns, frostbite, photosensitization, exogenous and endogenous eczemata, lesions of Foot and Mouth disease, Pox and Pseudo cow pox, furunculosis, traumatic wounds of skin and teats and Stephanofilariasis have been recorded (Dirksen, 1959). Skin gangrene of the udder and teat in milking cows was reported during the milking period (Gold, 1943; White et al. 1959).

Some other pathological conditions frequently encountered were non specific catarrh of the udder (Peterson, 1938), chronic oedema of the udder, heterophic presence of melanophores in the mammary gland, cystic

dilation of the lactiferous ducts and microscopic pseudoconcrements (corpora amylacea). Embolism, thrombosis and vericosis were also noticed in some mammary glands (Heidrich and Renk, 1967).

Reports on the incidence of mammary tumours are rare in cows and goats (Swett et al.1940). An intracanalicular pappilliferous fibroma (Trotter, 1909), fibrous cisternal polyps (Julian, 1948), and papillary cystadenoma (Renk, 1955) have been reported. It appears that only few cases of primary adenocarcinoma of the mammary gland have been recorded in bovines. These were carcinomas of the duct system with metastasis to the supramammary lymph nodes, lung, and liver (Cleland, 1908; Kenny, 1944; Elder et al. 1954). Drabble and Massy (1926) described a metastatic cornifying squamous cell carcinoma of the udder of an old dairy cow.

Kronberger (1961) reported four papillomas, four fibromas, two adenomas and two sarcomas in the udder of cows. Papillomas arising from the skin of the udder and teat in cows and goats were often encountered (Jackson, 1936; Davis and Kemper, 1936; Gottschalk, 1938; Heichlinger, 1953; Moulton, 1954; Grunder, 1959). Davis et al. (1933) described a carcinoma of the skin in the supramammary region of the udder of a six year old cow.

In chronic oedema of udder there was fibrous thickening of the sub-cutaneous connective tissue, particularly in the vicinity of the attachments of the teats and this form of oedema is the result of congenital weakness of connective tissue.

In non specific catarrh of the udder chronic processes of the lactiferous ducts and glandular tissue that lead to secretory changes and that are similar to the inflammatory processes caused by bacteria have been reported by many workers (Peterson et al. 1938).

In most of the cases of bovine ulcerative mammilitis, there was swelling of the teat wall, the skin became soft and slonghed off revealing irregularly painful deeply ulcerated areas which heal slowly. Microscopically there will be ballooning degeneration of epidermis, intercellular oedema and necrosis leading to vesiculation. Multinucleated giant cells form within the epidermis. Intra-nuclear inclusion bodies are numerous in the epithelial cells and giant cells (Martin et al. 1966; Pepper et al. 1966; Smith et al. 1972).

Corynebacterium infection in goats produced changes in teats and suprammary lymph nodes. There was degeneration and desquamation of the cisternal epithelium, leucocytic infiltration and fibroblastic proliferation in the sub-epithelial tissue (Jain, 1965).

In the fibrocystic disease (Cystic hyperplasia), histologically there was dilatation of interlobular ducts and ductules, causing atrophy or even disappearence of neighbouring acinar structures, surrounded by increased amount of dense connective tissue and infiltrated by lymphocytes (Singh and Iyer, 1973). They found that the fibro adenomas, microscopically revealed separation of the acini and interlobular ducts by the fibrous connective tissue. Quarters with lipofibroma showed multiple lobular structures containing fat, separated by dense connective tissue septae. In the case of intra ductal carcinoma the neoplastic growths were multicentric in origin and had involved a large number of lobules either partially or completely and originated from inter lobular and intra-lobular ducts and ductules. The cells were round, oval or polyhedral in shape. In some the central core showed necrosis and occassionally dystrophic calcification.

Cells in Milk

Comparatively little work has been done on the cytology of milk, or the physiological process which govern the numbers and types of cells in milk. This is one of the fields of investigation which is likely to give most useful results in the control of mastitis.

Cells in milk of healthy cows were of two types, leucocytes and epithelial cells. Christansen (1929) found true plasma cells also in milk with a high total count and he divided the cells into lymphocytes, large mononuclear cells, transitory forms, eosinophils, basophils, neutrophils, mononuclear cells containing fat and epithelial cells. Blackburn and Macadam (1954) could differentiate agranulocytes and granulocytes with a special staining technique.

The polymorph is the main cell seen in abnormal milk. Its function is to phagocytose bacteria and other foreign particles. Animals with low cell counts were easily infected with small doses of bacteria (Schalam and Lasmanis, 1963). Kaneko et al. (1964) using labelled neutrophils found that most of these cells found in milk had originally been stored or retained in various body sites.

The lymphocytes in milk may be contributing gamma globulins, which may be immunologically specific under certain conditions. Jensen and Eberhast (1975) found that the highly vacuolated mononuclear cells in milk from normal bovine mammary glands usually designated as epithelial cells, were able to phagocytose viable Streptococcus agalactiae and Staphylococcus aureus and confirmed the properties of phagocytes both invitro and invivo.

In 1905, Doane divised a method for enumeration of the cells in milk by centrifuging 10 ml of milk and by placing the deposit in a Thoma-Zeiss haemocytometer. Prescott and Breed (1910) introduced their method, of spreading milk on a slide and counting which gained general acceptance and has been used with minor modifications even since. Cullen (1965) investigated electronic counters. Paape et al. (1965) and Janzen (1968) compared various methods of leucocyte count in milk. Blackburn (1965) suggested a solution for use in assessing the cell count of cow's milk.

In mastitis the cell count is raised and the differential count is altered (Cullen, 1966). Normal milk had a leucocyte formula similar to that of blood, that is a lymphocyte: polymorph ratio of about 2 : 1. In mastitis milk nearly all the cells were polymorphs. Under pathological conditions the higher the cell count higher the percentage of polymorphs upto a maximum of about 90% (Waite and Blackburn, 1957). The increase in number of cells is thought to be due to chemotactic stimuli which result from the presence of micro-organisms in the udder (Cullen, 1966).

Significance of different levels of cell count in milk

The cell count alone is not a criterion for diagnosis of mastitis. A cell count varying from

300,000 to 10,000,000 per ml. was found to be normal depending upon the age of the animal and stage of lactation (Little, 1938). Milk from normal quarters rarely contain more than 500,000 leucocytes per ml. and the milk from infected quarters usually exceeds this number (Plastridge, 1958). Diurnal variation in cell count of cow's milk had been noted depending upon the time of lactation (White and Rattray, 1965). The cell count itself is of limited value in mastitis diagnosis. So it must be combined with cultural examination and confirmed as mastitic if any of the pathogen present regardless of the cell count and if there are 1,000,000 cells per ml. with or without organisms (Grees and Pearson, 1973). They found that there was a good correlation between the Streptococcal isolation and the high cell count.

Of the various indirect tests like Mastaid, Sodium Lauryl Sulphate Teepol test, Plate milk granulation, Cathalase test and leucocyte count, the leucocyte count showed the highest percentage of agreement with the bacteriological examination (Chander and Baxi, 1975).

The differential count along with the value of total count can give very useful informations (Blackburn et al. 1955). Dattatraya (1971) studied the cells in

milk of cows and buffaloes in different stages of lactation to get an idea of differential picture of milk from healthy as well as infected quarters of udder. He correlated the cell count with differential count, bacteriological examination and Sodium Lauryl Sulphate Teepol Test. A count of 100,000 to 500,000 cells per ml. raised suspicion and a count above 500,000 cells per ml. was suggested as a conclusive evidence of mastitis.

MATERIALS AND METHODS

MATERIALS AND METHODS

Whole udders of cows and goats were collected from the Municipal slaughter house, Trichur; Veterinary College slaughter house, Mannuthy and from animals brought for autopsy. The specimens were kept in 10% formalin for few hours just to harden which facilitated easy dissection. The tissues were examined grossly by dissecting through the lactiferous ducts and by slicing the tissues. Then small pieces of tissues from different fixed protions from each quarters were collected and preserved in 10% neutral formalin. Eight pieces of tissues were examined routinely from all quarters in addition to specific areas showing lesions. After fixing, the tissues were processed by the paraffin embedding method and sections of 5 micron thickness were taken. The sections were stained with haematoxylin and eosin. Selected sections were stained by the following methods: Van Gieson (Luna, 1968), Gram Weigert method (Davis, 1957) and Ziehl- Neelsen (Disbrey and Rack, 1970). When required some sections were stained with Sudan III and IV and with vonKossa for calcium salts.

A total of 300 cows and 500 goats were examined for udder abnormalities by gross examination and by palpation. Out of these 200 quarters from cows and

sixty seven halves from goats were selected for a detailed histopathological study.

Milk Samples

Milk samples were collected from 105 quarters of cows and 84 halves from goats for somatic cell count, bacteriological examination and testing for mastitis using the mastaid reagent (Glaxo). Cows and goats admitted to the Veterinary hospital, Trichur and those in the livestock farm, Mannuthy were clinically examined. The examination of the udder was intended to detect any lesion of the mammary gland. The size, shape and consistency of the mammary glands were checked by visual examination and palpation. Milk was collected both from clinical cases, sub-clinical cases and from apparently normal udders, which were detected by the mastaid reagent. All these samples were collected in sterilized vials in a sterile manner.

Detection of Sub-clinical mastitis by mastaid reagent

Procedure

Mastaid solution supplied by Glaxo was used. Approximately 3 ml of milk was collected in the receptecles of the plastic paddle supplied with the testing solution. Added equal volume of the testing

solution and mixed by slow circular movements (Chander and Baxi, 1975).

The results were noted as

Negative	(-)	no precipitate
Doubtful	(+)	distinct precipitate with a tendency to disappear
Positive	(++)	thick mixture with precipitation and gelatinisation
Strongly positive	(+++)	distinct gel formation which tend to adhere to bottom with a central peak on mixing.

Cultural examination for micro-organisms

All the milk samples were streaked in blood agar plates for detecting bacterial organisms if present (Merchant and Packer, 1971) and in Sabouraud's dextrose agar for fungal organisms (Davies, 1957). The blood agar plates were examined after 24 hours incubation at 37°C and then the colonies subcultured on blood agar slants and identified.

The samples cultured on Sabouraud's dextrose agar were examined upto a period of one month.

Somatic cell count of milk

Total and differential count of these milk samples were also studied.

Total cell count

0.01 ml. of milk using a standard platinum loop was spread on a slide on one square cm. area and dried (Prescott and Breed, 1910).

The stain used was modified Broadhurst Paley Stain (Schalm et al. 1971) which was prepared as follows:

Dissolved 1.5 gram of methylene blue in 250 ml. of hot 70% ethyl alcohol. Added 10 ml. of saturated alcoholic basic fuchsin solution. Added 5 ml of aniline and mixed well while keeping the solution warm. Added 5 ml. of dilute sulphuric acid (5 ml. of concentrated acid in 95 ml. of distilled water). Mixed well, warmed and filtered. To every 100 ml. of the filtrate added 50 ml of hot distilled water and shook well. Stored the stain in glass stoppered bottle in the refrigerator.

Procedure

1. Immersed the dried smear in xylene for 2 minutes drained and dried
2. Kept in absolute ethyl alcohol for 5 minutes
3. Immersed in Broadhurst Paley stain for 5 seconds or longer to obtain the proper intensity of staining. Rinsed the slide in water, dried and examined using an oil immersion microscope with a total magnification of 900.

Milk solids stained pink, cells blue and pale blue and bacteria either deep or light blue.

Counting and calibration of microscope

A minimum of 50 microscopic fields were examined and the average cell count per field was noted (Schalm et al. 1971).

The average cells counted multiplied by the working factor gave the number of cells per ml of milk. The working factor was calculated by dividing the microscopic factor by the number of fields counted. The microscopic factor was obtained by using the formula Πr^2 where r is the diameter of the field seen through oil immersion microscope at a magnification of 900. Since 0.01 ml (1/100) of milk was spread over an area of 1 cm^2, the possible number of such fields which can be counted in 1 cm^2 was calculated. This is the microscopic factor.

Differential count of cells in milk

About 10 ml of milk sample was centrifuged at 200 x G for 10 mts. Discarded the supernatent 9 ml and removed the fat layer with cotton. Mixed the sediment and prepared films on slides and dried. Treated the slides for 2 mts in xylene to remove the fat and then placed in methanol for 2 - 5 minutes.

After drying the smears were stained with wright's stain. Drained, dried and counted the different type of cells (Schalm et al. 1971).

RESULTS

RESULTS

The results of the histopathological examination of the tissues from the udders of cows and goats are summarised in table 1.

Cows

A total of 200 mammary quarters were examined histologically out of which 18 were found to be apparently normal. 61 quarters were in various stages of involution. The glandular elements were atrophic but the ductal system was more or less intact. There was increased interstitial connective tissue formation. Lymphoid infiltrations, were occasionally found. Fatty infiltration was noticed in certain cases.

Catarrhal Mastitis and Galactophoritis

Thirty two quarters showed various changes associated with catarrhal mastitis. The gross appearance of the glandular tissue showed great variation. The affected part was reddish, moist or slightly raised above the level of normal tissue from which it was sharply demarcated. Granular firm secretary lobules were found interspersed with soft yellowish white elastic lobules. Small nodules showing connective tissue induration and flakes of exudate in the ducts and cisterns were also observed. Frequently hardened areas of various sizes were found.

The histological changes in the glandular tissue and lactiferous ducts, even though well defined in some cases and could be classified as acute and chronic, were mostly cases which showed both those type of changes in the same quarter.

In eight cases the inflammatory changes were not strictly confined to the glandular tissue. In these, the cisterns and lactiferous ducts were also affected.

The changes in the epithelial lining of the cistern and ducts showed extreme variation. In some areas there was only slight separation and vacuolation of the epithelial cells, (Fig.1) but in others they were partly or completely desquamated. Stratification of the lining cells with attempted squamous metaplasia was also noticed. In some cases the cisternal and ductal epithelium showed polypous thickening (Fig.2). Because of this papilliform projection the lumen especially of the cisterns appeared irregular. There was collagenous thickening of the ductal wall.

Infiltration of cells was either scanty or massive. The infiltration was seen in the cisterns and ducts as well as in the parenchyma and the cells were mostly lymphocytes, neutrophils and macrophages (Fig.3). Plasma cells were also present. Occasion-

ally in the slightly oedematous sub-epithelial tissue, along with leucocytes, fibroblasts and angioblasts were present.

The glandular epithelium was tense in many cases and filled with colloid like secretion. The exudation when present in the alveoli consisted mainly of neutrophils and desquamated cells (Fig.4). The quantity of exudate varied between lobules. The quantity of neutrophils were massive in some cases to present a catarrho-purulent picture (Fig.5). The alveolar epithelium showed varying grades of degeneration. The degenerative changes were very minimal in some alveoli. This was manifested as minute droplets of fat in the epithelium. In other cases there was hydropic degeneration and nuclear pyknosis and disintegration. Frank necrosis and desquamation were encountered in more severe cases. The alveolar septa was widened and infiltrated with inflammatory cells.

In five quarters the changes in the gland tissue were not very pronounced and the reaction was more marked in the duct system. In these cases there was slight increase of collagenous tissue. Polypous thickening with or without desquamation of the epithelium was very often observed. The cellular reaction was more of the

lymphoid type even though a few macrophages were also seen. In well defined chronic cases, the lactiferous ducts and the cistern also showed proliferative reaction. In many of these cases the secreting and pieces had completely been replaced or become partially atrophic because of fibrous tissue overgrowth (Figs. 6 and 7). The gland lobule on the whole had become small with well dilated hyperplastic ducts prodominating. Cicatrization was found very prominent around some lactiferous ducts. In these cases where the cisternae or a few acini were retained, they revealed cystic alterations (Fig. 8).

Suppurative Mastitis

Ten quarters from 4 animals showed suppurative reaction. There were multiple abscesses with pus with a foul odour. Some of the larger abscesses gave a palpable lumpiness to the gland. Usually they were found in the vicinity of lactiferous ducts. Standards of connective tissue were found radiating into the surrounding udder tissue.

In addition to the large abscesses visualised grossly there were numerous microabscesses. Many of these microabscesses were surrounded by involuted lobules. The disseminated foci of suppurative areas could be seen encircled by granulation tissue (Fig.9). The

granulation tissue showed varying amounts of fibrous tissue formation with corresponding destruction of normal tissue. In the larger abscesses which were seen in chronic forms the proliferative processes were very pronounced. The interstial tissue around these areas were also markedly thickened. The inflammatory cells within the abscess showed complete disintegration (Fig.10) and the whole abscess was filled with liquified or inspissated material. Around the fibrous tissue there was cellular reaction, consisting mainly of lymphocytes and mononuclear cells. Many of the lobules were involuted. In addition, because of the proliferative reaction around the cisterns, there was a reduction in the size of the lumen. In many cases they were almost obliterated. The ducts cisterns and some alveoli in the vicinity of the abscesses were found to contain exudate mainly consisting of degenerated neutrophile (Fig.11). The lining epithelium of the ducts showed degeneration and necrosis. Desquamation was seen in some areas while apparent repair as evidenced by hyperplastic reaction was noticed at some location in the ducts (Fig.12). Corpora amylacea or frank concretions were also noticed.

Specific granulomatous mastitis

Udders of two animals were found affected with tuberculosis. In one all the quarters were involved

while only two quarters were involved in the other. Both these animals had tuberculous lesions in other organs also (No.44 also had tuberculous lesions in the uterus and lungs while No.50 had lesions in kidney, liver and lungs).

The mammary tissue was slightly firm and when cut in some parts the lobulated structure appeared pronounced while other areas had a mottled appearance. Most of the lobules varied from greyish red to white in colour and the surface was dry. Well developed strands of fibrous tissue could be seen ramifying the udder tissue. Small caseous areas of irregular size and shape were noticed. Some areas when cut had a slight gritty feeling.

The affected quarters showed numerous caseated areas involving many lobules (Fig.13). In some areas the lobular structure was completely obliterated. Occasionally a few lobules were found to have retained their histological integrity. But in these there was massive infiltration of lymphoid cells and the acini were found atrophic. In addition to the glandular structure both interlobular and intralobular ductal systems were heavily affected. The cellular reaction around the caseous areas was found mostly consisting of epithelioid cells and giant cells. A few plasma

cells were seen. Away from the caseating areas the cells encountered were predominantly lymphocytes and monocytoid cells of the macrophage type (Fig.14). Generally, giant cells of the Langhans type were very few. While there was heavy collagen deposition with marked fibrous tissue predominance in the interlobular septa and within the lobules, in other locations only proliferating fibroblasts were noticed. A few of the caseated areas showed foci of calcification.

Acid fast bacteria could be demonstrated in these udders.

Non-purulent interstitial mastitis

Forty six quarters from 22 animals revealed various degrees of interstitial mastitis. The glands were more firm than normal and the interlobular septa appeared more accentuated.

The essential histological changes were diffuse and focal cellular infiltration with varying grades of fibroblastic proliferation. The cell types were mostly lymphocytes, plasma cells and macrophages. Occasionally neutrophils were also seen.

In twelve of these udders the reactions were classified as chronic interstitial mastitis because of

the excessive fibrous tissue reaction and massive lymphoid infiltration (Fig.15). Attached to one of these quarters on the periphery of the glandular tissue was a lymphoid noduble of 1 mm. diameter. Histologically this revealed fibrous encapsulation in which was found lymphoid accumulation with germinal centre formation (Fig.16). These lymphocytes were found to be in a net work of argyrophil fibres. Two of the quarters had a focal interstitial mastitis with a predominant lymphoid cell reaction rather than fibroblastic proliferation and fibrous tissue deposition. In one of these the glandular tissue showed stasis of eosinophilic colloid in the alveoli and ducts (Fig. 17), fatty replacement of the parenchyma and subsequent overall reduction of the secreting element. Cellular infiltration of the septa was often accompanied by narrowing of the alveoli which showed signs of atrophy. Occasionally here and there were seen few macrophages and lymphocytes in the lumina of the alveoli. In advanced cases most of the secretory end pieces were completely obliterated by the proliferated fibrous tissue and cell proliferation (Fig.18). In many of them, the ducts appeared cystic (Fig.19). Concretions which were positive for calcium were seen in many of the lobules either many or few in number.

Four quarters were having only very slight interstitial reaction. There was slight oedema and the cellular reaction was minimal. Corpora amylacea and concretions were seen but only in limited numbers.

Acute diffuse mastitis

Eight quarters were found to be affected with the acute diffuse type of mastitis. Grossly the udders were enlarged and dense. When incised the cut surface was moist in two cases while the others showed marked hyperaemia. The cavitary system of the udders showed contents of abnormal colour and consistency.

Microscopically varying degrees of oedema and hyperaemia were seen in the inter lobular and intralobular connective tissue. There was marked dialation of lymph vessels and blood vessels and in some specimens thrombosis could also be demonstrated. The alveoli and interstitial tissue contained desquamated epithelial cells. The destruction of the epithelial cells was minimum or very severe. The exudate was either serous or fibrinous (Fig.20 and 21). Smaller or larger groups of acini were seen filled with strands of fibrin.

Thecisterns and the duct system also showed varying degrees of degenerative and inflammatory reaction (Fig.22).

The leucocytic reaction was very severe in some and consisted mostly of neutrophils. Marked monocytoid reaction was also seen in the interstitial tissue.

Necrotising mastitis

Two quarters showed multiple disseminated areas of necrosis which were demarcated by fibrous tissue. The capsule was thick and in the inner layers of the capsule progressive nuclear degeneration was observed. The necrotic areas revealed a structureless mass with degenerating leucocytes in the periphery (Fig.23). The vicinity of the necrotic areas the interstial connective tissue shows pronounced thickening resulting in extensive atrophy of the intervening glandular lobules.

Corpora amylacea and concretions

Very often most of the mastitic udders revealed numerous oval, round laminated or homogenous or slightly granular bodies varying in size from 15 microns to 200 microns in diameter in the alveoli and interalveolar tissues (Fig.24). They were seen at all locations, but mostly within the alveoli. The associated tissues were either normal or they showed regressive changes or were completely necrotic. Many of them were bright eosinophilic with a smooth or a slightly wavy contour.

In some others the eosinophilic structure was fringed with basophilic laminations which were positive for calcium by the Von Kossa method. When fat stains were employed the eosinophilic material did not reveal any positive reaction. Some of the bodies were completely basophilic and more dense. These structures did not reveal any calcium salts in them. Completely calcified concretions with numerous laminations or only with a laminated shell were also encountered. There was no cellular reaction around these bodies.

Goats

Out of the 67 halves examined from 35 goats eight halves were normal secreting glands. Of these two were showing moderate thickening of the interstitial tissue and two halves had slight epithelial hyperplasia and focal dilatation of the alveoli.

Twenty four halves were in various stages of involution as in cows. Many of the udders with normal regressive changes as well those with lymphoid reaction showed partial fatty replacement of glandular tissue (Fig.25). Depending upon the degree of fatty replacement the glandular portions showed corresponding reduction. In almost all these regressing glands corpora amylacea were present in large numbers and in some there was

very extensive calcification of these bodies. Two halves were showing hyperplasia of the duct epithelium in addition to all the above mentioned changes. Another gland was having focal interstitial and catarrhal mastitis along with the regressive changes in both halves. The interstitial tissue was infiltrated with neutrophils and lymphoid cells and the alveoli and the ducts also had desquamated epithelial cells and neutrophils along with the secretion.

Six animals showed interstitial mastitis in all the halves along with galactophoritis in some halves. Two halves had multifocal lymphoid reaction in the interstitial septa and destruction of acini in some areas (Fig.26). Others had a mixture of cellular infiltration consisting of neutrophils, lymphocytes, plasma cells and macrophages. Degeneration of the alveolar epithelium, collagenous thickening of the septa, galactophoritis and corpora amylacea of varying stages were also noticed. There was desquamation of the duct epithelium and collection of lymphocytes in the subendothelial area of the ducts. Another four halves also showed the same type of reaction with the characteristic lymphoid infiltration in the septa. The other inflammatory changes were not so prominent as the first one. Interlobular fibrosis, oedema and focal acinar reaction were also noticed. The acini

were filled with secrection along with desquamated epithelial cells and few lymphocytes and plasma cells (Fig.27). In these two galactophoritis was not so prominent. One animal had galactophoritis and mild interstitial mastitis characterised by the lymphoid reaction along with regressive changes.

Two halves were having interstitial mastitis and galactophoritis and there was numerous corpora amylacea and squamous metaplasia of the alveolar and duct epithelium. Another gland in these group was having chronic interstitial mastitis. The interstitial septa was very much thickened with fibroblastic proliferation and infiltration with lymphoid cells and revealed corpora amylacea and fatty replacement of parenchyma and subsequent reduction of glandular portions.

Six quarters were from known cases of Johne's disease. They showed diffuse lymphoid reaction along the interlobular septa (Fig. 28). The infiltration was found more intense near lymphatics and blood vessels. The lymphoid cells were found infiltrating into the intralobular connective tissue also more or less in a centripetal fashion. In most locations the alveolar epithelium did not manifest severe degenerative changes except in one, where masses of lymphoid cells were found replacing a few acini near the septa. Even though a few number of macrophages were seen, epithelioid cells

were absent. It was not possible to demonstrate acid fast bacteria in the tissue.

One animal had necrotic mastitis in one half alone. The other half was normal. The affected half showed complete necrosis of the tissues. The normal architecture of the gland was lost and the ducts and alveoli were filled with inflammatory cells which were completely or partially necrotic. Remnants of neutrophils and macrophages were seen. Clumps of bacteria with coccoid features were seen in sections.

Two animals had gangrenous mastitis in their right half alone. The udder was swollen bluish red in colour and the secretion was bloody. There was complete destruction of the mammary tissue and necrosis (Fig.29). The structure of the mammary gland was lost. The lining epithelium of ducts and alveoli was destroyed and tissue debris was present in the lumen. There were numerous corpora amylacia which showed calcification.

One animal showed acute suppurative mastitis in both the halves of the udder. In this case also the udder was enlarged and had purulent exudate. Microscopically there were multiple suppurative areas and interstitial fibrous thickening. There was loss of epithelium of the alveoli and the lumen was filled with cellular debris (Fig. 30). Neutrophils could be identified among degenerated cells and around necrotic tissue

and some of the large cisterns and ducts showed squamous metaplasia of the lining epithelium and focal destruction of the epithelium (Figs. 31 and 32).

Six halves had general interstitial fibrosis and two halves had glandular atrophy (Fig.33), and focal catarrhal mastitis. Two halves and severe loss or atrophy of alveolar and pieces, prominent development of duct system and severe interstitial collagenous thickening (Fig. 34). Six halves had interstitial oedema, fibrosis, focal lymphocytic accumulation in the interstitial area and focal dilation of the alveoli. There were areas of congestion and numerous corpora amylacea.

Another two halves were having interstitial fibrosis and oedema with stages of involution.

Six halves from 3 animals showed varying grades of degeneration and calcification of the arterial wall. In one case fhe sections of artery showed severe diffuse calcification in the media in a tracheal ring fashion (Fig. 35). In the other cases also blood vessels showed calcification but in a milder degree. Calcification was seen either as granular deposit or linear or ribbon like streaks sub endothelially and in the media (Figs. 36 and 37).

Milk Samples

Samples of milk from 105 quarters from cows and from 84 halves goats were examined. On the basis of the

mastaid reaction, the sub clinical cases of mastitis were classified as +, ++, +++ depending upon the intensity of reaction. Milk samples from clinical cases of mastitis were also collected.

Twenty samples of normal milk each from cows and nineteen goats were examined. None of them showed any bacterial growth. The somatic cell count varied from 150,000 to 500000 in cows while it was 300,000 to 500,000 in goats. The absolute number of neutrophils per ml varied from 70,000 to 220,000 in cows and 60,000 to 620,000 in goats. The differential count was 15 to 43% of neutrophils in cows and 12 to 31% in goats, were as that of lymphocytes was 50 to 82% and 44 to 78% in cows and goats respectively. The percentage of epithelial cells was 0 to 6% in cows and 0 to 36% in goats. No monocytes, eosinophils or basophils could be idenfified in normal milk samples (Fig. 38).

In the subclinical mastitis (diagnosed on the basis of the mastaid reaction) all the 40 samples from cows were positive in bacterial culture and the organism included Staphylococcus 62.50%, Streptococcus 2.5%, Staphylococcus and Streptococcus 15%, Coliforms 10%, Staphylococcus and Coliforms 7.5% and Corynebacterium 2.5%.

Out of 35 samples examined from goats 34 were found positive on bacteriological examination. The organisms

were 88.24% Staphylococcus, 8.82% Streptococcus, 2.94% Staphylococcus and Streptococcus. None of these samples revealed fungi.

The somatic cell count in subclinical mastitis varied from 500,000 to 30,000,000 in cows and 1,000,000 to 24,900,000 per ml in goats. The absolute number of neutrophils was 130,000 to 18,700,000 in cows and 470,000,000 to 16,560,000 per ml in goats (Figs. 39 and 40).

The differential count in subclinical mastitis showed great variation. Neutrophils ranged from 19% to 92% in cows and 18% to 76% in goats, lymphocytes, 7% to 69% in cows and 19 to 69% in goats. Epithelial cells, 0 to 38% in cows and 0 to 45% in goats. Monocytes ranged from 0 to 2% both in cows and goats and Eosinophils 0 to 12% in cows and 0 to 14% in goats.

Bacteria could be isolated from all clinical cases of mastitis. 45 samples from cows and 30 samples from goats were examined. The organisms were Staphylococcus 57.78%, Streptococcus 4.44%, Staphylococcus and Streptococcus 20.00%, Coliforms 6.67%, Corynebacterium 8.89% and Staphylococcus and Corynebacterium 2.22% in cows. In goats, Staphylococcus 63.33%, Streptococcus 10%, Staphylococcus and Streptococcus 16.67%, Coliforms 6.67% and Staphylococcus and Coliforms 3.33%. Here the total somatic cell count ranged from 1,250,000 to 30,000,000 in

cows and 1,000,000 to 30,000,000 per ml in goats. In few samples there was only clumps of bacteria and clear or light yellow fluid without a single cell. The absolute number of neutrophils ranged from 400,000 to 26,400,000 in cows and 640,000 to 23,400,000 in goats. The differential count was neutrophils, 12% to 88% and 24% to 88%, lymphocytes, 10% to 82% and 10% to 57%, epithelial cells, 0 to 37% and 0 to 40%, Monocytes, 0 to 6% and 0% and eosinophils 0 to 10% and 0 to 7% for cows and goats respectively.

The results of the examination of milk samples are summarised in table II and III.

Results of the Histological Examination of Tissues

Cows

Sl.No.	Histological diagnosis	Remarks
1	Stages of involution with intense lymphocytic reaction	All the four quarters.
2	Catarrhal mastitis (desquamative type)	All the four quarters.
3	Galactophoritis and mastitis	All the four quarters
4	Stages of involution and focal galactophoritis with fatty replacement	All the four quarters
5	Stages of involution	All the four quarters
6	Galactophoritis and mastitis	All the four quarters
7	Suppurative mastitis	Only in two quarters
8	Stages of involution with fatty replacement	All the four quarters
9	Galactophoritis	Only in two quarters
10	Mild catarrhal mastitis	All the four quarters
11	Interstitial mastitis	All the four quarters
12	Interstitial mastitis	All the four quarters
13	Mild catarrhal mastitis	All the four quarters
14	Stages of Involution	All the four quarters
15	Interstial mastitis with cystic dialatation of ducts and galactophoritis	All the four quarters
16	Stages of involution	All the four quarters

Sl.No.	Histological diagnosis	Remarks
17	Focal galactophoritis and stages of involution	In right fore quarter and involution in other quarters
18	Interstitial mastitis (subacute)	All the four quarters (focal squamous metaplasia in left hind quarter)
19	Stages of involution	All the four quarters
20	Stages of involution with numerous corpora amylacea	All the four quarters
21	Stages of involution	All the four quarters
22	Suppurative mastitis	All the four quarters
23	Suppurative mastitis	All the four quarters
24	Acute diffuse mastitis	All the four quarters
25	Normal	-
26	Mild interstitial mastitis with oedema and corpora amylacea	All the four quarters
27	Stages of involution	All the four quarters
28	Mild interstitial mastitis (cellular)	All the four quarters
29	Catarrhal mastitis	All the four quarters
30	Interstitial mastitis	Only in two hind quarters
31	Stages of involution	All the four quarters
32	Mild catarrhal mastitis	All the four quarters
33	Mild catarrhal and interstitial mastitis	All the four quarters
34	Interstitial mastitis (Chronic)	All the four quarters (Squamous

Sl.No.	Histological diagnosis	Remarks
		metaplasia and ectopic lymphnode in the left fore quarters
35	Interstitial mastitis (mild)	All the four quarters
36	Catarrho-purulent mastitis	All the four quarters
37	Stages of involution	All the four quarters
38	Galactophoritis	All the four quarters
39	Catarrhal mastitis	All the four quarters
40	Interstitial mastitis	All the four quarters
41	Interstitial mastitis	All the four quarters
42	Interstitial mastitis	All the four quarters (focal squamous metaplasia in the right fore quarter)
43	Stages of involution	All the four quarters
44	Tuberculous mastitis (without calcification)	All the four quarters
45	Cystic dilatation of ducts, and involution	All the four quarters with prominent fatty replacement in two quarters
46	Oedema	All the four quarters
47	Necrotising mastitis	Only in two quarters
48	Catarrhal mastitis	All the four quarters
49	Acute diffuse mastitis	All the four quarters
50	Tuberculous mastitis (with calcification)	All the four quarters

Goats

Sl.No.	Histological diagnosis	Remarks
1	Interstitial mastitis	Both halves (known case of Johne's disease)
2	Atrophy of glandular portions	Both halves
3	Interstitial fibrosis	Both halves
4	Interstitial fibrosis	Both halves
5	Stages of involution with lymphoid reaction	Both halves
6	Interstitial mastitis	Both halves (known case of Johne's disease)
7	Stages of involution with lymphocytic and fibroblastic reaction	Both halves
8	Stages of involution	Both halves
9	Normal gland	-
10	Necrotising mastitis	Only one half
11	Gangrenous mastitis	Only one half
12	Stages of involution	Both halves
13	Suppurative mastitis with squamous metaplasia	Both halves
14	Stages of involution	Both halves
15	Oedema and congestion	Both halves
16	Stages of involution with lymphocytes and calcified concretions	Both halves
17	Stages of involution	Both halves
18	Interstial mastitis	Both halves (known case of Johne's disease)
19	Stages of involution	Both halves
20	Oedema and congestion	Both halves
21	Interstitial mastitis (calcification of vessel wall)	Both halves

Sl.No.	Histological diagnosis	Remarks
22	Gangrenous mastitis	Only one half
23	Stages of involution	Both halves
24	Stages of involution (Oedema and calcification of vessel wall)	Both halves
25	Interstitial mastitis and galactophoritis with squamous metaplasia)	Both halves
26	Oedema and congeston with cystic dilation of alveoli	Both halves
27	Focal catarrhal mastitis	Both halves
28	Mild catarrhal and interstitial reaction with stages of involution	Both halves
29	Stages of involution with lymphoid reaction	Both halves
30	Degeneration and calcification of arterial wall (stages of involution)	Both halves
31	Normal gland	-
32	Galactophoritis and interstitial fibrosis	Both halves
33	Normal gland	-
34	Normal gland	Both halves
35	Catarrhal mastitis	Both halves

Table II

Results of Examination of Milk Samples

a) Somatic cell count - Total number of cells/ ml

	Cows (10^6)	Goats (10^6)
Normal	0.15 to 0.50	0.30 to 0.50
Subclinical mastitis	0.50 to 30.00	1.00 to 24.90
Clinical mastitis	1.25 to 30.00	1.00 to 30.00

b) Somatic cell count - Range of neutrophils / ml

Normal	0.07 to 0.22	0.06 to 0.62
Subclinical mastitis	0.13 to 18.70	0.47 to 16.56
Clinical mastitis	0.40 to 26.40	0.64 to 23.40

c) Somatic cell count - Range of percentage of cell components

	Cows	Goats
i) Neutrophils		
Normal	15% to 43%	12% to 31%
Subclinical mastitis	19% to 92%	18% to 76%
Clinical mastitis	12% to 88%	24% to 88%
ii) Lymphocytes		
Normal	52% to 82%	44% to 78%
Subclinical mastitis	7% to 69%	19% to 69%
Clinical mastitis	10% to 82%	10% to 57%

	Cows	Goats
iii) Epithelial cells		
Normal	0% to 6%	0% to 36%
Subclinical mastitis	0% to 38%	0% to 45%
Clinical mastitis	0% to 37%	0% to 40%
iv) Monocytes		
Normal	Nil	Nil
Subclinical mastitis	0% to 2%	0% to 2%
Clinical mastitis	0% to 6%	Nil
v) Eosinophils		
Normal	Nil	Nil
Sub clinical mastitis	0% to 12%	0% to 14%
Clinical mastitis	0% to 10%	0% to 7%

Table III

Incidence of Various Bacteria

a) Clinical mastitis

	Cows	Goats
Staphylococcus	57.78%	63.33%
Streptococcus	4.44%	10.00%
Staphylococcus and Streptococcus	20.00%	16.67%
Coliforms	6.67%	6.67%
Staphylococcus and Coliforms	Nil	3.33%
Staphylococcus and Corynebacterium	2.22%	Nil
Corynebacterium	8.89%	Nil

b) Subclinical mastitis

Staphylococcus	62.5%	88.24%
Streptococcus	2.5%	8.82%
Staphylococcus and Streptococcus	15.00%	2.94%
Coliforms	10.0%	Nil
Staphylococcus and Coliforms	7.5%	Nil
Corynebacterium	2.5%	Nil

DISCUSSION

DISCUSSION

During the present investigation a total of 200 quarters from cows and 67 halves from the udders of goats were subjected to detailed histological study. Except for 18 quarters in cows and eight halves in goats, all the others showed varying grades of pathological alterations - from degeneration and desquamation of a few epithelial cells to advanced cases of necrosis and gangrene. Most of the materials in this investigation were collected from slaughter house from animals which were apparently healthy. This study of the nature of the lesions in the udder dosnot truly reflect the different pathological processes that are commonly seen in the mammary glands of animals since only a particular group of animals, mainly non-lactating, are brought for slaughter. However, detailed histopathological examination of the udder revealed varying degrees of involvement of the udder.

The commonest lesion observed in the glands of both cows and goats was mastitis. Varying types and intensity were encountered; from subtle changes to rather intensive involvement with complete obliteration and glandular atrophy. The tissue reaction was more in the nature of a proliferative type than exudative. Fibrous tissue proliferation and lymphoid reaction

were significant features, and this can be considered quite natural since most of the materials collected were not from clinical cases of mastitis. In a similar study from specimens obtained from Trichur slaughter house Pillai (1970) found 25% of the udders examined had chronic mastitis.

The duct system was invariably involved in most of the cases of mastitis. The subtle distinction between galactophoritis and mastitis was not possible always but in a few cases, only the lactiferous duct and cisterns were found involved. It is generally accepted that many types of infections take place through the duct system. Clinically detectable changes may not inevitably follow the passage of the organism through the teat canal. The intact epithelium of the cistern and lactiferous system of the gland and possibly also bactericidal and bacteriostatic substances of milk assist in preventing the multiplication of the organisms. (Heidrich and Renk, 1967). On the other hand, epithelial defects in the cistern and previous inflammatory changes, such as non-specific catarrh of the udder may favour the establishment of organisms that have entered the udder.

The changes in the duct system were, in addition to cellular infiltration and epithelial damage, proliferation of connective tissue and hyperplasia and

metaplasia of lining cells. The moderate thickening of the epithelium and small rounded polypoid proliferations into the lumen of the cistern and large ducts in many cases in the present study were also encountered by Pattison and Smith (1953) in experimental mastitis induced by Streptococcus dysgalactiae. Low grades of progressive fibrosis and lobular atrophy in some of the udders could be cases similar to those described by Stabenfeldt and Spencer (1966) in lesions caused by non-haemolytic coagulase negative Staphylococci. Classical cases of suppurative mastitis with abscess formation seen in this study were similar to those caused by Corynebacterium pyogenes and Pseudomonas aeruginosa.

Four cases of gangrenous mastitis two in cows and two in goats were observed. Organisms with the morphology of Staphylococci could be demonstrated in tissue sections. The alpha toxin of Staphylococcus is responsible for the development of gangrenous form of mastitis in cows, goats and sheep. The toxin produces vasoconstriction leading to ischaemia and death of tissue (Brown and Scherer, 1958). Staphylococcal antitoxins in blood and milk resulting from previous exposure to infection may be adequate to prevent the occurrence of gangrene, but extensive areas of suppuration will result

from uninhibited multiplication of Staphylococcus with the possible formation of large abscesses (Schalm et al. 1971).

The acute diffuse types are characterised by oedema, and catarrhal and fibrinous inflammation. Such reaction was found only in eight cases. Usually a variety of organisms are incriminated out of which Coliforms are very important.

Chronic interstitial mastitis was a common occurrence in a large number of glands (21.72%) examined. These appeared as lightly scattered area of lymphoid proliferation with minimal alveolar damage to large areas of interstitial thickening associated with degeneration and atrophy of the glandular end pieces. Most of the reactions were indicative of a past infection and consequent reparative phenomenon rather than ongoing inflammatory process. The disseminated lymphoid infiltration and lymphoid nodules observed in some of the glands could be due to Brucella infection. Emminger and Schalm (1943) reported that gross lesions are not seen in the parenchyma, but histologically few to many small inflammatory foci varying in degree from acute or sub-acute and chronic are present. The lesion is a non-purulent interstitial mastitis.

Another pathological aspect that was brought out during the course of this investigation is the lymphoid

reaction which was specifically distributed along the course of the interlobular and intra lobular connective tissue in the udder of goats suffering from Johne's disease. Even though no organisms could be detected in the glands by microscopical examination of sections stained by Ziehl-Neelsen method or from ground tissue smears, the pattern of lesion was very characteristic in animals having paratuberculous infection. It may be quite likely that the lymphoid cells, with deep staining cytoplasm, could be 'T' type of lymphocytes which are concerned with cellular immune mechanisms which might be operating in animals infected with Mycobacterium Johnei. The absence of plasma cells in these locations further substantiates this point.

Tuberculous mastitis was encountered in this investigation and numerous acid fast organisms were demonstrated in the infected tissue. Caseous and caseo-calcific lesions were seen. Giant cells were observed but were not a constant feature. Under natural conditions mammary tuberculosis is usually haematogenous but small proportion of cases could be galactogenous via teat canal (Schalm et al. 1971). The association of tuberculous lesions in other organs along with mammary tuberculosis in these cases may suggest a haematogenous infection in these cases. The public health significance of tuberculous

infection of the udder needs no over emphasis. Tuberculous mastitis was not encountered in goats.

Spherical structures which are generally referred to as corpora amylacea were very often encountered both in normal alveoli as well as in diseased portions. Their number was more in mastitic glands. These bodies were laminated indicating a progressive genesis by gradual deposition. They were basophilic or eosinophilic. It is likely that these originated from inspissated milk proteins which later became hyalinised and very often calcified. The process of calcification always proceeded, from the periphery. Ahmed (1975) when studying the calcification in human breast carcinomas observed that mammary epithelium is capable of concentrating calcium ions in milk and that calcification is the result of an active secretory process rather than deposition of calcium in degenerative and necrotic cells. It is likely that the phosphoproteins may be the most likely focus of calcification.

In six cases, varying grades of arterial calcification were noticed in goats which were very similar to "Monckeberg's sclerosis". It is described as a degenerative process involving the media and internal elastic lamina of muscular arteries. Experimentally epinephrine has produced similar lesions supporting the suggestion

that the lesion results from endogenous or exogenous vasotonic agents (Gore, 1966). However, similar arterial lesions occur with hyper parathyrodism with hypervitaminosis D and for unknown reasons. Since only the udder tissue was examined histologically it was not possible to ascertain whether this process of calcification was only a local dystrophic phenomenon or manifestation of a generalised reaction.

A large number of glands with chronic indurative changes showed cystic dilation of the ductal system. The lobular pattern and acinar structure have been obliterated and the only histologic unit remaining is the ductal system with severe periductal fibrotic reaction. In many locations there is marked over growth of connective tissue into the lactiferous ducts with partial or complete occlusion. Such partial or complete obliteration of the ductal lumen could very well explain the dilatation of the remaining ducts with active looking lining cells. Singh and Iyer (1972) have referred to these conditions as separate entities as "Cystic disease" and "epitheliosis". From the observations made in this study it seems such cases could be considered more as reparative and compensatory tissue reactions rather than as separate pathological entities.

Eight glands, both from cows and goats, with lesions of mastitis showed squamous metaplasia of ductal system. Singh and Iyer (1972) also observed cases of squamous metaplasia of the ductal lining cells in association with chronic mastitis. The factors responsible for this remains conjectual.

No neoplastic lesions could be observed in any of the tissues examined in the present study.

Normal milk always contains numbers of cells, but the limits of normalcy are not precise. Cell content in milk varies in the different stages of lactation and in the different fractions of milk (Waite and Blackburn, 1957). Diurnal variations have also been reported in the somatic cell count of milk (Schalm et al. 1971). In the present study the total somatic cell count was below 500,000 per ml. in both cows and goats. This observation is in general agreement that made by many previous workers. Cole et al.(1965) suggested that the leucocyte level of 1,000,000 cells per ml. was indicative of mastitis. The British Veterinary Association (1965) observed that quarter sample leucocyte counts of 500,000 per ml. or over are generally indicative of sub-clinical mastitis. In the present investigation the values were above 500,000 in sub-clinical and clinical mastitis. While the percentage

of neutrophils in normal milk was below 31% in goats and 43% in cows, the corresponding maximum values in mastitic cases were 88% and 92% respectively.

It was found that both in cows and goats in clinical as well as in sub-clinical cases Staphylococcus was found to be the chief etiological agent. Other organisms encountered were mixed infection by Staphylococcus and Streptococcus, Streptococcus, Coliforms, Staphylococcus and Coliforms and Corynebacterium in that order. Even though all the samples of milk were cultured in Sabauraud's media, fungi could not be isolated from any of the sample.

SUMMARY

SUMMARY

During the present study a total of 267 mammary quarters from cows and goats were selected for detailed histopathological study.

Eighteen quarters in cows eight halves in goats were apparently normal.

Sixty one quarters from cows and twenty four halves from goats were in various stages of involution. The changes associated with involution were atrophy of glandular parenchyma, fatty replacement and collagenous thickening of the interstitial tissue.

Mastitis was the important type of pathological lesion encountered.

Catarrhal mastitis and galactophoritis of varying grades and types were found in 45 quarters in cows and two halves in goats. In many of these cases the cisterns and the lactiferous ducts manifested productive inflammatory lesions.

Fifty five quarters from cows and 12 halves from goats revealed interstitial mastitis. The reaction was mainly characterised by fibroblastic proliferation and lymphoid reaction. The cisterns and the alveoli were secondarily involved.

There were twelve cases of suppurative mastitis out of which two were from goats. They were seen either as frank abscesses or as microabscesses. In most of these cases the duct system was also involved. Reparative attempts of duct epithelium was manifested in the form hyperplastia of the lining cells and dilatation.

Eight quarters from cows had acute diffuse mastitis characterised by oedema, congestion and leucocytic infiltration.

Necrotising mastitis was seen in two quarters from a cow and one half from a goat.

Six quarters from 2 cows revealed tuberculous mastitis.

Two mammary halves from two goats had gangrenous mastitis. The mechanism of gangrene formation in relation to the alpha toxin of Staphylococcus has been discussed.

Other non-specific lesions encountered were oedema, congestion, focal lymphocytic reaction, interstitial fibrosis, cystic dilatation of alveoli and ducts, arterial calcification, squamous metaplasia of the alveolar and ductal epithelium.

Pseudo concretions and true concretions were constantly observed in many of the mammary glands examined. It was suggested mammary epithelium could concentrate calcium and that it got deposited on inspissated and hyalinised milk proteins.

The varying grades of arterial calcification seen in the mammary glands of goats were very similar to 'Monckeberg's selerosis' described in human beings.

The characteristic feature of the mammary gland in goats with Johne's disease was the distribution of lymphoid cells in the inter lobular and intra lobular septa. It was postulated that these cells might be 'T' lymphocytes which are concerned with cellular immunity.

The cystic changes encountered in the mammary glands were not considered as a separate entity- 'fibrocystic disease' - but only compensatory processes as a result of fibrosis and atrophy of some part of the glandular and ductal system.

A total of 189 quarter samples of milk were also examined for total somatic cell count, differential count and for the presence of pathogenic bacteria and fungi of cows and goats by cultural examination.

The total cell count in normal milk varied from 150,000 to 500,000 /ml. In sub-clinical and clinical

cases upto 30,000,000 cell per ml could be counted.

The percentage of neutrophils in normal milk was below 31% in goats and 43% in cows, the corresponding maximum values in mastitic cases were 88% and 92% respectively.

In both cows and goats the chief etiological agent for mastitis was Staphylococcus. Other organisms encountered were Streptococcus, Coliforms and Coryne-bacterium.

No fungi could be isolated from the milk samples taken from Clinical cases of mastitis.

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* Reference not consulted in original.

Fig. 1. Lining cells of the udder cistern showing vacuolar degeneration. Cow (H & E, 400x).

Fig. 2. The wall of the udder cistern showing polypous thickening. Separation of the 2 cell layers seen in some areas. Cow (H & E, 400 x).

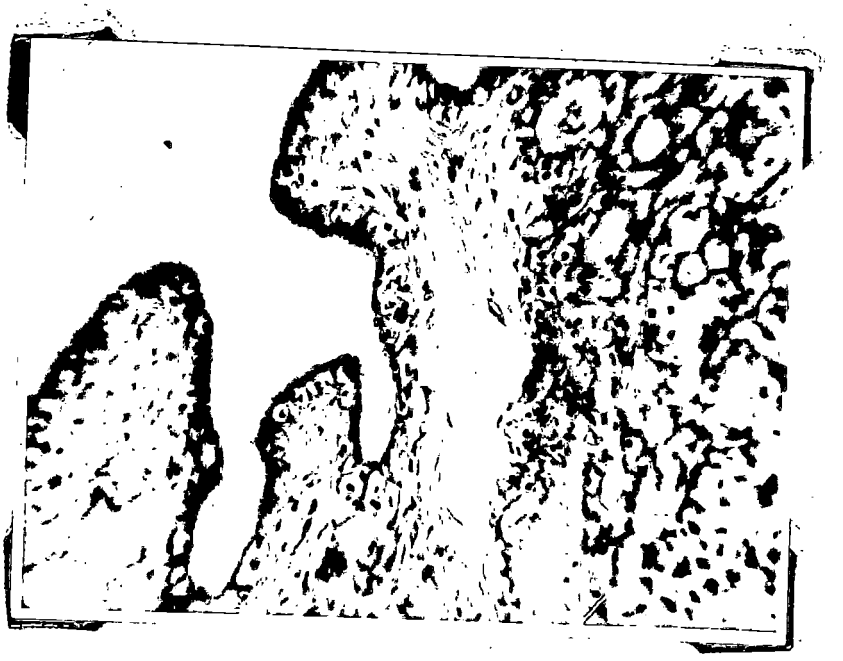
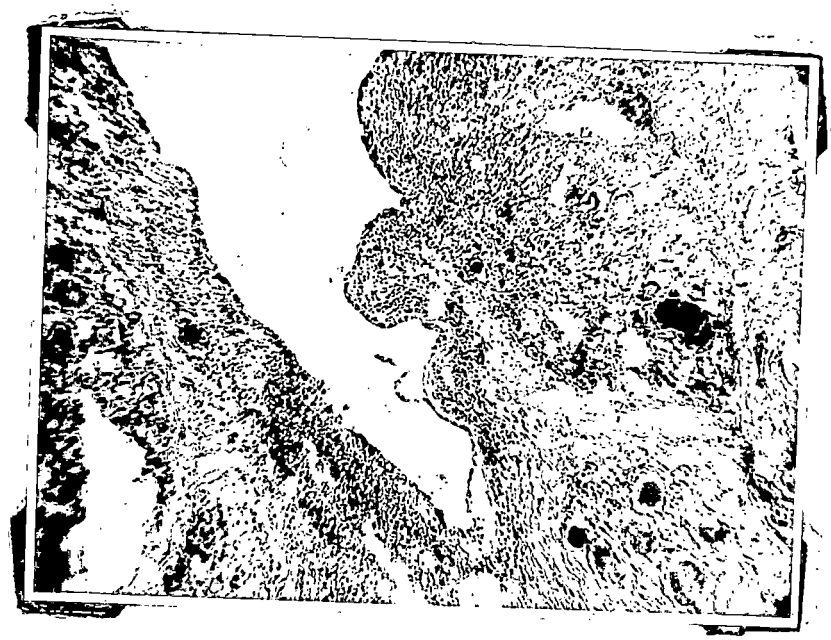


Fig. 3. Udder cistern showing massive infiltration of inflammatory cells with extension into the glandular tissue. Cow (H & E, 100 x).

Fig. 4. Mammary alveoli with leucocytes and desquamated epithelium. Cow (H & E, 400 x).



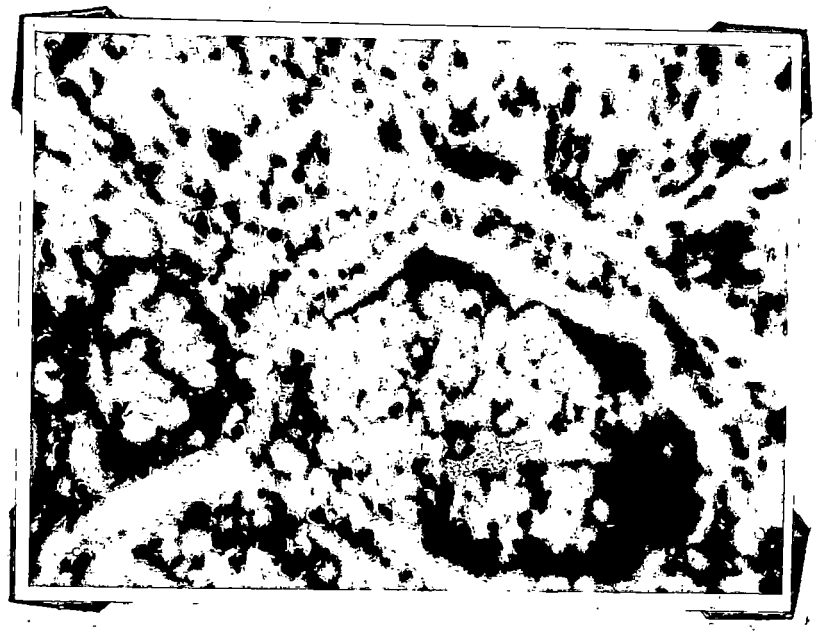
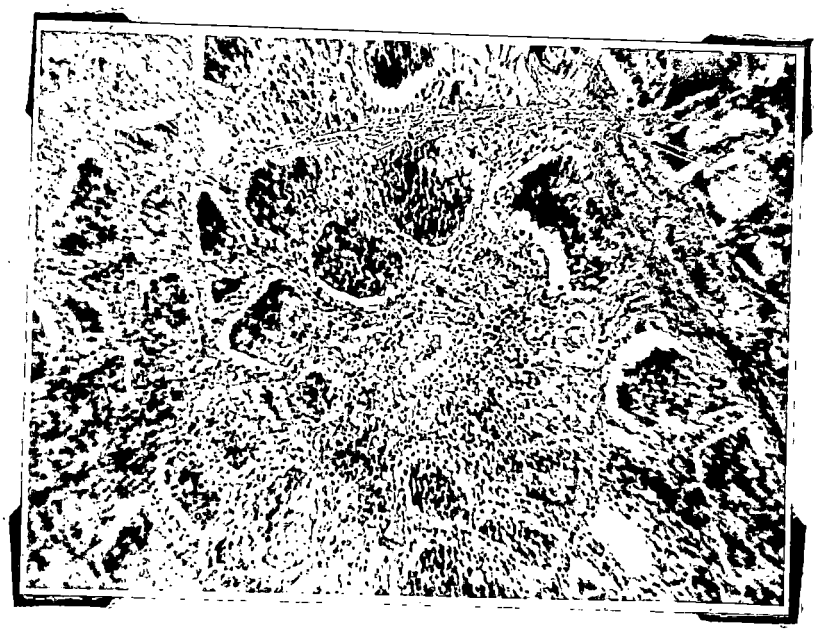


Fig. 5. Most of the alveoli packed with disintegrating inflammatory cells most of which are neutrophils. Cow (H & E, 100 x).

Fig. 6. Periductal fibrosis and lymphoid infiltration. Atrophy of secreting end pieces. Cow (H & E, 90 x).



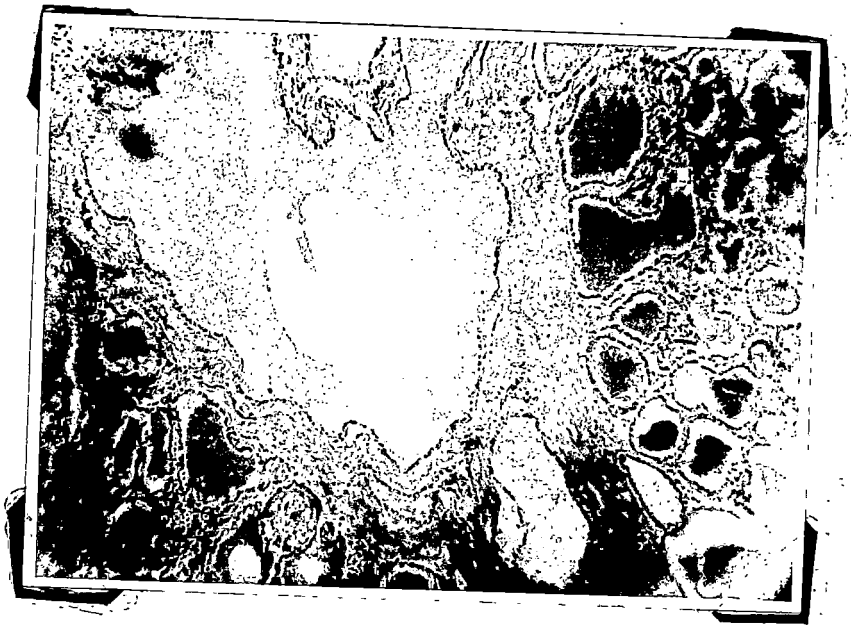


Fig. 9. Disintegrating abscess in the mammary gland surrounded by cellular granulation tissue. Cow (H & E, 400 x).

Fig. 10. Portion of an abscess showing disintegrating content and fibrous encapsulation. A few lymphocytes are seen in the capsule. Cow H & E, 400 x).

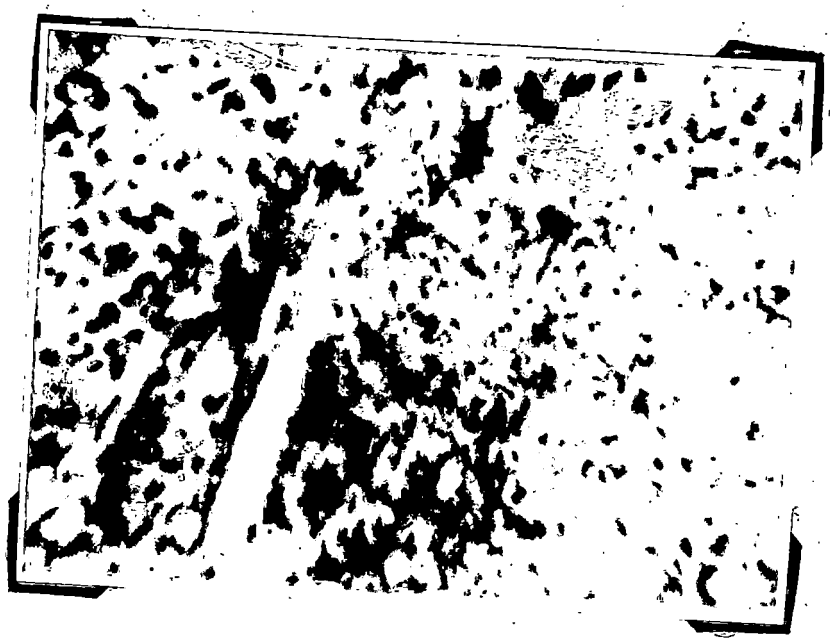
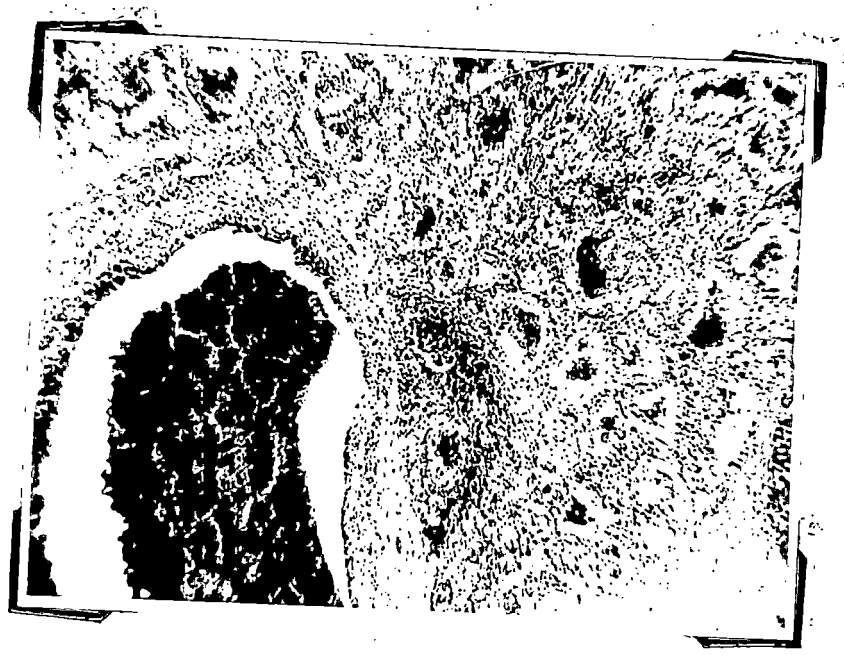


Fig. 11. Alveoli, ducts and cistern containing exudate consisting mainly of neutrophils. Cow (H & E, 100 x).

Fig. 12. Lining epithelial cells of a large duct showing focal desqumation. Hyperplasia seen in other areas. Cow (H & E, 100 x)



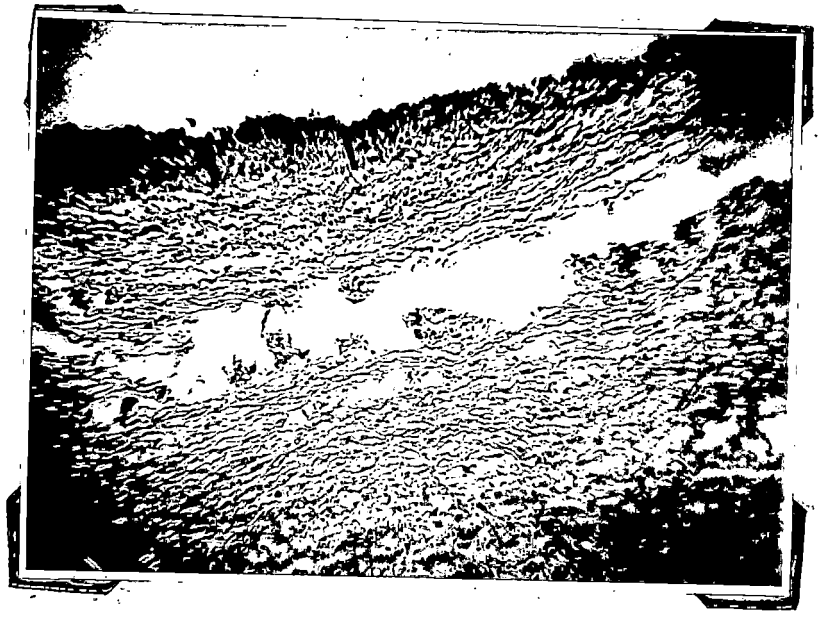


Fig. 13. Tuberculous mastitis showing an area of caseation. Cow (H & E, 100 x).

Fig. 14. Tuberculous mastitis - early lesion showing cellular reaction consisting mostly lymphoid cells and macrophages. Cow (H & E, 100 x).

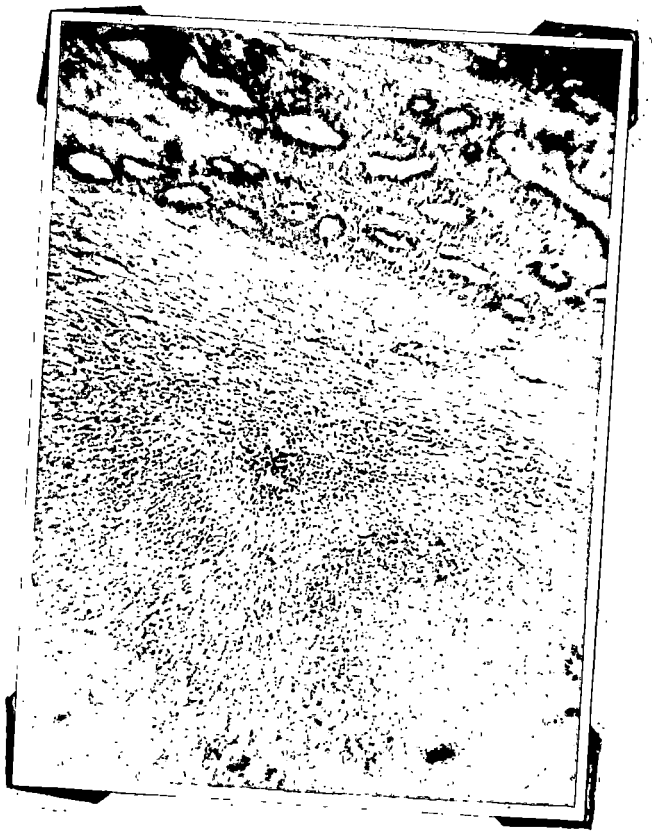


Fig. 15. Interstitial mastitis characterised by intense lymphoid infiltration. Cow (H & E, 400 x).

Fig. 16. Lymphoid nodule with germinal centre at the periphery of mammary parenchyma. Cow (H & E, x100 x)

Fig. 17. Alveoli filled with eosinophilic colloid like material. Cow (H & E, 100x).

Fig. 18. Advanced case of glandular atrophy. Only the duct system remains patent. Cow (H & E, 400 x).

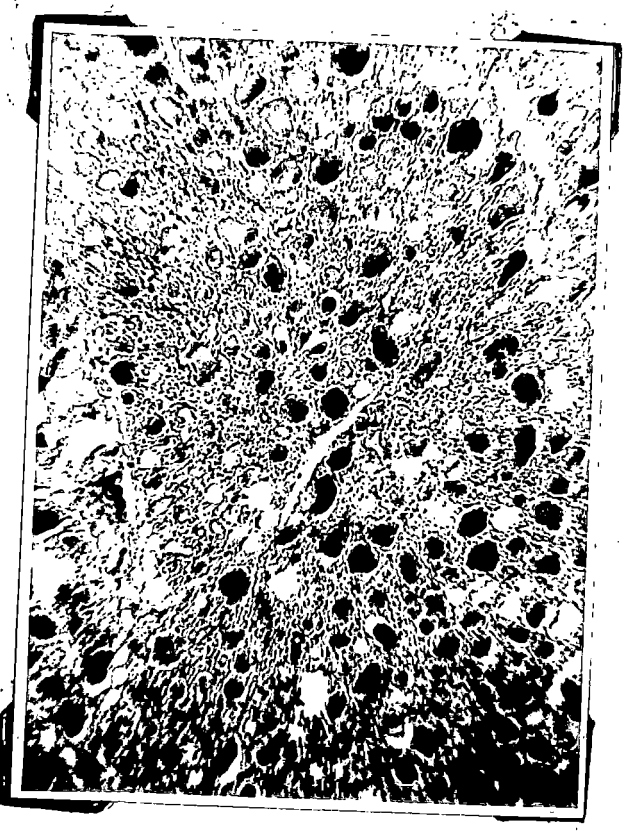


Fig. 19. Obliteration of the secretary end pieces and cystic dilatation of the ducts. Cow (H & E, x 100 x).

Fig. 20. Alveoli contain serofibrinous exudate contain leucocytes and desquamated epithelial cells. Cow (H & E, x 100 x).

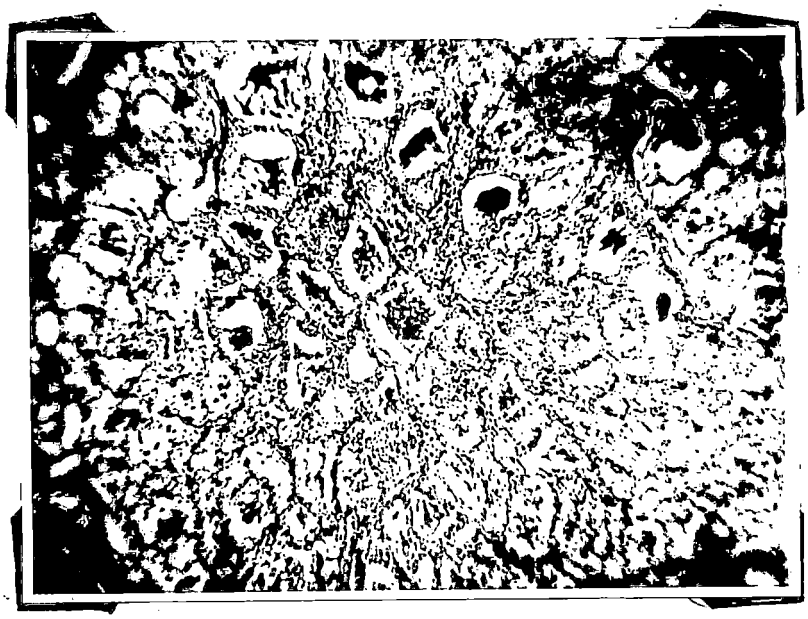
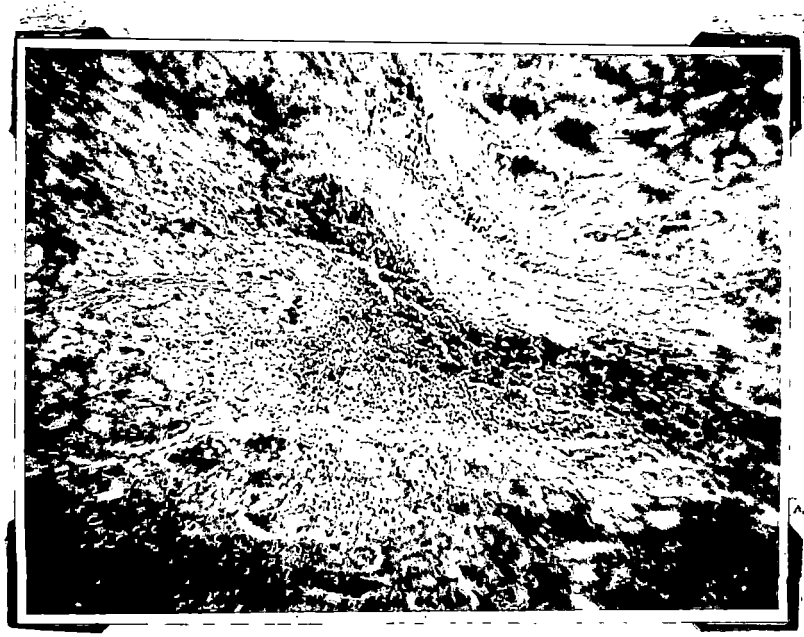


Fig. 21. Alveoli contain serous fluid and few inflammatory cells and epithelial cells Cow (H & E, 400x).

Fig. 22. Involvement of the duct system - inflammatory cells and oedema in the epithelial and subepithelial layer Cow (H & E, 100x).

Fig. 23. Degenerated inflammatory cells at the periphery of a necrotic area in the mammary gland. Cow (H & E, x 100x)

Fig. 24. Micro concretions. Most of these bodies are partially or completely calcified. Cow (H & E, 100 x).



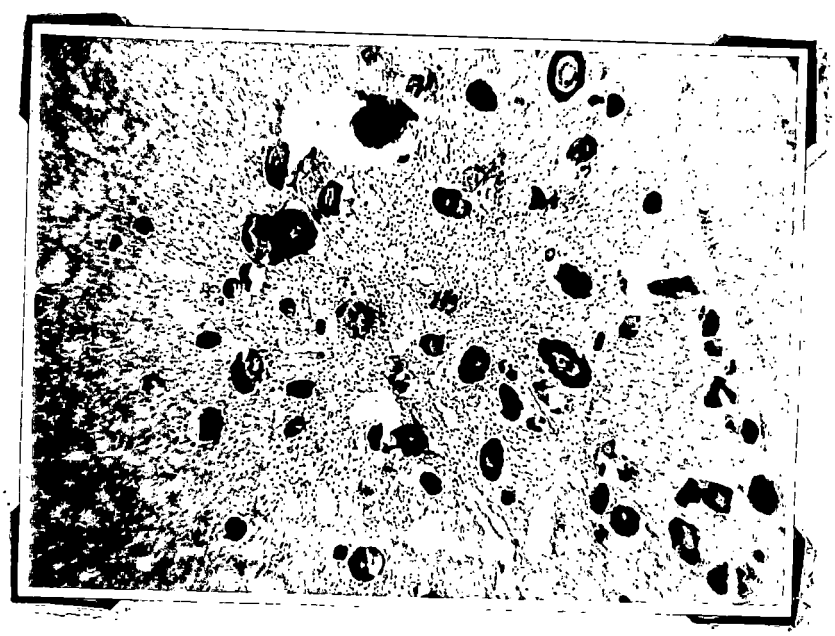


Fig. 29. Gangrene of the udder - complete loss of glandular architecture with vacuolations. Concretions also present. Goat (H & E, 100 x).

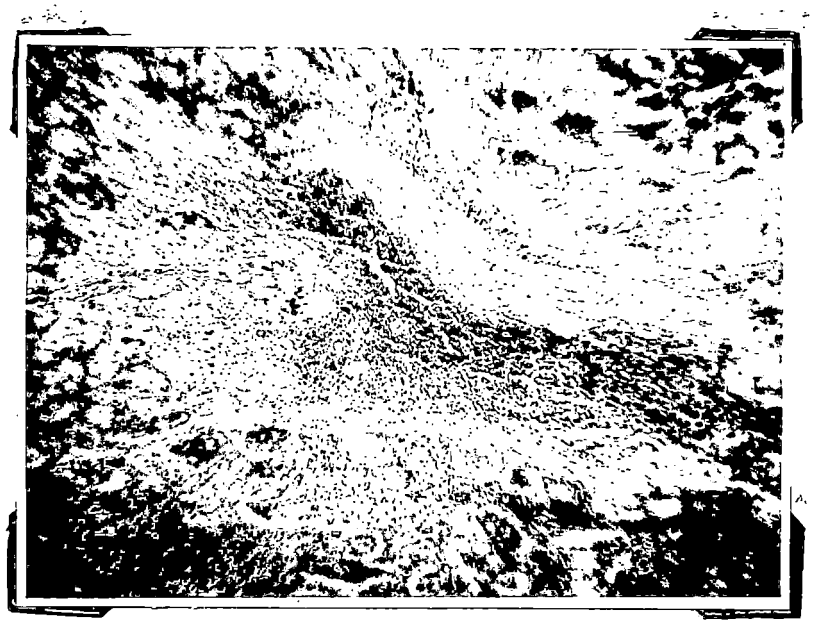
Fig. 30. Lobule showing destruction of many alveoli and containing purulent exudate. Goat (H & E, 100 x).

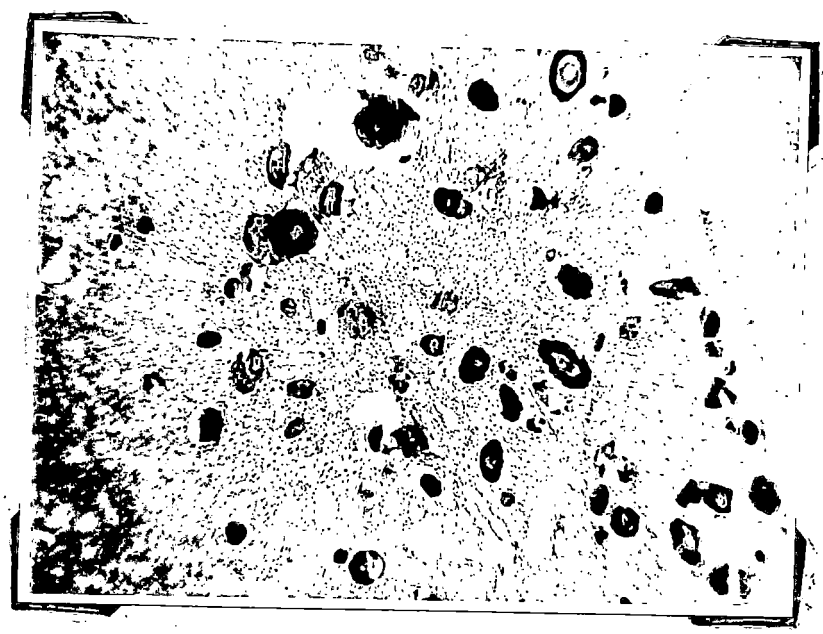
Fig. 25. Udder showing fatty replacement of glandular tissue. Goat (H & E, 100 x).

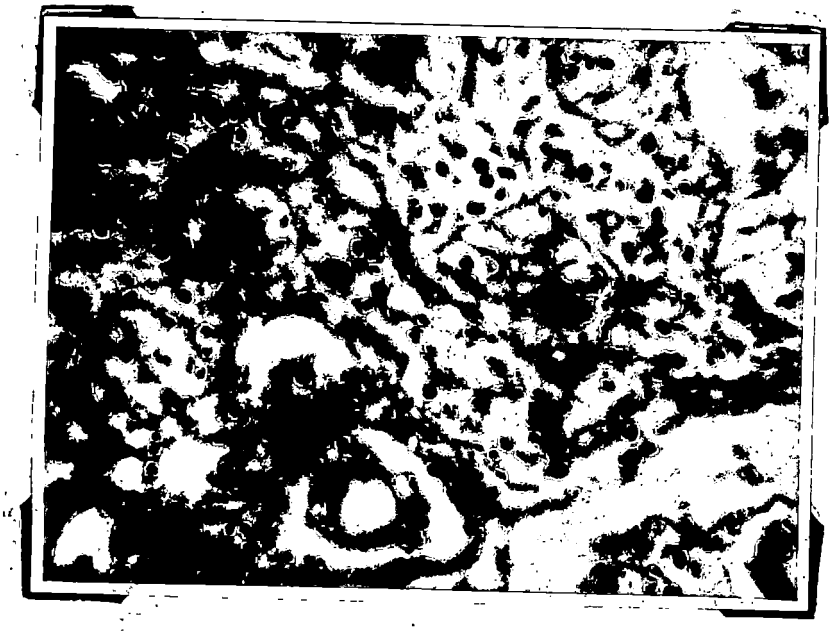
Fig. 26. Degeneration and desquamation of alveolar epithelium. Interstitial lymphoid infiltration. Goat (H & E, 400x).

Fig. 23. Degenerated inflammatory cells at the periphery of a necrotic area in the mammary gland. Cow (H & E, x 100x)

Fig. 24. Micro concretions. Most of these bodies are partially or completely calcified. Cow (H & E, 100 x).







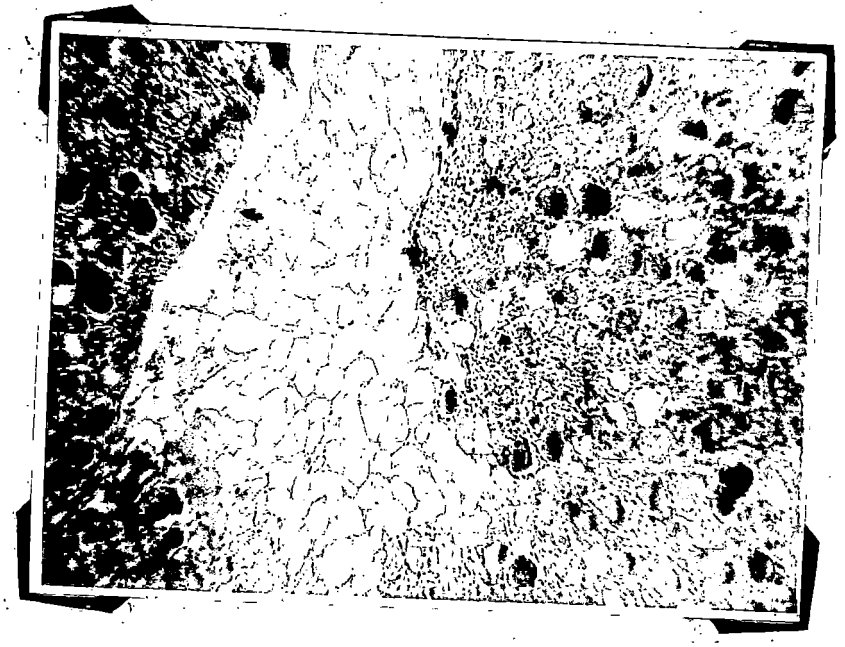
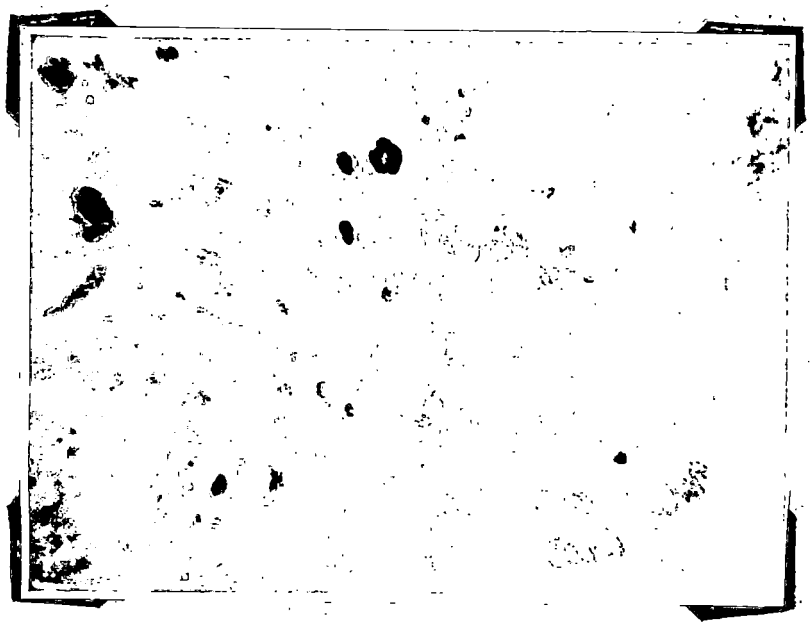


Fig. 27. Mammary acini filled with secretion containing lymphocytes and epithelial cells. Goat (H & E, 100 x).

Fig. 28. Infiltration of lymphocytes along the interlobular and intralobular septa. Goat (H & E, 400 x).



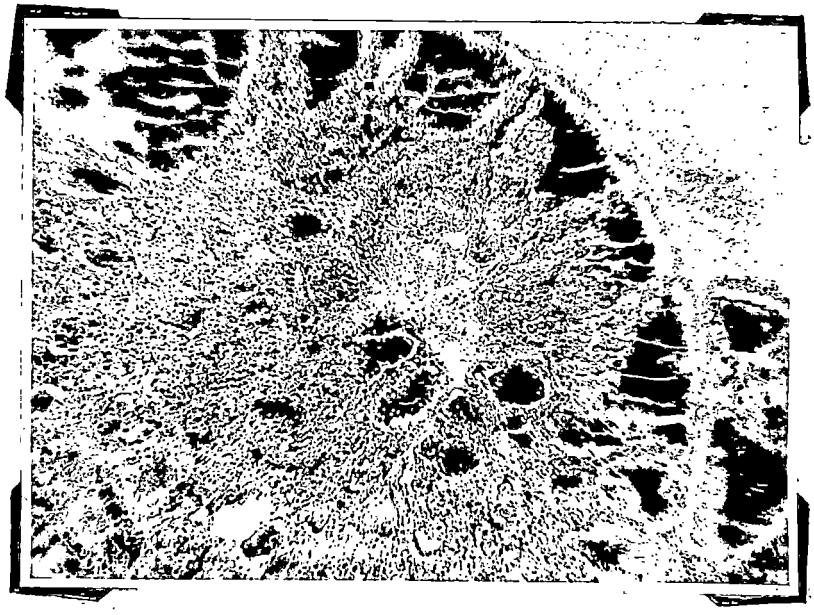
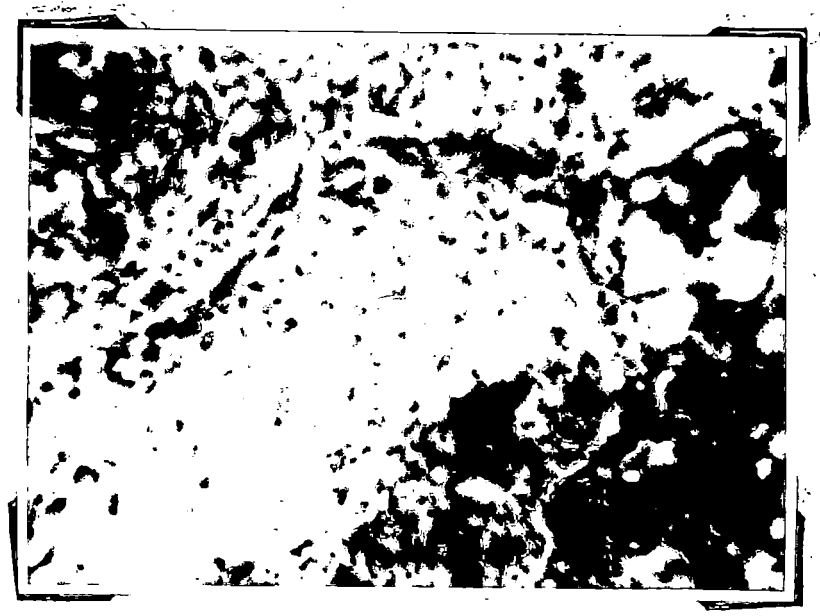


Fig. 31. Epithelium of cistern showing squamous metaplasia. Goat (H & E , 400 x).

Fig. 32. Udder cistern containing cellular debris. Lining cells show squamous metaplasia. Goat (H & E, 400 x).



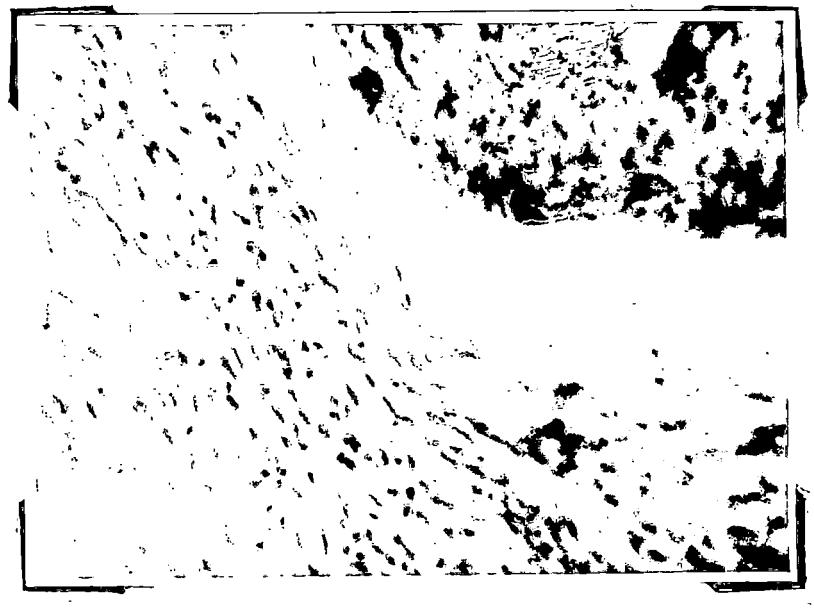
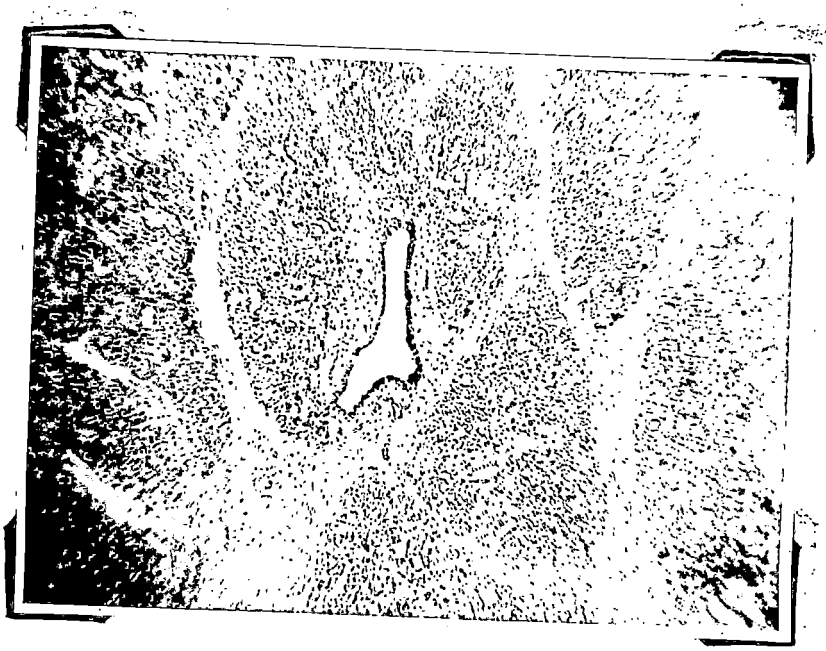


Fig. 33. Massive glandular atrophy and interstitial fibrosis. Goat (H & E, 100 x).

Fig. 34. Interstitial fibrosis and prominent duct system. Goat (H & E, 400 x).



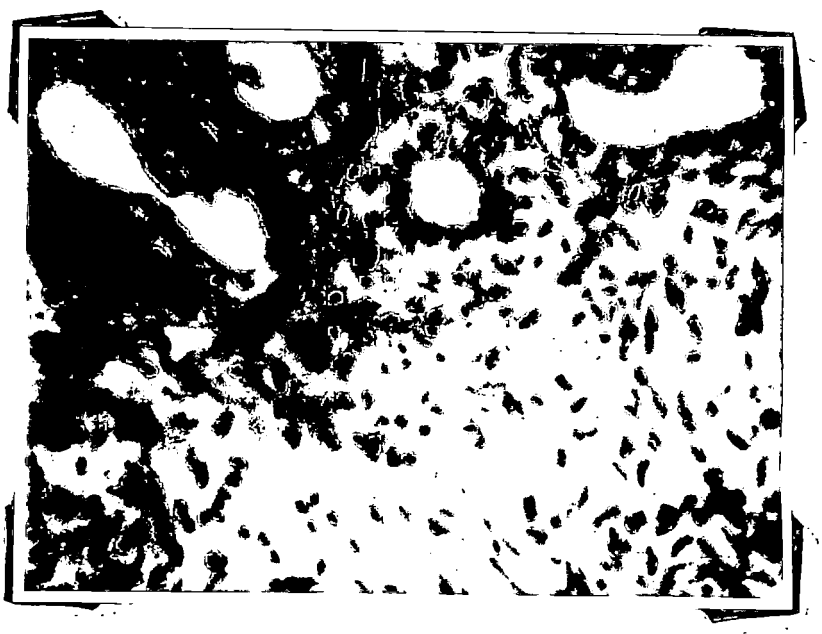
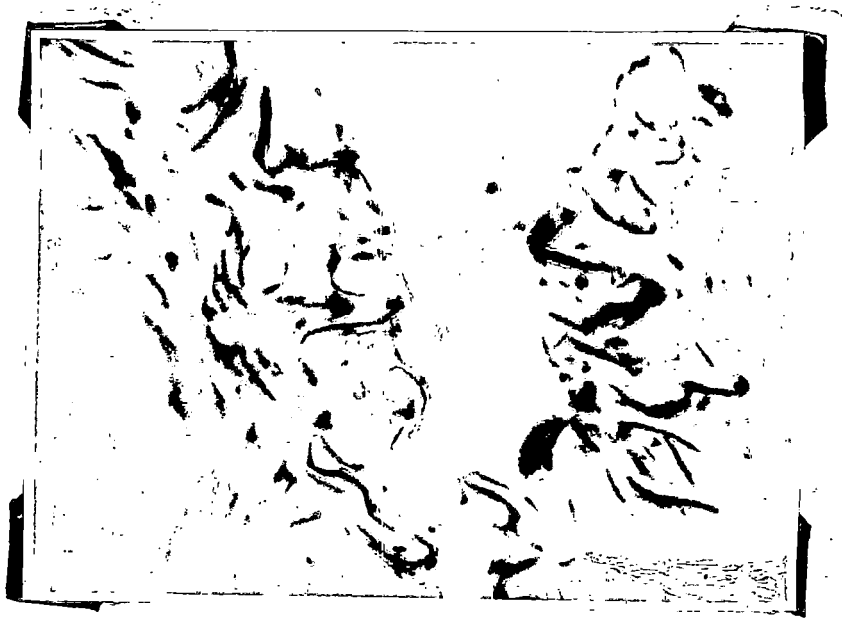


Fig. 35. Artery in the mammary gland showing calcification. Goat (H & E, 400 x).

Fig. 36. Artery in the mammary gland showing foci of calcification. Goat (H & E, 100 x).

Fig. 37. Streaks of calcification in the arterial wall. Goat (H & E, 400 x).

Fig. 38. Somatic Cells in the normal milk of goat. Cells are predominantly lymphocytes. 900x.



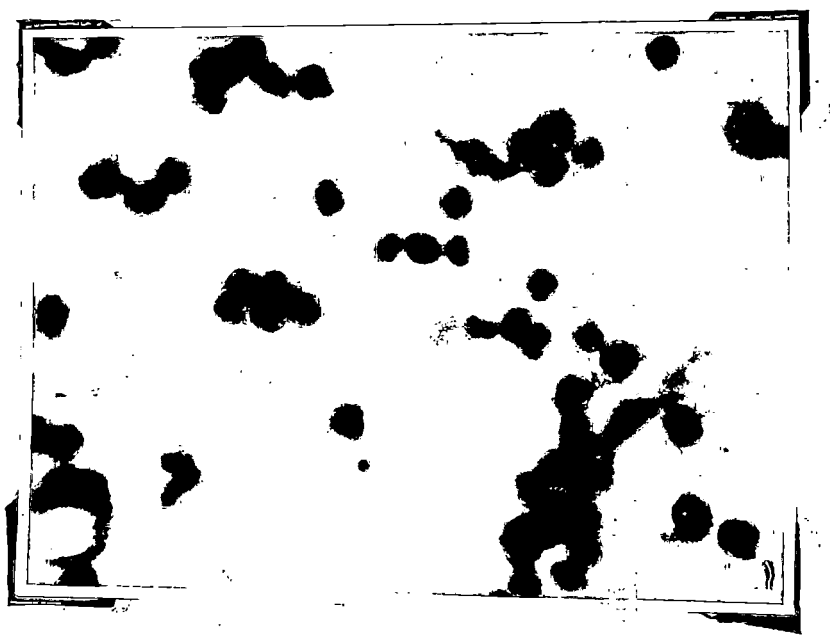


Fig. 39. Somatic cells in a sub clinical case of mastitis. Large number of neutrophils are present. Cow 900 x.

Fig. 40. Neutrophils and Lymphocytes in mastic goat milk. 1200 x.

APPENDIX

Appendix I (a)

Results of examination of milk sample (Cow)

Normal milk samples

Sl.No.	Reaction to Mastaid solution	Cultural examination	Total cell count/ml. (10^6)	Absolute number of neutrophils/ml. (10^6)	Differential count.					
					N	L	EPI	E	M	B
* 1	Negative	Negative	0.30	-	-	-	-	-	-	-
* 2	-do-	-do-	0.40	-	-	-	-	-	-	-
* 3	-do-	-do-	0.35	-	-	-	-	-	-	-
* 4	-do-	-do-	0.42	-	-	-	-	-	-	-
5	-do-	-do-	0.50	0.1	20	74	6	-	-	-
* 6	-do-	-do-	0.45	-	-	-	-	-	-	-
* 7	-do-	-do-	0.20	-	-	-	-	-	-	-
*8	-do-	-do-	0.15	-	-	-	-	-	-	-
* 9	-do-	-do-	0.18	-	-	-	-	-	-	-
*10	-do-	-do-	0.27	-	-	-	-	-	-	-
*11	-do-	-do-	0.39	-	-	-	-	-	-	-
12	-do-	-do-	0.50	0.19	38	60	2	-	-	-
*13	-do-	-do-	0.50	-	-	-	-	-	-	-
*14	-do-	-do-	0.50	-	-	-	-	-	-	-

/Contd./

Sl.No.	Reaction to Mastaid solution	Cultural examination	Total cell count/ml. (10^6)	Absolute number of neutrophils/ml. (10^6)	Differential count					
					N	L	EPI	E	M	B
15	Negative	Negative	0.50	0.07	15	74	11	-	-	-
16	-do-	-do-	0.50	0.09	18	82	-	-	-	-
17	-do-	-do-	0.50	0.22	43	52	5	-	-	-
18	-do-	-do-	0.50	0.09	19	76	5	-	-	-
*19	-do-	-do-	0.45	-	-	-	-	-	-	-
20	-do-	-do-	0.50	0.14	27	68	5	-	-	-

* Differential cell counts not made since the number of cells were very few.

Appendix I (b)

Sub clinical mastitis

Sl.No.	Reaction to Mastaid solution	Cultural examination	Total cell count/ml. (10^6)	Absolute number of neutrophils/ml. (10^6)	Differential count					
					N	L	EPI	E	M	B
1	+	Staphylococcus and Coliforms	0.75	0.35	47	53	-	-	-	-
2	+	Staphylococcus	0.50	0.13	25	69	6	-	-	-
3	+	Staphylococcus	0.50	0.27	53	39	8	-	-	-
4	+	Staphylococcus	0.75	0.15	20	74	6	-	-	-
5	++	Coliforms	2.25	0.79	35	55	-	-	-	-
6	++	Staphylococcus and Coliforms	2.65	1.33	50	42	8	-	-	-
7	++	Staphylococcus	1.00	0.48	48	51	1	-	-	-
8	++	Staphylococcus and Streptococcus	1.50	0.72	48	46	6	-	-	-
9	++	Staphylococcus	1.30	0.53	41	53	6	-	-	-
10	++	Coliforms	1.80	1.08	60	40	-	-	-	-
11	++	Staphylococcus and Coliforms	5.00	2.15	43	52	5	-	-	-
12	++	Staphylococcus	1.00	0.60	60	34	6	-	-	-
13	++	Staphylococcus	1.20	0.60	50	39	11	-	-	-
14	++	Coliforms	1.70	0.71	42	54	4	-	-	-

/Contd./

Sl.No.	Reaction to Mastaid solution	Cultural examination	Total cell count/ml. (10^6)	Absolute number of neutrophils ml. (10^6)	Differential count					
					N	L	EPI	E	M	B
15	++	Staphylococcus and Streptococcus	3.60	2.09	58	42	-	-	-	-
16	++	Staphylococcus	1.65	0.74	45	38	17	-	-	-
17	+++	Staphylococcus	30.00	18.60	62	26	12	-	-	-
18	+++	Staphylococcus	15.00	9.30	62	29	9	-	-	-
19	+++	Staphylococcus	30.00	18.60	60	28	10	2	-	-
20	+++	Staphylococcus	1.25	0.40	32	49	3	6	-	-
21	+++	Staphylococcus	3.50	1.40	40	35	22	-	-	-
22	+++	Staphylococcus	1.50	0.60	42	36	10	12	-	-
23	+++	Staphylococcus	1.35	0.51	38	40	20	10	2	-
24	+++	Staphylococcus and Streptococcus	13.80	7.18	52	11	37	-	-	-
25	+++	Staphylococcus	20.60	14.42	70	26	4	-	-	-
26	+++	Staphylococcus and Streptococcus	29.20	16.35	56	34	10	-	-	-
27	+++	Staphylococcus	18.60	5.58	30	64	6	-	-	-
28	+++	Staphylococcus	19.90	9.55	48	38	14	-	-	-
29	+++	Staphylococcus	2.50	1.73	69	28	-	3	-	-
30	+++	Staphylococcus and Streptococcus	15.00	12.60	84	9	-	7	-	-

/Contd./

Sl.No.	Reaction to Mastaid solution	Cultural examination	Total cell count/ml. (10^6)	Absolute number of neutrophils ml. (10^6)	Differential count.					
					N	L	EPI	E	M	B
31	+++	Staphylococcus	16.50	11.22	68	24	7	1	-	-
32	+++	Staphylococcus	7.55	6.04	80	14	6	-	-	-
33	+++	Staphylococcus	24.60	18.70	76	23	1	-	-	-
34	+++	Staphylococcus and Streptococcus	19.20	17.67	92	7	-	-	-	-
35	+++	Staphylococcus	9.80	8.43	86	12	2	-	-	-
36	+++	Staphylococcus	10.50	9.45	90	9	1	-	-	-
37	+++	Staphylococcus	6.50	3.58	55	40	5	-	-	-
38	+++	Corynebacterium	4.60	2.85	62	31	17	-	-	-
39	+++	Coliforms	11.00	4.29	39	42	17	2	-	-
40	+++	Staphylococcus	2.60	1.30	19	43	38	-	-	-

Appendix I (c)

Clinical mastitis

Sl.No.	Reaction to Mastaid solution	Cultural examination	Total cell count/ml. (10^6)	Absolute number of neutrophils ml. (10^6)	Differential count.					
					N	L	EPI	E	M	B
1	Clinically positive	Staphylococcus and Corynebacterium	12.00	5.64	47	30	24	-	-	-
2	-do-	Staphylococcus	2.40	1.73	72	12	15	1	-	-
3	-do-	Coliforms	1.63	1.30	80	14	2	2	2	-
4	-do-	Staphylococcus	6.35	3.56	56	30	11	3	-	-
5	-do-	Staphylococcus	1.53	0.78	51	39	8	2	-	-
6	-do-	Staphylococcus	14.00	6.72	48	37	6	6	3	-
7	-do-	Staphylococcus and Streptococcus	9.00	4.40	49	38	8	1	4	-
8	-do-	Staphylococcus	2.95	1.59	54	24	8	8	6	-
9	-do-	Staphylococcus	1.50	0.78	52	28	4	1	6	-
10	-do-	Staphylococcus	3.00	1.71	57	25	8	7	3	-
11	-do-	Staphylococcus	1.35	0.69	50	35	8	5	2	-
12	-do-	Staphylococcus	8.50	6.63	78	17	4	1	-	-
13	-do-	Staphylococcus	7.00	4.69	67	23	6	2	2	-
14	-do-	Corynebacterium	30.00	11.40	36	40	16	6	2	-

/Contd./

Sl.No.	Reaction to Mastaid solution	Cultural examination	Total cell count/ml. (10^6)	Absolute number of neutrophils ml. (10^6)	Differential count					
					N	L	EPI	E	M	B
15	Clinically positive	Staphylococcus	1.50	0.45	30	70	-	-	-	-
16	-do-	Corynebacterium	12.00	1.92	16	67	16	1	-	-
17	-do-	Staphylococcus and Streptococcus	30.00	22.80	61	20	15	2	2	-
18	-do-	Staphylococcus	30.00	18.30	61	28	11	-	-	-
19	-do-	Staphylococcus	30.00	18.60	62	20	10	2	4	3
20	-do-	Corynebacterium	No cells to count, light yellow watery fluid.							
21	-do-	Corynebacterium	1.50	0.50	33	54	13	-	-	-
22	-do-	Staphylococcus	1.25	0.40	32	47	18	3	-	-
23	-do-	Coliforms	No cells to count, light yellow watery fluid.							
24	-do-	Staphylococcus	3.50	1.4	40	35	22	3	-	-
25	-do-	Coliforms	5.35	3.85	72	21	-	3	4	-
26	-do-	Staphylococcus	1.50	0.63	42	36	10	10	2	-
27	-do-	Staphylococcus	1.35	0.51	38	40	20	10	2	-
28	-do-	Staphylococcus and Streptococcus	13.80	7.18	52	11	37	-	-	-
29	-do-	Staphylococcus and Streptococcus	20.50	4.10	20	38	27	8	7	-

/Contd./

Sl.No.	Reaction to Mastaid solution	Cultural examination	Total cell count/ml. (10^6)	Absolute number of neutrophils ml. (10^6)	Differential count					
					N	L	EPI	E	M	B
30	Clinically positive	Staphylococcus	3.61	2.53	70	20	8	2	-	-
31	-do-	Staphylococcus and Streptococcus	23.50	16.92	72	28	-	-	-	-
32	-do-	Staphylococcus and Streptococcus	27.50	18.17	66	20	4	6	4	-
33	-do-	Staphylococcus	20.60	14.42	70	26	4	-	-	-
34	-do-	Staphylococcus and Streptococcus	29.20	16.35	56	34	10	-	-	-
35	-do-	Staphylococcus	24.90	8.96	36	50	9	3	2	-
36	-do-	Staphylococcus	26.50	13.51	51	44	3	2	-	-
37	-do-	Staphylococcus	17.60	8.62	49	46	5	-	-	-
38	-do-	Staphylococcus	18.60	5.58	30	64	6	-	-	-
39	-do-	Staphylococcus	19.90	9.55	48	38	14	-	-	-
40	-do-	Streptococcus	25.70	12.85	50	40	3	2	2	-
41	-do-	Streptococcus	27.50	3.30	12	72	9	4	3	-
42	-do-	Staphylococcus	2.50	1.73	69	20	5	4	2	-
43	-do-	Staphylococcus and and Streptococcus	30.00	24.00	80	12	8	-	-	-
44	-do-	Staphylococcus	30.00	24.60	82	16	-	2	-	-
45	-do-	Staphylococcus and Streptococcus	30.00	26.40	88	10	2	-	-	-

Appendix II (a)

Results of examination of milk sample (Goat)

Normal milk samples

Sl.No.	Reaction to Mastaid solution	Cultural examination	Total cell count/ml. (10^6)	Absolute number of neutrophils ml. (10^6)	Differential count.					
					N	L	EPI	E	M	B
* 1	Negative	Negative	0.30	-	-	-	-	-	-	-
* 2	-do-	-do-	0.50	-	-	-	-	-	-	-
* 3	-do-	-do-	0.50	-	-	-	-	-	-	-
* 4	-do-	-do-	0.40	-	-	-	-	-	-	-
* 5	-do-	-do-	0.45	-	-	-	-	-	-	-
* 6	-do-	-do-	0.35	-	-	-	-	-	-	-
7	-do-	-do-	0.50	0.08	16	65	19	-	-	-
8	-do-	-do-	0.50	0.06	12	64	24	-	-	-
9	-do-	-do-	0.50	0.10	20	68	12	-	-	-
*10	-do-	-do-	0.38	-	-	-	-	-	-	-
11	-do-	-do-	0.50	0.11	22	78	-	-	-	-
*12	-do-	-do-	0.30	0.06	20	44	36	-	-	-
*13	-do-	-do-	0.30	-	-	-	-	-	-	-
*14	-do-	-do-	0.50	-	-	-	-	-	-	-

/Contd./

Sl.No.	Reaction to Mastaid solution	Cultural examination	Total cell count/ml. (10^6)	Absolute number of neutrophils ml. (10^6)	Differential count					
					N	L	EPI	E	M	B
* 15	Negative	Negative	0.45	-	-	-	-	-	-	-
* 16	-do-	-do-	0.25	-	-	-	-	-	-	-
* 17	-do-	-do-	0.35	-	-	-	-	-	-	-
18	-do-	-do-	0.50	0.10	20	62	18	-	-	-
* 19	-do-	-do-	0.30	-	-	-	-	-	-	-

* Differential cell counts not made since the number of cells were very few

Appendix II (b)

Sub clinical mastitis

Sl.No.	Reaction to Mastaid solution	Cultural examination	Total cell count/ml. (10^6)	Absolute number of neutrophils ml. (10^6)	Differential count					
					N	L	EPI	E	M	B
1	+	Negative	1.50	0.47	31	69	-	-	-	-
2	+	Staphylococcus	2.00	0.88	44	56	-	-	-	-
3	++	Staphylococcus	4.50	1.26	28	72	-	-	-	-
4	++	Staphylococcus	4.05	1.38	34	62	4	-	-	-
5	++	Staphylococcus	8.20	3.54	48	56	-	-	-	-
6	++	Staphylococcus	1.00	0.61	61	39	-	-	-	-
7	++	Staphylococcus and Streptococcus	2.90	0.73	25	60	15	-	-	-
8	++	Staphylococcus	4.80	2.06	43	57	-	-	-	-
9	++	Staphylococcus	5.00	2.25	45	52	3	-	-	-
10	+++	Staphylococcus	9.95	7.06	71	26	3	-	-	-
11	+++	Staphylococcus	30.00	15.90	53	37	6	2	2	-
12	+++	Staphylococcus	13.95	7.81	56	44	-	-	-	-
13	+++	Staphylococcus	5.50	2.20	40	42	18	-	-	-
14	+++	Staphylococcus	9.00	6.12	68	24	6	2	-	-
15	+++	Staphylococcus	8.20	5.74	70	21	9	-	-	-
16	+++	Staphylococcus	2.30	1.49	65	23	7	2	3	-

/Contd./

Sl.No.	Reaction to Mastaid solution	Cultural examination	Total cell count/ml. (10^6)	Absolute number of neutrophils ml. (10^6)	Differential count					
					N	L	EPI	E	M	B
17	+++	Staphylococcus	4.90	2.20	45	35	18	2	-	-
18	+++	Staphylococcus	11.50	4.83	42	45	13	-	-	-
19	+++	Staphylococcus	4.00	3.04	76	19	5	-	-	-
20	+++	Streptococcus	13.00	5.13	38	40	18	14	-	-
21	+++	Staphylococcus	12.00	6.12	51	36	6	6	-	-
22	+++	Staphylococcus	23.00	16.56	72	26	2	-	-	-
23	+++	Staphylococcus	8.70	2.44	28	30	34	8	-	-
24	+++	Streptococcus	21.90	3.94	18	26	45	11	-	-
25	+++	Staphylococcus	18.70	4.49	24	50	26	-	-	-
26	+++	Staphylococcus	23.70	15.88	67	33	-	-	-	-
27	+++	Staphylococcus	18.00	7.56	42	54	4	-	-	-
28	+++	Staphylococcus	28.00	14.56	52	34	11	-	-	-
29	+++	Streptococcus	24.90	8.96	36	57	4	3	-	-
30	+++	Staphylococcus	18.60	5.58	30	64	6	-	-	-
31	+++	Staphylococcus	19.90	9.55	48	38	14	-	-	-
32	+++	Staphylococcus	2.50	1.73	69	28	3	-	-	-
33	+++	Staphylococcus	21.00	7.14	34	54	12	-	-	-
34	+++	Staphylococcus	24.40	8.78	36	58	6	-	-	-
35	+++	Staphylococcus	19.00	9.95	51	44	5	-	-	-

Appendix II (c)

Clinical mastitis

Sl.No.	Reaction to Mastaid solution	Cultural examination	Total cell count/ml. (10^6)	Absolute number of neutrophils ml. (10^6)	Differential count					
					N	L	EPI	E	M	B
1	Clinically positive	Streptococcus	20.50	7.79	38	51	11	-	-	-
2	-do-	Staphylococcus	16.00	5.92	37	55	8	-	-	-
3	-do-	Staphylococcus	23.00	5.52	24	32	14	-	-	-
4	-do-	Staphylococcus	15.40	5.85	38	54	8	-	-	-
5	-do-	Staphylococcus	17.40	6.44	37	48	15	-	-	-
6	-do-	Staphylococcus	7.20	1.73	24	36	40	-	-	-
7	-do-	Staphylococcus	9.00	4.50	50	37	13	-	-	-
8	-do-	Staphylococcus and Streptococcus	30.00	23.10	77	23	-	-	-	-
9	-do-	Staphylococcus	30.00	21.10	70	27	3	-	-	-
10	-do-	Staphylococcus and Streptococcus	30.00	23.40	78	22	-	-	-	-
11	-do-	Staphylococcus	25.00	21.75	87	13	-	-	-	-
12	-do-	Staphylococcus and Streptococcus	30.00	20.40	68	28	-	4	-	-
13	-do-	Staphylococcus	12.70	11.18	88	10	-	2	-	-

/Contd./

Sl.No.	Reaction to Mastaid solution	Cultural examination	Total cell count/ml. (10^6)	Absolute number of neutrophils ml. (10^6)	Differential count					
					N	L	EPI	E	M	B
14	Clinically positive	Staphylococcus	13.80	7.18	37	52	-	1	-	-
15	-do-	Coliforms	1.00	0.64	64	31	-	3	-	-
16	-do-	Staphylococcus and Coliforms	12.00	4.56	38	48	14	-	-	-
17	-do-	Staphylococcus	16.70	7.85	47	22	21	-	-	-
18	-do-	Staphylococcus	13.80	4.69	34	41	18	7	-	-
19	-do-	Staphylococcus	18.40	8,83	48	42	6	4	-	-
20	-do-	Staphylococcus and Streptococcus	25.20	7.05	28	46	26	-	-	-
21	-do-	Staphylococcus and Streptococcus	No cells to count							
22	-do-	Staphylococcus	20.40	7.34	36	51	7	4	2	-
23	-do-	Staphylococcus	24.90	8.96	36	57	4	3	-	-
24	-do-	Staphylococcus	26.50	13.51	51	44	3	2	-	-
25	-do-	Staphylococcus	17.60	9.50	54	41	5	-	-	-
26	-do-	Staphylococcus and Streptococcus	25.70	12.85	50	46	4	-	-	-
27	-do-	Streptococcus	27.50	22.55	82	12	6	-	-	-
28	-do-	Coliforms	30.00	11.10	37	62	-	1	-	-
29	-do-	Staphylococcus	27.00	16.20	60	34	-	6	-	-
30	-do-	Staphylococcus	16.50	7.92	48	36	16	-	-	-

STUDIES ON PATHOLOGICAL CONDITIONS IN THE MAMMARY GLANDS OF CATTLE AND GOATS

By

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ABSTRACT OF A THESIS

Submitted in partial fulfilment of the requirement for the degree

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ABSTRACT

An investigation was carried out to study the pathological lesions encountered in the mammary glands of cattle and goats from specimens selected from slaughter house and autopsy cases. The somatic cell count in milk from clinical and sub-clinical cases of mastitis was also undertaken.

Two hundred quarters from cows and 67 halves from goats were subjected to detailed histopathological investigation. Mastitis was found to be the important type of lesion encountered. Lesions varied from subtle changes to intensive involvement with complete obliteration and atrophy. The lesions encountered were catarrhal mastitis and galactophoritis, suppurative mastitis, acute diffuse mastitis, necrotising mastitis, gangrenous mastitis, interstitial mastitis and tuberculous mastitis. Other significant alterations associated with or without mastitis were congestion and oedema, squamous metaplasia of lining epithelium of ducts and cisterns and calcification of vessel walls. The calcification of the vessels was similar to that observed in 'Monckeberg's sclerosis' in human beings.

No tumours were found.

It was suggested that lymphoid cells found in the

interstitial septa of the mammary glands of goats affected with Johne's disease might be the 'T' type of lymphocytes which are concerned with cellular immunity. The cystic changes encountered in the glands were not considered as the separate entity- Fibrocystic disease- but only compensatory processes as a result of fibrosis and atrophy of some part of the glandular and ductal system.

Micro concretions were constantly observed and it was suggested that calcium got deposited over inspissated and hyalinised milk proteins.

A total of 189 quarter samples of milk was also examined for total somatic cell count, differential count and by culturally for the presence of pathogenic bacteria and fungi. The total cell count in normal milk of cows and goats varied from 150,000 to 500,000 per ml. In sub-clinical and clinical cases upto 30,000,000 cells per ml could be counted. The percentage of neutrophils in normal milk was below 31% in goats and 43% in cows. The corresponding maximum values in mastitic cases were 88% and 92% respectively. In both cows and goats the chief etiological agent for mastitis as found in the present study was Staphylococcus. Other organisms encountered were Streptococcus, Coliforms and Corynebacteria.