BOVINE MASTITIS

ITS DIAGNOSIS AND TREATMENT

A thesis submitted to the
KONKAN KRISHI VIDYAPEETH, DAPOLI
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by

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To Lord Shrikrishna

C E R T I F I C A T E

Certified that the thesis entitled "BOVINE MASTITIS : ITS DIAGNOSIS AND TREATMENT" submitted by SHRI VIVEK HINDURAO KSHEERSAGAR in partial fulfilment of the requirements for the degree of MASTER OF VETERINARY SCIENCE in VETERINARY MEDICINE, which embodies the results of the record of bonafide research work carried out by him under the guidance of University teacher Dr.L.K.KADVEKAR, is to our satisfaction.

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C O N T E N T S

Introduction

I N T R O D U C T I O N

The term mastitis refers to inflammation of the mammary gland regardless of the cause. It is characterised by physical, chemical and usually bacteriological change in the milk and by pathological changes in the glandular tissue as described by Blood, et al. (1983).

In India the prevelence of subclinical mastitis in cows is 54% and in buffaloes 23.86% (Singh & Baxi, 1980). The subclinical mastitis is undetectable externally but now-a-days the diagnosis of mastitis depends largely on indirect tests which depend, in turn, on the leukocyte content of the milk.

Although mastitis occurs sporadically in all species, it assumes major economic importance in dairy cattle. In milking buffaloes, the epidemiological pattern is similar and similar patterns of loss could be expected.

Singh et al. (1982) noted that, loss of milk as a result of subclinical mastitis was 0.23 Kg. per day in cattle and 0.35 Kg. per day in buffaloes.

Morris (1973) reported that infections lasting over three months depressed the relative yield of affected quarters by an average of about 35%, while transient infections depressed yield later in the same lactation by 13%.

There is an additional danger that the bacterial contamination of milk from affected cows may render it unsuitable for human consumption, or interfere with manufacturing process or, in rare cases, provides a mechanism of spread of disease to humans e.g. tuberculosis, streptococcal sore throat and brucellosis.

Blood et al. (1983) suggested that the total losses caused by mastitis are composed of 1) Value of milk production lost (70%), 2) Value of cows lost by premature culling (14%). 3) Value of milk discarded or downgraded (7%) and 4) Treatment and veterinary expenses(9%).

Higgins (1981) reported that reproductive inefficiency was the major cause of culling (39%), followed by planned culling, principally for poor milk yield (24.7%) and mammary disorders (21.2%). 90% of mammary problems were mastitis.

Occurence of mastitis varies with the species of bacteria. Organisms like, Staph aureus, E. coli and lesser coliforms cause loss of life; Corynebacterium pyogenes causes complete loss of quarters; staphylococci and streptococci cause acute clinical mastitis, particularly the latter, but their principal role is in causing subclinical mastitis resulting in reduction of milk produced and a downgrading of its quality. (Blood et al. 1983).

It was, therefore, thought to undertake a multifaceted study on subclinical mastitis, along with few cases of clinical mastitis; its diagnosis; cultural and sensitivity tests and treatment with recent chemotherapeutic agents e.g. povidone iodine, ampicillin cloxacillin and gentamicin.

Review Of Literature

PART-I

REVIEW OF LITERATURE

The review of literature concerning bovine mastitis is dealt with in four parts.

Part - I : Epidemiology of bovine mastitis.

Part - II : Cultural examinations.

Part- III : Sensitivity tests.

Part - IV : Treatment.

PART - I : EPIDEMIOLOGY.

Rathore (1970) analysed a data on 200 cows and showed that the average annual decline on solids-not-fat was 0.037% in cows without clinical mastitis and 0.067% in those with mastitis.

Bozhkova (1973) made a survey in 1970 and 1971 involving over 19,000 cows on 54 farms in 12 regions of Bulgaria. Mastitis was found in 3,836(20%) of cows. Frequency in different breeds was 15% in Bulgarian Brown, 20% in Bulgarian Simmental, 23% in Red and 26% in Black Pied cows. Catarrhal mastitis was the commonest form (53% of the cases). 28% of cases occurred in the first month of lactation, 13% in the second, 15% in the third and 8% in the fourth month.

Walser et al. (1973) analyzed a data for 994 cows and showed that 93% of cases, only one quarter of the udder was affected. Hind quarter was affected in 67% and a fore quarter in 33% of these cases.

Yurkov (1977) compared the occurence of subclinical mastitis among cows of different breeds (Bulgarian brown, Bulgarian Simmental, Black Pied and Bulgarian red). Tests on the milk of over 12,000 cows belonging to four breeds showed that breed had little influence on the occurrence of subclinical mastitis in Bulgaria. Hygienic and bacteriological factors were more important than breed.

Batra et al. (1977) observed the incidence of clinical mastitis in 760 lactations of 331 Holstein cows and studied effects of sire, parity, and month of calving. Incidence of clinical mastitis was higher in hind quarters, than fore quarters. Results indicated that occurrence of mastitis in one quarter was associated with above average frequencies of mastitis in the other quarters of the same cow. Clinical mastitis increased with increase in lactation number upto the fourth lactation. Cows calving in January and February had mastitis more frequently than those calving in March and April.

Verma (1978) carried out the California Mastitis Test, with confirmation by cell count and bacteriological examination, in 245 cows of four breeds at a breeding station. Losses attributable to subclinical mastitis were greatest among Gir cows (9%) than Holstein - Friesian x Thari cross breds (3%) with Danish Red and Tharparkar intermediate. It is acknowledged that managemental factors were probably more important than breed.

Kapur and Singh (1978)[b] reported that clinical mastitis was present in one or two quarters in 37% of 63 cows, 40% of 60 buffaloes. Rear quarters were infected most frequently, especially in buffaloes. Occurrence of clinical mastitis was highest in the third lactation in cows, and the fourth lactation in buffaloes, also in the third and fourth months of lactation.

Hegemann (1978) stated that the bacterial contamination of the mammary gland and it was influenced by following factors:

1) Age : Contamination reached a maximum at seven years.
2) Lactation : During the year, contamination reached a maximum at the tenth month.
3) Milk yield : Cows producing over five litres per milking were more susceptible to contamination.
4) Size of the farm : The smaller the farm, the less was the risk of contamination.

Misra (1978) screened, 346 buffaloes (1,112 milk samples from various farms in Uttar Pradesh and observed that 28 were found positive (8%); 6% due to streptococci and 1.7% due to staphylococci. The incidence of subclinical, clinical and chronic mastitis in the affected quarters was 89%; 7% and 3.6% respectively. The incidence of streptococcal mastitis (79%) was higher than that of staphylococcal mastitis (19%). Clinical cases were mainly due to staphylococci, whereas subclinical and chronic cases were due to streptococci.

Streptococcus equisimilis was the most prevalent. Mastitis occurred during the first to sixth lactations and was almost equally distributed in three different stages of lactation.

Rojo Vazquez et al. (1979) reported that milk samples from 464 quarters from 116 cows in full lactation from various herds were examined bacteriologically and for cell counts. Sixty cows showed subclinical mastitis, which was less frequent in cows in their first lactation or in the first four months of any lactation.

Noori and Tauro (1979) screened, milk samples from 50 cows (200 samples) and 40 buffaloes (160) for the California Mastitis Test. 35% and 27.5% of cases respectively were positive.76% of cows and 56.5% of buffaloes had subclinical mastitis. Of 70 and 44 CMT positive milk samples from cows and buffaloes, 44 (63%) and 30 (68%) contained S.aureus; however the actual number of quarters infected by S.aureus in cows and buffaloes was only 20% and 21% respectively. It was concluded that S.aureus was not a major cause of subclinical mastitis in these animals.

Ruffo et al. (1979) carried out cell counts, on quarter samples collected regularly from a herd of 170 Holstein cows during two years. Graphs demonstrate the increase in cell count with each month of lactation; such increases were greater during the second and subsequent lactation than during the first lactation.

Park (1980) isolated 148 cultures of gram-negative bacilli from clinical cases of mastitis over a two year period in six dairy herds. Most of these isolates (75%) were obtained from rear quarters.

Verma et al. (1980) observed that 562 quarters of 141 apparently healthy cows on the Government Cattle Farm, Patna, 73 (13%) quarters of 43 (30%) cows proved to have subclinical mastitis.

Prandzhev et al. (1980) detected subclinical mastitis in 68% of 2,377 cows of eight herds. On average, only one quarter was affected. Abnormal milk secretion (non-specific mastitis) was present in one-third of the cases; while the remainder had bacterial infection which was more often subclinical mastitis than latent infection.

Singh and Baxi (1980) studied on the incidence and diagnosis of mastitis in milch animals. Bacteriological and other techniques applied to milk samples from 50 cows, 88 buffaloes revealed subclinical mastitis in 27 cows and 21 buffaloes. According to quarters examined in cows 27.75% and in buffaloes 42.57% incidence. They found the incidence increasing according to lactations i.e. first lactation (cow 20.57%; buffalo 8.33%), second lactation (cow 42.86%; buffalo 11.11%), third lactation (cow 60%; buffalo 23%), fourth lactation (cow 66.67%; buffalo 60%), fifth lactation (cow 100%; buffalo 75%), sixth and above (cow 80% and buffalo 83.33%). Distribution of incidence with number of infected quarters along with percentage, one (cow, 40.74%; buffalo, 42.86%), two(cow, 33.33%; buffalo 23.81%),

three (cow, 14.91%; buffalo 14.28%), four (cow, 11.11%; buffalo 19%) and percentage of different quarters affected with subclinical mastitis was LF (cow, 18.97%; buffalo, 20.43% LH (cow, 26.41%; buffalo, 29.54%), RF (cow, 22.64%; buffalo, 15.91%), RH (cow, 32.07%; buffalo, 34.09%).

Gonzalez et al. (1981) stated that, thirty dairy farms, 18 with machine milking and 12 with hand milking were visited on a single occasion in Cordoba province in 1977. Milk sample of cows from 4,168 quarters showed, 2,388 (57.3%) positive to the California Mastitis Test. A lower frequency of subclinica mastitis was seen in cows in first lactation. A higher percentage of Staph. aureus and the three streptococcal specie in the hand milked farms were considered to be due to a low level of hygiene.

Rupasinghe and Kulasegaram (1981) observed in four Government farms with purebred European breeds of cattle and in five private dairies with crossbred cows in smaller units the prevalence of subclinical mastitis ranged from 27.8% to 92.2% cows (11.8% - 97.3% of quarters).

Singh et al. (1982) detected subclinical mastitis in 258 (18%) of 1,400 cows and 67 (13%) of 508 buffaloes. Staphylococci, were associated with 78% of cases of mastitis in cows and 61% in buffaloes. Streptococci were associated with 20% and 23% of cases, respectively. Clinical examination of 1,908 udders revealed blind teats in 10%.

Egan (1982) carried out a California Mastitis Test (CMT) on quarter milk samples of 3,029 lactating cows on 30 farms in Dublin (1980). A CMT reaction of ++ or higher was found in 1,252 (10.3%) quarters. Signs of clinical mastitis were observed in 66 (0.5%) quarters. Quarters showing a CMT reaction of +++ or signs of clinical infection were sampled aseptically for bacteriological examination. Pathogenic bacteria were isolated from 416 (76.3%) of the quarters showing a +++ CMT reaction. Staphylococcus aureus was the principal bacterium isolated from quarters.

Brooks et al. (1982) stated that the proportion of cows from which a bacterial pathogen was isolated, increased with age but not with the stage of lactation.

Daniel et al. (1982) found that there was a highly significant relationship between cow age group and the proportion of affected quarters and it was calculated that the change with each year of age was a 55% increase over the previous year.

Bakken (1982) studied subclinical mastitis in Norwegian dairy cows, prevalence rates and epidemiological assessments. From November, 1976 to December, 1979 quarter samples were collected twice a year from 328 commercial dairy herds. A total of 1,40,243 quarter samples from 35,464 cow units were examined. The average prevalence rate of subclinical mastitis was 31% of the cows and 11.6% of quarters. Almost 50% of the

cases were nonspecific mastitis. Staph. aureus was the main pathogen isolated. The average prevalence of S. aureus was 10.6% of cows and 3.3% of quarters, while the prevalence of all infectious mastitis was 19.9% of cows and 6.5% of quarters. The mastitis prevalence varied in relation to the number of lactation, lactation stage and geographical origin of the herds.

Yass et al. (1983) studied mastitis in 181 buffaloes in 1981-82, of which 70 were from the Government farm at Missan and 81 were from four private farms near Baghdad (Iraq). The prevalence of clinical mastitis was 25.17% (12.08% on quarter basis) and that of subclinical mastitis 31.94% (11.68% on quarter basis). Rear quarters were affected more frequently than the fore quarters. Staphylococcus aureus was the chief aetiological agent in both clinical and subclinical mastitis, followed by Streptococcus agalactiae, Str. - dysagalactiae and Corynebacterium pyogenes. Prevalence of mastitis increased with lactation number and age; it was highest during the fifth lactation and at nine to ten years of age. Incidence was higher during the first two months of lactation, declining during subsequent months.

Wulfmeyer (1983) found that there was a close correlation between increase in age and frequency of subclinical mastitis which was highest in cows six years old. There was also a very close correlation between subclinical mastitis and milk yield in both groups.

Blood et al. (1983) related mastitis susceptibility of cow, to 1) Stage of lactation (first two months)

2) Age of cow (older).

3) Level of inherited resistance, possibly related to teat shape and anatomy of the teat canal.

Rahman et al. (1984) diagnosed subclinical mastitis in 113 (59.2%) of 191 cows and 32 (36.8%) of 87 buffaloes in six dairy herds. Incidence was high in second and third lactation cows (four to six years old) and in third and fourth lactation buffaloes (six to eight years old). Four quarter infections were common in cows (35, 31.0%) an single quarter infection frequent in buffaloes (14, 43.8% 240 milk samples from 103 cows and 60 milk samples from 3 buffaloes were examined for bacteria. 95 (92.2%) cows an 22 (68.8%) buffaloes were positive.

Review Of Literature

PART-II

PART - II : CULTURAL EXAMINATIONS

McDonald et al.(1970) isolated 70 cultures of aerobic gram-negative rods from bovine udder infections in a mastitis research herd comprising of 20 dairy cows, over a period of eight years. Nearly one-half were Escherichia coli and most of remaining isolates were Citrobacter, Klebsiella and Proteus spp.

Sharma and Singh (1970) stated that out of 128 mastitic quarters, half harboured Staphylococcus aureus, 29%, Streptococcus spp., 1.6%, Pseudomonas spp. 2.3% Corynebacterium spp., 7% mixed infections; from 3.9% no bacteria could be isolated.

Fuh and Chang (1971) examined milk samples bacteriologically from 490 quarters of 139 cows in 14 herds in the Taipei district and isolated Str. agalactiae from 28% of the quarters, 46% of the cows, and 86% of the herds; Staph aureus was isolated from 14% of the quarters, 32% of the cows, and 79% of the herds.

Hoare and Barton (1972) examined a total of 2,212 samples positive to the rapid mastitis test from 113 problem herds; and found Staph aureus in 34% of samples and 97% of herds. Str. agalactiae was isolated from 13% of samples and 63% of herds.

Kral et al.(1973) studied in for a four year period; 6,27,770 milk samples from 3,52,351 cows; at 19 state veterinary institutes. Mastitis was diagnosed in 33.8% of the cows, while bacteria were isolated from about half of these cases i.e. from 16.9% of the total number of cows. They were Strep. agalactiae from 9.0%, Staph. aureus from 3.3%, Strep. dysagalactiae, Str. uberis and other streptococci2.8% E.coli from 0.6% and Corynebacterium pyogenes 0.21%.

Todorov et al.(1974) surveyed 26 herds around Sofia for 30 months, involving tests on 7,570 cows, showed that subclinical mastitis was present from 6% to 39% (av 14%) of cows. Bacteria isolated included staphylococci from 45% of bacteriologically positive samples, streptococci (32%) and corynebacteria (15%), Staphylococcus aureus accounted for 63% of the 435 isolated obtained and Staph epidermidis for the remainder, 43% of the 308 isolates of Streptococcus spp. were Str. agalactiae, 20% Str. uberis and 12% Str. dysgalactiae.

* Misra et al (1974) observed Staphylococcus aureus in 33.1% of the infected quraters, S.albus in 15.3% and Klebsiella spp. in 8.4%. Other pathogenic bacteria isolated were Corynebacterium pyogenes, Pseudomonas aeruginosa, Escherichia coli and Streptococcus agalactiae. Mixed infections were encountered in 36% of quarter samples.

Kohler - Samouilidis (1977) obtained 170 milk samples, from cows with suspected mastitis, Staphylococcus aureus (71 strains), S.epidermidis (82 strains) and Micrococcus (17 strains) were isolated. All the Staph. aureus were coagulase positive and the other two were coagulase negative. The formation of a blood agar toxic zone was apparent in 36 (51%) of the Staph. aureus, except for one S.epidermidis strains, all remainder were negative.

Havelka (1977b) examined 54,824 milk samples from 28,737 cows in the year, 1975. Milk from 18.9% of the cows contained mastitis producing bacteria Streptococcus agalactiae was present in 13.9% of the cows, S.aureus in 2.9%, other streptococci in 1.3%, E.coli in 0.13%, Klebsiella in 0.13% and Corynebacterium pyogenes in 0.15%.

Kohler - Samouilidis (1978) isolated 102 strains of Corynebacterium spp. from milk samples, eight were C.pyotenes, two C.renale , 45 C.bovis,45 C.striatum; two strains could not be identified. The biochemical characteristics of the strains were variable. The percentage of gelatin liquefying strains was high (49%).

Yurkov and Todorov (1978) observed mixed infections of Corynebacterium spp. and Micrococcus spp. particularly with Staphylococcus spp.were present in about 5% of samples. Mixed infections of Staphylococcus spp. and Streptococcus spp. were rare.

Gil [redacted] (1978) obtained the milk supplied by each of the 129 dairy herds. All but six yielded potential mastitis agents, the commonest was Staphylococcus epidermidis (68 isolates), followed by Streptococcus dysgalactiae (58), Staph. aureus (38), Str. uberis (14) and Str. agalactiae (4).

Hamir et al.(1979) recorded that Staphylococcus aureus and Streptococcus spp. were the most frequently isolated pathogenes followed by Escherichia coli and Klebsialla pneumoniae more than (20%) of cases had more than one pathogen.

Rojo Vazquez et al.(1979) isolated organisms from 53 cows included Staphylococcus aureus (17.8%) S.epidermidis (39.6%), Streptococcus agalactiae (4%), Corynebacterium (5.7%) and E.coli and other gram-negatives (15.5%).

Park (1980) isolated 148 cultures of gram - negative bacilli from clinical cases of mastitis over a two year period in six dairy herds 81 (55%) were Escherichia coli, 30 (20%) Enterobacter aerogenes, 18 (12%) Klebsiella pneumoniae, 12 (8%) Progeus spp. four Alcaligenes faecalis and three Psendomonas aeruginosa.

Prandzhev et al.(1980) stated that among the bacteria isolated (subclinical mastitis (68%) of 2,377 cows of eight herds) 72%, were Staphylococcus spp.

14% were Micrococcus spp. and 8.4% were Streptococcus spp.

Verma et al. (1980) studied that the three commonest pathogenes isolated from subclinical mastitis cow milk samples were Staphylococcus aureus (34%), Escherichia coli (27%), and S.epidermidis (23%). They also isolated Alcaligenes faecalis (7%), Streptococcus dysgalactiae (5.5%), Micrococcus luteus (4%) and Proteus morganii (4%).

Madariaga Aguilar and Lopez-Alva Rez (1981) isolated organisms from milk samples giving reactions of 3 + in California Mastitis Test. The organisms most frequently isolated were, Staphylococcus aureus (27%), Streptococcus agalactiae (19%), Bacillus spp. (other than B.cereus) (12%), Str.epidermidis (9%) and E.coli (9%).

Tessi et al.(1981) isolated Staphylococcus aureus (54%) from 320 milk samples from mammary quarters of cows representing 40 herds; Streptococcus agalactiae from 23% and Pseudomonas aeruginosa from 13%.

Gonzalez et al.(1981) recorded that the milk samples from 300 quarters giving a positive CMT reaction of 2 + or 3 + were cultured for bacteria. Isolations were made of Staphylococcus aureus (43% of samples), Staph. epidermidis (21%), Streptococcus uberis (19%), Str. agalactiae (13%), Str. dysgalactiae (9%) Corynebacterium bovis (7%), Coryynebacterium pyogenes (1.3%) and Coliforms (1.7%).

Char et al. (1983) analyzed 175 milk samples from buffaloes with mastitis, bacteriologically between 1977-81 showed that Staphylococcus aureus was the chief pathogen (27%), followed by Streptococcus spp.(14%), Corynebacterium spp. (10%), Staph. epidermidis (4%), Diplococcus and Bacillus subtitis (each 3%) and Proteus vulgaris (1%).

Rahman and Baxi (1983) studied various aspects of Staphylococcal mastitis in bovines. A total 565 mastitis milk samples of cows and buffaloes suffering from clinical and subclinical mastitis were investigated bacteriologically Staphylococci could be isolated 61.97% of bacteriologically positive samples which proved to be the chief aetiological agents of bovine mastitis in India.

Stem et al. (1984) stated that milk samples for aerobic culturing were collected from 72 quarters of cows with clinical mastitis that had not responded to *intramammary antimicrobial treatment. Mastitis pathogens* were isolated from 56 samples (78%).

Rahman et al. (1984) examined 240 milk samples from 103 cows and 60 milk samples from 32 buffaloes for bacteria. 95 (92.2%) cows and 22 (66.8%) buffaloes were positive bacteriologically. Staphylococcus aureus (79 cows, 36.4%; 11 buffaloes, 31.4%), Staph. epidermidis (54 cows, 24.9%; 9 buffaloes, 25.7%) and Streptococcus

agalactiae (36 cows, 16.6%; 1 Buffalo, 2.7%) were most frequently isolated. No mycoplasma was isolated.

Aydin and Coskuner (1984) isolated 564 agents from mastitis milk samples, 23% were coagulase positive staphylococci and 7% coagulase negative staphylococci, 11% were Streptococcus agalactiae, 9% Streptococcus uberis, 2% Streptococcus dysgalactiae, 4% Streptococcus faecalis, 9% Escherichia coli, 7% Corynebacterium pyogenes and some fungi were also isolated.

Fagliari et al. (1984) carried out comparison were made between CMT results and bacteriological examinations in 272 milk samples from crossbred cows in Ilha Solteira sp. region of Brazil. In samples classified as CMT +, ++, and +++, respectively bacteria were detected in 22.4, 74.4 and 85.6% of cases. Streptococci and coagulase positive stphylococci were the most frequent isolates.

Review Of Literature

PART-IV

PART - IV : TREATMENT

Sharma and Singh (1970) stated that comparison of three proprietary intramammary infusions revealed that Mastalone (a combination of oxytetracycline, oleandomycin, neomycin and prednisolone) was effective in 87%, Strypen Forte (benzylpenicillin plus dihydrostreptomycin) in 70% and Aureomycin (chlortetracycline) in 63% of cases.

Rosenzuaig and Mayer (1970) examined bacteriologically individual quarters (including dry cows) of a herd of 150 milking cows under poor management and milking conditions. Each cow was again examined at drying off, after which all quarters were treated, whether infected or not, with 0.5 g. benzathine cloxacillin, and the teats were dipped in 5,000 ppm iodophor. Further bacteriological examinations were carried out 12-14 days after calving, and a month after the last calving in the herd. The incidence of Staph. aureus infection in the herd fell from 25% to 13% between the first and final examinations. The incidence of staphylococcal infection of the udder decreased by 61% between drying-off and 12-14 days after calving in treated cows and by 13% in untreated controls, and the incidence of new staphylococcal infection in the same period increased by 4.7% in treated cows and 22.3% in controls.

Bratlie (1973) carried out bacteriological examination of milk samples from 746 dry cows, it revealed that 240 (32%) were infected in one or more quarters; the total infected quarters being 504 (52.5%). All the four quarters of infected cows were treated with either placebo, a combination of cloxicillin, neomycin and hexachlorphane,or penicillin plus neomycin. Examined four days after parturition, a cure rate of 59.7% was detected in placebo treated group and 78% in the antibiotic treated groups. At the same time new infections were seen in noninfected quarters, as follows:

12.6% in placebo treated, 9.9% in antibiotic treated and 5.9% in health untreated quarters. It was suggested that only the infected quarters of dry cows should be treated, except where infection was due to beta-haemolytic streptococci.

Merck et al.(1974) carried out drying off half of 325 cows in three herds at the Max Planck Institute, with the injection of 500 mg. benzathine-cloxacillin into each teat canal. Bacteriological and cytological studies showed that, in the treated group, the incidence of subclinical mastitis after calving was reduced by 64% from the incidence prior to drying off, while it increased by a half in the untreated group. The number of infected quarters was

reduced by 53% by the dry period therapy, and it cured 75% of the quarters which had been affected with subclinical mastitis.

Dannemann (1974) carried out the trial in 18 herds on cows which had shown raised cell counts on bucket samples and/or bacteriological cultures from quarter samples in the preceding lactation. Dry period therapy was given to 79 cows (314 quarters) with benzathine oxacillin, and to 79 cows (310 quarters) with benzathine-cloxacillin. Bacteriological and cytological investigation of milk from the treated quarters two and six weeks after calving showed clear and sustained improvement after either treatment, with slightly more favourable results with benzathine-oxacillin.

Roychoudhury et al.(1976) conducted a trial with Pendistrin-SH (squibb) in clinical cases of staphylococcal mastitis; and found that a combination of procaine, benzyl penicillin, streptomycin, sulphamerazine and hydrocortisone, was 94-100% effective in coagulase positive staphylococcal infection. In chronic cases due to mixed infection the efficacy of the infusion was 33% to 55%. This less effectivity was due to (i) resistance of the organisms to the penicillin therapy which might develop from injudicious, inadequate and repeated use of the drug.

(2) Fibrosis and cellulitis in chronic infections which might prevent infusion of the drug to the hidden foci of infection and (3) Presence of mixed microbial infection outside the spectrum of antibiotic activity of the penicillin.

Bywater (1976) stated that, subclinical mastitis may be treated during lactation by examining each quarter bacteriologically to identify those infected and then treating simultaneously with appropriate therapy. Though, a more practicable approach to subclinical mastitis was the use of therapy at drying-off as part of a control programme.

Ungureanu et al.(1977) suggested therapy at drying-off, involving the injection of six ml. (500 mg) cloxacillin. The injection was given into 363 teat canals, of which 139 were infected. It was observed that a total of 303 were free of infections at the subsequent calving and only 60 infected. There were 16% of new infections. The efficacy of the treatment was greatest against Streptococcus agalactiae and less against Staphylococcus aureus.

Os et al.(1977) stated that the activity of the combination ampicillin + cloxacillin against Streptococcus agalactiae was mainly due to ampicillin. Against penicillin-resistant staphylococci, the activity of the

combination was mainly due to the activity of cloxacillin. Against the other microorganisms both compounds contributed to the activity of the combination.

Nielsen (1978) carried out single intramammary treatments of bovine mastitis with penicillin preparations during lactation and at drying-off with seven preparations and their curative effects. The efficacy of seven commercial preparations was assessed in 693 cows and 1,305 infected quarters (1.88 infected udder/cow). Three preparations were of procaine penicillin, two of potassium penicillin, one of benzathine penicillin and one of penethamate hydroiodide. Different preparations also contained streptomycin, neomycin and bacitracin. Each preparation was used only once and only benzathine penicillin-streptomycin was used at drying-off. Of the total, 354 cows (51%) were cured (784 quarters, 60%) after three months. The average rate of cure was 54% (range 40-95%) of cows and 34% (range 25% to 62%) of quarters. The average cure rate in lactating cows was 60% and in drying-off cows 75%.

Kapur and Singh (1978a) reported that in 33 cows and 26 buffaloes, 88 infected quarters were treated with intramammary injection of Spiramycin, Pendistrin-SH, Mastalone; or Omnamycin. Mastalone cured 15 to 17 treated quarters, Corynebacterium pyogenes and Pseudomonas spp.

failed to respond to it. Spiramycin cured only 30 of 43 quarters. amd Streptococcus agalactiae, Escherichia coli and Pseudomonas spp. failed to respond. Pendistrin-SH cured 12 of 14 quarters, one quarter infected with Staphylococcus aureus and one with Bacillus species failing to respond. Omnamycin cured 12 of 14 quarters, the two resistant infections being with Staph. aureus and Elcoli.

Pearson and Mackie (1979) stated that 53% of 436 quarters responded to treatment, only 28% of S.aureus quarters responded. There was little relationship between in vitro sensitivity and cure rate.

Preez et al.(1981) treated the teat infections of Staphylococcus aureus with 1 g. chloramphenicol (three times at 12 hours intervals), it reduced infection rate by 66% by 14 days after the last treatment. The same treatment in subclinical reduced infections by 31%. Orbenin dry cow treatment, three times at 12 hours intervals, reduced infections 81 and 80% respectively. No chloramphenicol residues were detected in milk 12 hrs. after the last treatment. After Orbenin dry cow treatment residues were present in all quarters.

Poutrel and Rainard (1981) quoted that, selective treatment of all cows with atleast 1 CMT positive quarter at eight weeks before drying-off was suggested as the

simplest and most economic treatment for herds with a low mastitis infection rate. Systematic therapy was recommended for herds with high infection rate.

Garcia Partida et al.(1981) used gentamicin in the treatment of bovine mastitis. Out of 125 cases of bovine mastitis (11 subclinical, 50 acute and 64 chronic), 100 cases were treated with 50 mg. gentamicin, 1 mg. dexamethasone plus 1,00,00 i.u. procaine penicillin in 20 ml and 25 cases with 50 mg gentamicin, 1 mg dexamethasone plus 125 mg trimethoprim. Subclinical cases reveived a single dose, the remainder a second dose at 18 hours and if necessary, a third dose at 24 hours. After 30 days 91.2% were classed as "Cured"; all of these were negative to the California Mastitis Test. Results with the gentamicin/trimethoprim product were superior (96%) to those of the gentamicin/penicillin product (90%). Results were analysed in relation to the infecting organisms; all of 19 cases of E.coli mastitis, 47 of 49 Staph. aureus, and 44 of 49 cases of streptococcal mastitis were deemed cured.

Becker (1982) tested a proprietary combination of 200 mg ampicillin and 400 mg. oxacillin in 10ml. oil suspension in 107 cows with 153 mastitis quarters in 45 housed dairy herds over a 13 months period. Secretions became normal in 57% of cases within 10 days of the,

conclusion of treatment. Only 11% of affected udders remained diffusely indurated on palpation, though 54% had been before treatment. Mastitis due to gram-positive organisms responded more quickly to treatment then other forms.

Hamaver (1983) treated acute bovine mastitis with trimethoprim/sulfonamide intramammary. The nature, actiology was investigated. In 86 cases involving 91 quarters. Alternate cases received the experimental trimethoprim-sulfonamide treatment (25 ml. i/v and intramammary injection of a preparation in oil on two successive days). The remaining cases, for comparison received the antibiotic treatments currently in use, which included oxytetracycline, kanamycin, neomycin, penicillin, streptomycin and ampicillin. Judged by test results two weeks later, 88% of the experimental groups and 80% of the comparison groups were cured.

Storper et al.(1983) tested the efficacy of three new intramammary antibiotic combination products in eliminating streptococci and staphylococci from subclinically infected udders of lactating cows, and the duration of antibiotic residues in milk were determined. Products tested were Amclox (75 mg. ampicillin sodium and 200 mg. cloxacillin sodium), Kanapen (250 mg kanamycin sulphate and 3,00,000 units procaine penicillin G), Ampicet

(100 mg. ampicillin sodium and 200 mg. cephalothin sodium). Each product was infused twice, with 24 hours interval, into the quarters of the udder. The efficacy of each of the products in eliminating streptococcal infection was good and compared favourably with the reported efficacy of products containing only penicillin G, only cloxacillin or a combination of ampicillin and cloxacillin. With Amclox, the average cure rate of Staphylococcus aureus infection was 47.5%, ranging between 12.1 and 87.8% in three herds. Kanapen eliminated 79.2% and 88.6% S.aureus cow infections in two herds. Ampicet cured 66.0% of S.aureus cow infections in five herds although in one herd only 15.6% of the infections were eliminated. Antibiotic activity was found in milk upto 84 hours after Amclox therapy, whereas the required milk withholding periods were 108 hours and 48 hours after treatment with Kanapen and Ampicet, respectively, (all in herds milking three times daily).

Trapecar et al.(1983) carried out a field trial of "Kloksavet M" and "Ampivet K" for staphylococcal and streptococcal mastitis. "Kloksavet M" (PLIVA, Zagreb) was an intramammary preparation containing 200 mg. cloxacillin and 10 mg prednisolone, while "Ampivet K" contains 200 mg. ampicillin and 100 mg cloxacillin. One tube was inserted into an affected quarter on three

successive days. 17 cows with Staphylococcus aureus mastitis, 5 with Streptococcus pyogenes and 6 with S.uberis mastitis were treated with either preparation. The high efficacy of the antibiotic was confirmed.

Singh et al.(1984) conducted a study to know the efficacy of cloxacillin (Orbenin-LA) alone had its combination with ampicillin (Ampiclox L.C.) in acute mastitis. Further they stated that, these drugs had not been used routinely in our country and were still not available as intramammary infusion. The Orbenin L.A. was tried in 13 cases of acute mastitis and it was able to cure 61.53% of the animals and 56.75% of the quarters treated. Ampiclox L.C. was tried in 12 cases and it was able to cure nine cases completely and two partly. The percentage of cure was 75% of animals and 86.95% of the quarter treated. Orbenin L.A. was able to eliminate 54.83% of the staphylococci and 50% streptococci whereas Ampliclox L.C. cleared 100% streptococci, 90% staphylococci and 77.77% of E.coli infections.

Material And Methods

MATERIAL AND METHODS

Dairy unit of Bombay Veterinary College, Parel; Unit No.22 of Konkan Krishi Vidyapeeth at Aarey Milk Colony, Goregaon; Cattle Breeding Farms of the Bombay Gowrakshak Mandali at Kandivali and Betegaon; as well as a few buffalo stables at Malad, were selected for the study. These farms had purebred and cross-bred cattle and/or Murrah buffaloes.

In all 166 animals and their 644 quarters, were examined for mastitis. These farms and stables carried systemic rearing of cows and buffaloes and were hygienically managed. Hand milking was done twice a day.

History with relevant data of each animal like breed, age and no. of lactations was recorded. (Appendix No.1).

MATERIALS:

1) Modified California Mastitis Test (MCMT) Reagent. It was prepared in laboratory according to Bhatnagar and Mehrotra (1969).

2) Different Media were used for the isolation and identification of organisms.

3) About 16 antibiotic and antimicrobial sensitivity discs (Pasteur Biological Labs.) and Pivipol, Betadine discs (Prepared in Laboratory) were used for the sensitivity of organisms. (Appendix No.2).

4) Drugs used :-

(i) Pivipol supplied by AR-EX Laboratories Pvt. Ltd.

(ii) Betadine veterinary supplied by Wockhardt Pvt.Ltd.

(iii) Tilox (Vet.) supplied by Wockhardt Pvt.Ltd.

(Iv) Mastalone for Mastitis (Pfizer).

(v) Pendistrin-SH Veterinary (Sarabhai).

(vi) Albercilin Vet. (Albert).

(vii) Lykacetin - S (Lyka Labs.).

(viii) Terramycin (Pfizer).

(ix) Gentavet supplied by P.C.I. Pharmaceuticals Pvt.Ltd.

(x) Antrima (May & Baker).

Their contents, dosages and duration of treatments were given in Appendix No.3.

METHODS:

The quarters were termed as left fore (LF), left hind (LH), right fore (RF) and right hind (RH). After cleaning of udder, teats, and discarding first few strips, the Modified California Mastitis Test was carried out at the time of milking.

I MODIFIED CALIFORNIA MASTITIS TEST (MCMT) Bhatnagar & Mehrotra (1969).

MCMT Reagent :- Five hundred mg. of Det (Modern soapless detergent manufactured by Swastik Household and Industrial Products, Bombay-38) was added to 100 ml. of distilled water and mixed well. 1.5 gm. of sodium hydroxide and 10 mg. of bromo-cresol purple were added to the mixture and mixed well to dissolve the ingredients and was stored

in sterilized screw capped bottles.

Procedure :- About 3 ml. milk was drawn from each quarter into each cup of the plastic paddle. Then 3 ml. of MCMT reagent was poured in each milk sample and mixed gently by slow circular movements of the paddle in the horizontal plane for 10 to 20 seconds and results were recorded as below:

1) No change in the mixture.....(-) Negative.

2) Mixture showing slight precipitate and gel which dissolves later on, with continuous movement of the paddle..... (±) Suspicious.

3) Mixture showing definite precipitate and gel.....(+) Positive.

4) Mixture showing thick gel formation which even sometimes bulges the milk surface.....(++) Highly Positive.

For present study only highly positive milk samples were processed for cultural examination-Method of Fagliari et al. (1984).

II. CULTURAL EXAMINATION :-

From positive quarters about 5 c.c. milk was collected aseptically into a sterile test tube for cultural examination. Milk was centrifuged at 2000 rpm. for 10 minutes. Supernatent was discarded. A loopful sediment was inoculated on 5% bovine blood agar by streak method and incubated at 37°c, for twenty four hours. If samples were culturally negative at 24 hours, they were again incubated for one more day.

Identification of organisms was carried out by colony characters, staining reaction and haemolysis. Staining was done with Gram's staining and morphology, arrangements etc. were observed.

For Staphylococcus spp: Coagulase Test (Finegold et al.,1978) was performed as follows:-

Citrated rabbit plasma, 0.5 ml. of a 1:4 dilution, in a small (12 x 100 mm) tube was inoculated heavily with one or two drops of an overnight culture of the organism and incubated at 35°c in a water bath. Complete or partial coagulation in 1 to 4 hours, was interpreted as positive.

Typical strains of S.epidermidis and S. saprophyticu were coagulase negative, whereas pathogenic strains of S.aureus were coagulase positive.

For Corynebacterium sp. gelatin liquefication was observed to identify Corynebacterium pyogenes.

For gram-negative rods, MacConkey's agar, Triple Sugar Iron Media (TSI) were carried out to identify the genus and species.

III. SENSITIVITY TEST:

Sensitivity test was carried out by paper disc method as described by Finegold et al. (1978). This test can be performed easily and results can be obtained within 10-12 hours to determine the correct chemotherapeutic approach to the treatment.

Culture was prepared in nutrient broth and directly smeared on special media "Mueller-Hinton Agar" (HIMEDIA). The discs were placed on inoculated plates. The plates were incubated 12-18 hours at 37°c and sensitivity results were read according to the sensitive zones. (Appendix No.2).

IV. TREATMENT:

In most of the cases, antibiotics with a large inhibition zone on agar were selected for treatment (method of Weigt & Bleckmann 1978).

The antibiotics selected, their dosages and duration of the treatment was as shown in Appendix No.3.

* Follow up :- After completion of treatment, retesting with MCMT after 72 hours was done. From the quarters which were found positive to MCMT, samples were collected and again processed for cultural and sensitivity tests; and treatment was given. This was carried out till all selected cases were found to be negative.

* Negative cases :- Negative cases at first MCMT; Ist treatment; or later ones were selected randomly and their milk samples were processed for cultural examination.

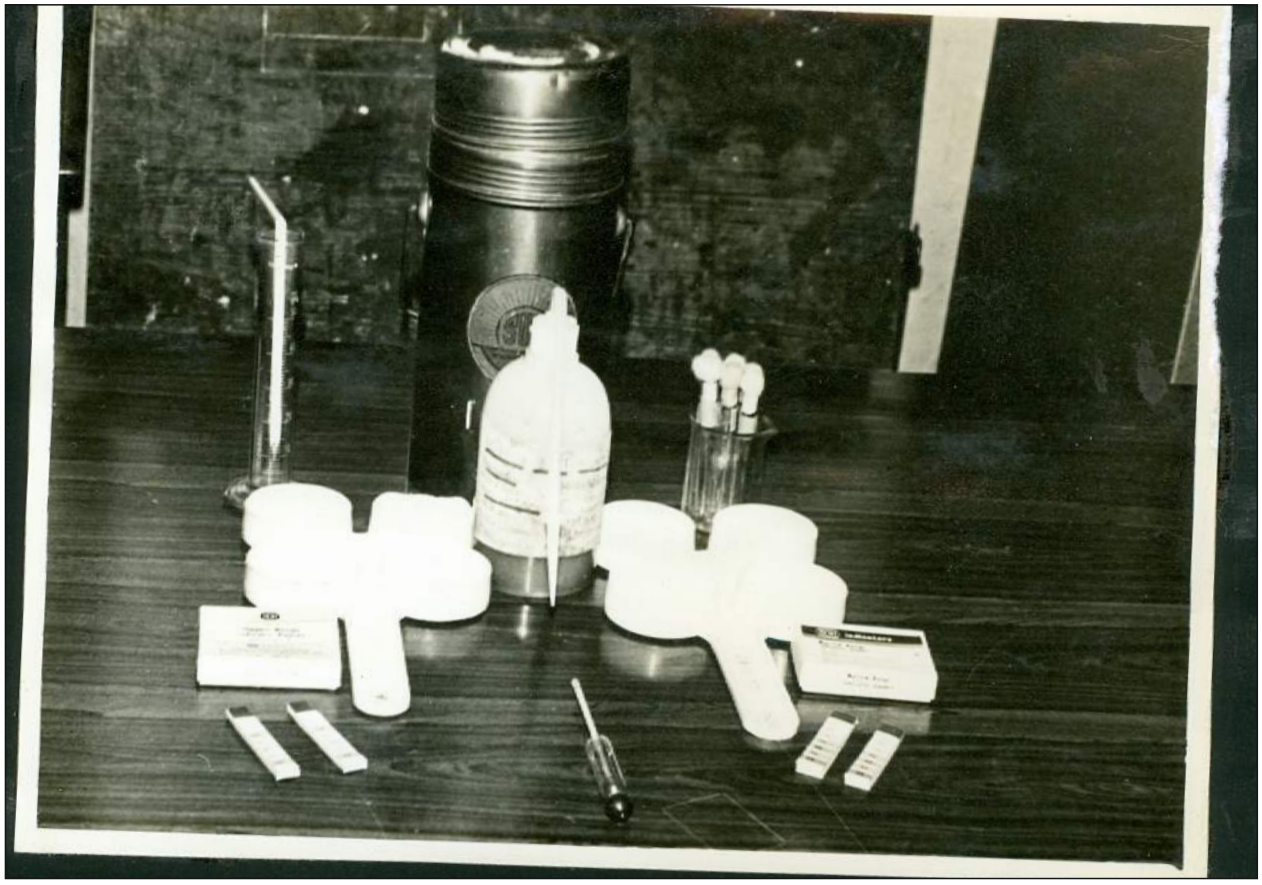

Fig-1- Showing Material required for MCMT and collection of milk samples.

Fig -2- showing a method of collection of milk sample for MCMT

Fig-3- Showing a negative MCNT reaction

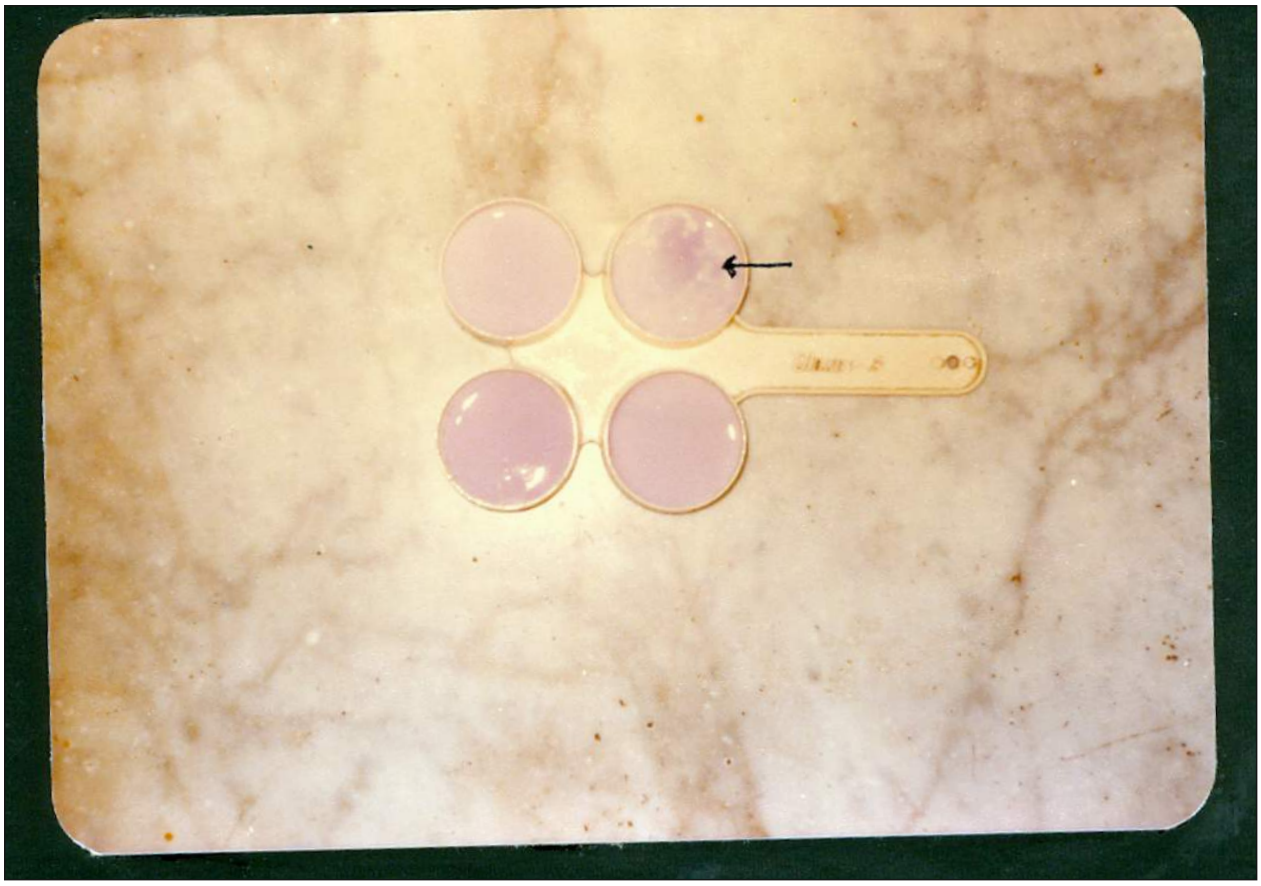

Fig -4- Showing a highly positive (++) MCMT, marked by an arrow.

Fig-5- Showing a method of cultural examination

Fig-6-

Showing a pattern of sensitivity test results with Pivipol disc indicating the orcanisms being resistant.

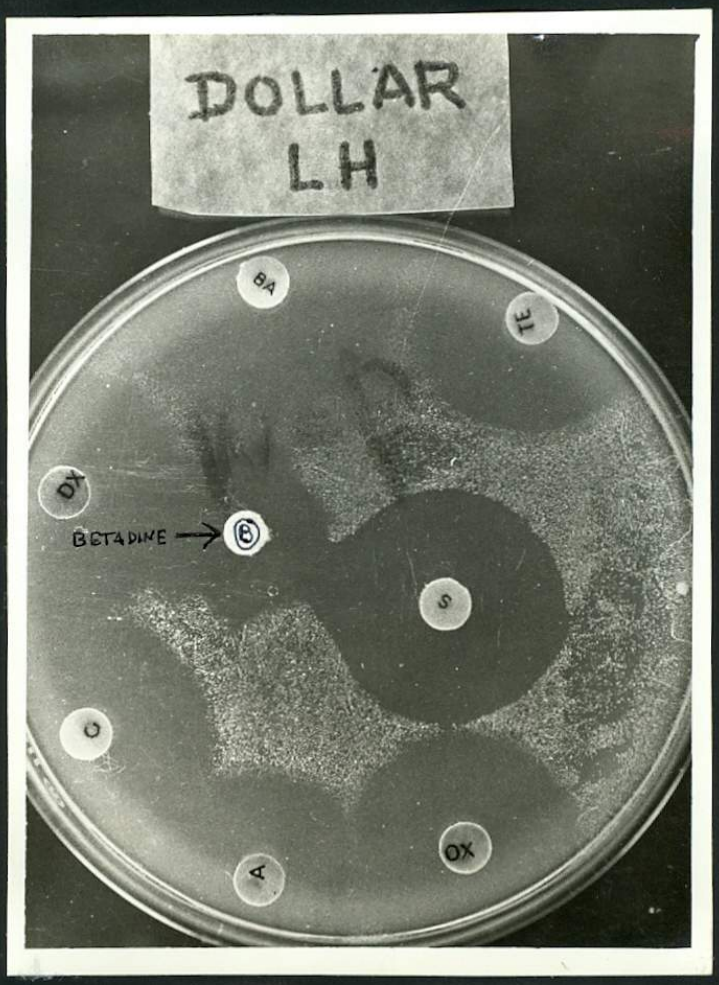

Fig-7-

Showing a pattern of sensitivity test results with Betadine disc showing a clear zone indicating the organisms being sensitive.

Fig-8-

Showing mhamotherapeutic agents (pivipol, Betadine and Tilox)

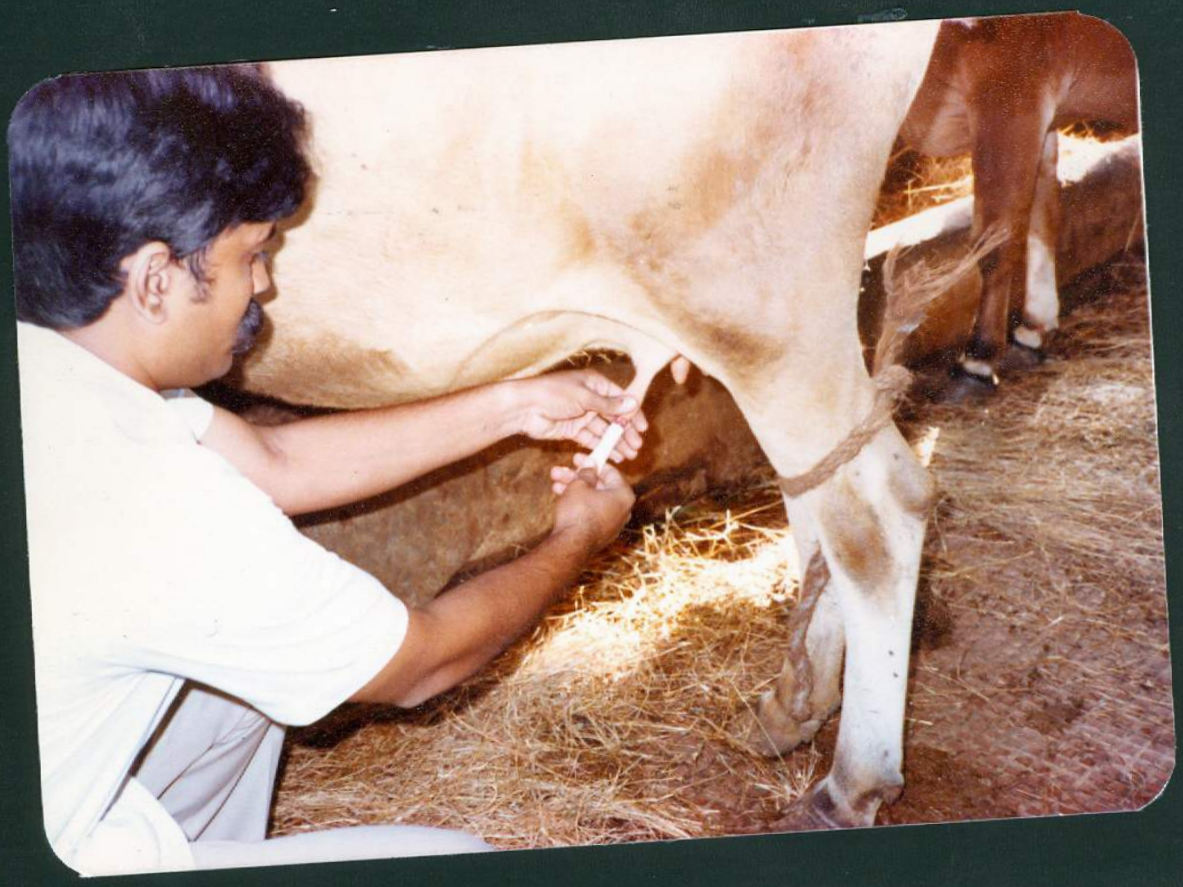

Fig-9-
Showing intramammary infusion of Tilox.

Fig-10-
Showing intramammary infusion of Pivipol

Results

R E S U L T S

General Observations on Incidence of Bovine Mastitis :

We had studied the incidence of mastitis (Epideomiological one aspect) on very limited number of cattle and buffaloes so whatever our findings regarding incidence of bovine mastitis were as follows.

1. Breed : In cattle, according to breedwise, Gir (41.86%) Gir X Holstein Friesian crossbreds (50.94%) and Gir X Jersey crossbreds (21.95%) incidence of mastitis was recorded.

2. Age : After 8 years, incidence of mastitis increased above 50 %, in cattle and buffaloes.

3. Number of Lactations : Out of 64 positive cases of bovine mastitis, in various lactations, 26 cases were in third and fourth lactations (40.62 %).

4. Number of Quarters : Incidence recorded as one quarter infected (65 %), two (24 %), three (9 %) and all four (2%) in cattle, while in buffaloes cases were with single quarter infections.

5. Type of Quarter : According to quarter types, incidence of mastitis was found nearly equal in hind and fore quarters in cattle. But in buffaloes, out of ten cases of mastitis, nine were from hind quarters.

Table : 1 : Incidence of Subclinical mastitis
(Modified California Mastitis Test)

Sr. No.	Farm	No.of Animals		No.of Quarters	
		Total	Positives	Total	Positives
1.	Dairy Unit at BVC (Cows).	11	7 (63.64)	44	9 (20.45)
2.	Aarey Colony Unit No.22 (Cows).	25	7 (28.00)	100	11 (11.00)
3.	Aarey Colony Unit No.22 (Buffaloes)	18	2 (11.11)	70	2 (2.86)
4.	Cattle Breeding Farm, Kandivali (Cows).	42	16 (38.10)	165	20 (12.12)
5.	Cattle Breeding Farm, Betegaon (Cows).	56	18 (32.14)	209	33 (15.79)
	Total	152	50 (32.89)	588	75 (12.76)

( ) Figures indicate percentages.

Table : 2 : Cultural Examinations of Subclinical Mastitis.

Total ++ MCMT samples	Culturally positive samples	Efficacy of MCMT
75	71	94.67 %

Table : 3 : Clinical Mastitis Cases.

Sr. No.	Farm	No.of Animals	No.of Quarters
1.	Dairy Unit at BVC (Cows)	2*	2*
2.	Aarey Colony Unit No.22(Cows)	2	2
3.	Cattle Breeding Farm, Betegaon (Cows).	2	3
4.	Buffalo Stables at Malad.	8	8
	Total	14	15

* 1 animal - 1 quarter culturally negative.

Table : 4 : Aetiology.

Sr. No.	Organisms	No.of Quarters	Percentage
1.	Coagulase positive staphylococci	35	35
2.	Coagulase negative staphylococci	22	22
3.	Haemolytic streptococci	3	3
4.	Non-haemolytic streptococci	14	14
5.	*Corynebacterium pyogenes*	9	9
6.	*Bacillus* spp.	5	5
7.	*E.coli*.	3	3
8.	*Klebsiella* spp.	2	2
9.	Unidentified gram-positive rods	7	7
	Total	100	100

* Mixed infections in 15 quarters (17.65 %).

Table-5. "SENSITIVITY RESULTS"

No.	ANTIBIOTIC → / ORGANISMS ↓	AM	C	A	CX	DX	FX	GM	N	FD	NY S	NY I	OL	OX	P	BA	S	TE	POVIDONE IODINE: PIVIPOL 1% S	PIVIPOL 1% I	BETADINE 5% S	BETADINE 5% I
1.	Coagulase Positive staphylococci	19	19	11	25	20	13	33	25	14	-	-	24	13	5	14	14	10	-	-	1	-
2.	Coagulase Negative staphylococci	15	14	8	17	14	6	16	13	8	-	-	13	9	8	6	8	9	1	-	2	-
3.	Streptococci	10	8	3	7	6	4	9	7	3	-	-	6	6	5	6	8	3	-	3	3	3
4.	Corynebacterium pyogenes	4	4	3	4	5	2	7	8	2	-	-	5	1	4	5	4	1	-	1	-	5
5.	Bacillus spp.	4	4	-	3	1	-	5	5	1	-	-	2	-	-	-	4	-	-	-	-	-
6.	E. coli.	2	2	2	-	2	1	3	2	-	-	-	1	2	1	3	2	2	-	-	1	1
7.	Klebsiella spp.	-	2	-	-	-	-	1	1	-	-	-	-	-	-	-	2	-	-	-	-	-
8.	Unidentified gram-positive rods.	3	1	2	3	4	-	4	4	2	-	-	4	4	2	1	2	3	-	-	-	1
	TOTAL:-	57	54	29	59	52	26	78	65	30	0	0	55	35	25	35	44	28	1	4	7	10

** Antibiotic short forms : Pl.see APPENDIX NO. 2

* S = Sensitive

* I = Intermediate.

According to sensitivity results for each type of organism; first three antibiotics were recorded as follows :

Table : 6 : Antibiotics of choice for various organisms.

Sr. No.	Organisms	Antibiotics		
		First choice	Second choice	Third choice
1.	Coagulase positive staphylococci.	GM (94.29)	CX, N (71.43)	OL (~~71.43~~) (68.57)
2.	Coagulase negative staphylococci.	CX (77.22)	GM (72.73)	AM (68.18)
3.	Streptococci	AM (58.82)	GM (52.94)	S (47.06)
4.	Corynebacterium Pyogenes	N (88.89)	GM (77.78)	DX, OL, BA (55.56)
5.	Bacillus spp.	N, GM (100)	C, S, AM (80)	CX (60)
6.	E. Coli.	BA, GM (100)	--	--
7.	Klebsiella spp.	C, S (100)	--	--
8.	Unidentified gram-positive rods.	DX, N, P, GM, OX (57.14)	--	--

* Antibiotic short forms : Pl.see Appendix No.2

( ) Figure shows percentage.

* Five antibiotics of choice for treatment of Bovine Mastitis.

1. Gentamicin - (78 %)
2. Neomycin - (65 %)
3. Cloxacillin - (59 %)
4. Ampicillin - (57 %)
5. Oleandomycin - (55 %)

( ) Figure shows percent sensitivity of organisms to antibiotic.

* Five antibiotics to be chosen as a last resort FOr the treatment of Bovine Mastitis.

1. Penicillin - (75 %)
2. Furazolidone - (74 %)
3. Tetracycline - (72 %)
4. Chlortetracycline - (71 %)
5. Nitrofurantoin - (70 %)

( ) Figure shows percent resistant of organisms to antibiotic.

Table : 7 : Treatment.

Sr. No.	Antimastitis preparations	No.of quarters treated	No.of quarters cured.	Efficacy %
1.	Tilox-vet	31	26	83.87
2.	Gentavet	16	16	100.00
3.	Lykacetin-S	7	7	100.00
4.	Terramycin	6	5	83.33
5.	Albercillin	5	4	80.00
6.	Mastalone	4	4	100.00
7.	Pendistrin-SH	2	2	100.00
8.	Antrima	2	2	100.00
	Total	73	66	90.41

REPEAT CASES :

Incidence of repeat cases (which did not respond to the treatment) was 13.73 % animalwise and 9.59 % quarterwise (Table-8).

TREATMENT WITH POVIDONE IODINE :

There was not a single reference available, regarding treatment of bovine mastitis with povidone iodine.

Pankey et al. (1983) tried povidone iodine as a teat dip under experimental challenge to Staphylococcus aureus and Streptococcus agalactiae.

Literature on Betadine (5 % povidone iodine) and Pivipol (1 % povidone iodine) stated that, it could be used in treatment of mastitis. In case of 'Betadine' a dose of 10-20 ml. per quarter as intramammary route was suggested but the course of treatment was not mentioned.

Though the sensitivity test for Betadine and Pivipol was carried out, the treatment was not given on the basis of sensitivity as the test results were not standerdised.

In this study povidone iodine was used in different doses and different durations of treatment. The detail treatment record is presented in Table-9.

MILK YIELD : After the treatment, there was no appreciable difference in milk yield of cattle and buffaloes.

NEGATIVE CASES :

Negative cases at first MCMT; Ist treatment; or later ones (Total 15 cases) were selected randomly and their milk samples were found to be culturally negative.

TABLE-8.

DETAIL RECORD OF REPEAT CASES.

NO.	FARM	Case No. Quarter & Type of Mastitis.	Ist Cultural Results.	Sensitive To	Treatment with	IInd Cultural Results.	Sensitive To	Treatment with	IIIrd MCMT Results.	Cultural Results	Sensitive To	Treatment with	IVth MCMT Results & Remarks.
1.	Dairy Unit at B.V.C., Parel.	C_{112}, RF clinical.	Nonhaemolytic Streptococcus +Bacillus spp.	AM,N,C,CX, GM, S.	TILOX	Corynebacterium pyogenes	N,OL,P,C, GM,S,BA.	LYKACE-TIN-S	NEGATIVE	-	-	-	-
2.	Aarey Colony, Unit No.22, Goregaon.	514,LH, Subclinical	Nonhaemolytic Streptococcus spp.	AM,A,DX, N,P,GM, OX,S,BA.	TERRA-MYCIN.	Coagulase Negative Staphylococcus sp.	A,DX,TE, C,GM,OX, BA.	-"-	NEGATIVE	-	-	-	-
3.	Cattle Breeding Farm, Kandivali.	Veena-III LH, Sub-clinical.	Corynebacterium pyogenes	AM,A,DX, N,P,C,CX, GM, S.	TILOX	Corynebacterium pyogenes.	N,OL,GM, OX,BA.	MASTALONE. with Terramycin 20ml. I/M-5 days.	POSITIVE (CLINICAL CASE).	Corynebacterium pyogenes	DX,N,OL, TE,CX, GM,OX.	GENTAVET	Quarter dried fibrosed & no milk production.
4.	Cattle Breeding Farm,Betegaon.	Tora-II, RH Subclinical	Coagulase positive Staphylococcus sp.	AM,N,FD, C,GM.	ALBER-CILLIN.	Coagulase positive Staphylococcus sp.	OL,FD,C, CX,GM, BA.	GENTAVET	NEGATIVE	-	-	-	-
5.	-"-	Krishna-IV, RF, Sub-clinical.	-"-	AM,A,DX, N,OL,CX, GM,OX,S	TILOX	-"-	OL,FD,C, CX,GM,FX, BA.	-"-	-"-	-	-	-	-
6.	-"-	Vanbala-II RH, Sub-clinical.	-"-	AM,N,OL, P,FD,CX, GM,S,FX, BA.	TILOX	-"-	A,DX,N,OL, TE,FD,C, OX.	MASTALONE.	-"-	-	-	-	-
7.	-"-	Asha-III LF, Sub-clinical.	-"-	AM,A,N,TE, FD,CX,GM, OX,FX.	TILOX	-"- & Bacillus sp.	TE,GM (both cultures)	GENTAVET	-"-	-	-	-	-

* Antibiotics short forms : Pl. see APPENDIX NO. 2.

* Treatment : Pl. see APPENDIX NO. 3.

Table-9.

TREATMENT RECORD WITH POVIDONE IODINE

NO.	PREPARATION	Dose per quarter.	Duration	Species & no.	Type of Mastitis.	No.of quarters.	Side effects and/or Results.	After 72 hrs. MCMT Results.	REMARKS.
1.	Pivipol	20 ml. b.i.d.	1 day	Cows (2)	Subclinical Mastitis.	2	Intense irritation observed, on an average 30% milk yield decreased. After 4 to 5 days milk yield came to normal.	POSITIVE	Avoid treatment with povidone iodine in subclinical mastitis in cows. These cases were treated with respective antibiotics after cultural and sensitivity tests and cured.
2.	Betadine	10 ml b.i.d.	1 day	Cows (3)	-"-	5			
3.	Pivipol	10 ml. b.i.d.	3 days	Buffalo (1)	Subclinical Mastitis.	1	No irritation,no depression in milk yield.	NEGATIVE	Povidone iodine preparations, can be used in treatment of subclinica mastitis in buffaloes.
4.	Betadine	10 ml. b.i.d.	3 days	Buffalo (1)	-"-	1	Slight irritation but no depression in milk yield.		
5.	Pivipol	10ml. o.i.d.	5 days	Cow (1)	Clinical Mastitis.	1	Quarter was dried completely.	-	-
6.	Betadine	10 ml. o.i.d.	5 days	Cow (1)	-"-	1	-"-	-	Cow was pregant. After parturation milk was completely normal and MCMT negative.
7.	Pivipol	40 ml. o.i.d.	2 days	Buffalo (1)	Clinical Mastitis.	1	No discharges was observed after IInd treatment.	-	The quarter dried as expected. It was not responding to any antibiotic therapy.
8.	Pivipol	20 ml. 1st day 10ml.2nd & 3rd day o.i.d.	3 days	Cow (1) Blood in milk & culturelly negative.	Clinical Mastitis.	1	No irritation was observed and bleeding stopped on 2nd day. After 3rd treatment normal milk was produced.	NEGATIVE	It can be used in the cases of blood in milk.

Discussion

D I S C U S S I O N

It is assumed that, the managemental factors are more important rather than breed as reported by Yurkov (1977) and Verma (1978). However, the current investigations revealed that breed also leads to a little variation in the incidence of subclinical mastitis, as the incidence was found to be comparatively high in Gir cows and also in Gir X Holstein - Friesian crossbreds as compared to Gir X Jersey crossbreds. The reason for this could be attributed to the teat shape and anatomy of the teat canal (Blood et al. 1983). As far as the shape, is concerned cylindrical shaped teats had a significantly higher incidence of mastitis (Rathore, 1977).

Besides this the incidence was found to be more in older cows [illegible] which is in agreement with the findings of Blood et al.(1983).

It was found that, the incidence of mastitis was comparatively high in the third and fourth lactations among the 166 cases studied. These findings are in accord with the findings of Kapur and Singh (1978).

The study of number of quarters infected indicates that, single quarter infections were common in cows, but the findings of this study are different from the findings of Rahman et al.(1984) who noted that infections of all four quarters were common in cows.

As to the type of quarter, the incidence was almost similar in hind quarters and fore quarters in cattle, however, this is not in agreement with the findings of Singh and Baxi (1980) who have reported that hind quarters were more infected (58.48%) than fore quarters. In case of buffaloes, hind quarters were more infected and these observations are similar to those of Kapur and Singh (1978b).

Bhatnagar and Mehrotra (1969) employed Modified California Mastitis Test (MCMT) for the diagnosis of subclinical mastitis. They recorded the efficacy of MCMT to be 94.3% as ascertained by cultural examinations. In the present study, the MCMT was found to be 94.67% efficient.

The pathogenic bacteria were isolated from 94.67% of the quarters showing ++ (Highly Positive) MCMT reaction where as similar investigations conducted on +++ CMT reaction and cultural examinations by Egan (1982) and Fagliari et al.(1984) revealed that 76.3% and 85.6% of quarters respectively, showed pathogenic bacteria.

Rahman and Baxi (1983) studied various aspects of staphylococcal mastitis in bovines. Staphylococci were isolated from 61.97% of bacteriologically positive samples which proved that staphylococci were the chief aetiological agent of bovine mastitis in India. Our findings are in agreement with this, as staphylococci were isolated from 57% of bacteriologically positive samples.

Among the antimicrobials examined for sensitivity test, it was remarkably noted that, none of the isolates were sensitive to nystatin. Contrary to this, Yadav et al (1972) have found that 51.9% coagulase positive strains of staphylococci were sensitive to nystatin.

Chakrabarty and Hazarika (1972) observed antibiotic sensitivity of a total of 185 strains of pathogenic microorganisms isolated from bovine mastitis cases. 30% strains were resistant to penicillin, 35% to streptomycin, 17% to oxytetracycline and 1.6% to chloramphenicol; all of them were sensitive to chlorotetracycline and tetracycline. But in contrast with above observations, in present study, out of 100 strains of pathogenic microorganisms 75% were resistant to penicillin, 72% to tetracycline, 71% to chlortetracycline, 65% to oxytetracycline, 54% to streptomycin and 46% to chloramphenicol. This may be due to more strains developing resistance to routine antibiotics.

Observations of Verma and Mishra (1977) revealed that, staphylococcal strains were sensitive to furazolidone (88.6%), chloramphenicol (75.9%), penicillin (74.6%) and streptomycin (62%). But these findings differ from the present study which revealed that staphylococcal strains were sensitive to furazolidone (33.33%), chloramphenicol (58%), penicillin (23%) and streptomycin (39%).

This might have resulted from constant and indiscriminate use of tetracyclines, strepto-penicillins nitrofurans for the treatment of bovine mastitis for the last decade.

In the present study, gentamicin showed highest inhibitory effect (78%) on different strains. The similar findings were noted by Kormendy (1977) who stated that, out of 205 strains, 199 were sensitive to gentamicin.

Singh et al.(1984) noted that Ampiclox L.C. was 86.95% effective in the quarters treated, which is similar to 83.87% efficacy of Tilox in present study. (Both preparations have same contents).

The activity of the combination of ampicillin + cloxacillin against streptococci was mainly due to ampicillin. Against penicillin resistant staphylococci, the activity of the combination was mainly due to the activity of cloxacillin. Against the other microorganisms, both compounds acted synergistically, (OS et al.1977).

Vangelov (1982) observed that 12,00,000 i.u. of gentamicin sulphate intramammary was well tolerated in cows and stated that it was the drug of choice of acute mastitis and where desired, for a shorter period of excretion in the milk. In present study, intramammary infusion of 50,000 i.u. (50 mg) gentamicin sulphate was given and was found to be 100% effective. It did not exihibit any side effects.

There was little relationship between the *in vitro* sensitivity and cure rate as noted by Pearson and Mackie (1979), however, the present study showed that, treatment response appeared to agree with the results of susceptibility test (90.41%). These findings are similar with the findings of Anderson *et al.*(1982) and Hamir *et al.*(1980).

In olden days tincture iodine was injected with glycerine to create chemical mastitis whenever the quarters were to be dried off and rendered functionless. The new products of povidone iodine (Betadine & Pivipol) are nonstaining, nonirritant and having multipurpose microbicidal action against bacteria, fungi, protozoa, yeasts and viruses. However, in present study irritation was observed specially in cows while treating subclinical mastitis. This product was found to be very useful in the clinical cases which do not respond to antibiotic therapy as well as for drying-off the quarters in chronic cases of mastitis at higher dosages. Blood in milk can also be treated successfully. This study was limited to very few cases and it is felt that this study should be carried out further to establish its exact use in bovine mastitis.

In present study, milk production did not increase appreciably after the treatment. Similar findings were noted by Morris (1973) and Jacob (1980) who stated that, when infections were successfully treated during a lactation,

the yield did not return to normal until the next lactation.

Among the cases showing positive MCMT inspite of treatment, one cow infected with Corynebacterium pyogenes did not respond to any treatment. Similar findings were recorded by Blood et al.(1983) that, even with intensive therapy at least 50% of the quarters are rendered useless and many of those which respond are greatly reduced in productivity. In similar manner, Seffner (1982) stated that full recovery was never observed in mastitis due to Corynebacterium pyogenes.

Summary And Conclusions

S U M M A R Y

I. Epidemiology : Incidence.

1. Breed : Gir and Gir X Holstein Friesian cross-breds had higher incidence of mastitis than Gir X Jersey crossbreds.

2. Age : After eight years of age, incidence of mastitis was found to be increased above 50%, in cattle and buffaloes.

3. Number of lactations : There was higher incidence of bovine mastitis in third and fourth lactation.

4. Number of quarters : In bovine mastitis, single quarter infections were more common than two, three and four quarters infections.

5. Type of quarter : In buffaloes, hind quarter infections were more common than fore quarter infections.

6. Subclinical mastitis : Incidence of subclinical mastitis was recorded as 32.89% animalwise and 12.76% quarterwise.

II. Modified California Mastitis Test :

On the basis of cultural tests, efficacy of MCMT was found to be 94.67% when ++ milk samples were studied.

III. Cultural examinations:

The chief aetiological agent of bovine mastitis was coagulase positive staphylococci (35%), followed by coagulase negative staphylococci (22%) and streptococci (17%).

IV. Sensitivity Test :

Microorganisms were found, highly sensitive to gentamicin (78%), neomycin (65%), cloxacillin (59%), ampicillin (57%) and oleandomycin (55%). Microorganisms were found highly resistant to penicillin (75%), furazolidone (74%), tetracycline (72%), chlortetracycline (71%) and nitrofurantoin (70%).

V. Treatment :

Tilox vet. (ampicillin + cloxacillin) was found to be 83.97% effective and Gentavet (gentamicin) was found to be 100% effective, quarterwise.

VI. Repeat cases :

Incidence of cases which did not respond to the treatment; was 13.73% animalwise and 9.59% quarterwise. In all, seven cases repeated and out of this six responded to second treatment and one mastitis case due to Corynebacterium pyogenes did not respond to third treatment and resulted into permanent loss of that quarter.

VII. Treatment with povidone iodine :

This chemotherapeutic agent was found to be very useful in clinical cases which did not respond to antibiotic therapy as well as to dry off the quarters in chronic cases of mastitis.

VIII. Milk yield :

After the treatment, there was not appreciable difference in milk yield of cattle and buffaloes, during the same lactation.

C O N C L U S I O N S

Breed, age, number of lactations, number of quarters and type of quarter of the animals have bearing on the incidence of bovine mastitis.

The MCMT reagent is economical and can be prepared in laboratory or in any dispensary. The reagent is 94.67% efficient in highly positive milk samples of subclinical mastitis.

It was observed that, the chief aetiological agent was staphylococci followed by streptococci.

The microorganisms were found highly sensitive to gentamicin, neomycin, cloxacillin, ampicillin and oleandomycin, while they were highly resistant to tetracyclines, streptopenicillins and nitrofurans. This concludes that routine antibiotic preparations available in the market are of little use as they contain streptopenicillin, tetracycline and nitrofurans.

New preparations- Gentavet and Tilox were found to be very effective in the treatment of bovine mastitis.

This study has shown that 13.73% cases did not respond to first treatment. To ensure whether the animal is responding to the treatment or not, MCMT should be repeated 72 hours after last treatment. As a follow-up,

they must be treated according to cultural and sensitivity tests. Those ca-ses which do not respond after third treatment, their fate should be decided, on economy.

In treatment with povidone iodine 1% and 5% were tried in some clinical and subclinical cases, the response was inconclusive. So more research has to be carried out to decide its exact use.

After the treatment of bovine mastitis, there are little chances of increase in milk production during the same lactation.

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Appendices

A P P E N D I X - 1.

DATE_____ FARM_____ PLACE_____

TIME_____ CASE NO._____ SPECIES_____

COLOUR_____ BREED_____ CROSS %_____

DATE OF BIRTH_____ AGE_____

NO. OF LACTATIONS_____ PREGNANT/EMPTY_____

DATE OF LAST CALVING_____ MONTH OF LACTATION_____

TODAY'S AVERAGE MILK YIELD_____

MANAGEMENT_____

(Teat dips; milking techniques etc.)

ANY HISTORY_____

_____

-.-

MODIFIED CALIFORNIA MASTITIS TEST:

1) Left fore quarter _____

2) Left hind quarter _____

3) Right fore quarter _____

4) Right hind quarter _____

RESULTS: _____

* Type of mastitis :- CLINICAL/SUBCLINICAL.

* Inflamation :- ACUTE/SUBACUTE/CHRONIC.

I. CLINICAL OBSERVATIONS:-

* ANY INJURY/ABNORMALITY _____

* PALPATION OF UDDER/LYMPH NODE _____

* ANY OTHER _____

II. MILK-PHYSICAL TESTS:

* COLOUR _____ * CONSISTENCY _____ *FLAKES ____

* PH _____ *ANY OTHER _____

III. CULTURAL TESTS:-

NO.	AGAR	COLONY CHARACTERS	STAINING REACTIONS

FINAL RESULT: _____

IV. SENSITIVITY TEST (ZONE SIZE & REMARKS)

1.	AM _____	2.	C. _____
3.	A _____	4.	CX _____
5.	DX _____	6.	FX _____
7.	GM _____	8.	N _____
9.	FD _____	10.	NY _____
11.	OL _____	12.	OX _____
13.	P _____	14.	BA _____
15.	S _____	16.	TE _____
17.	PIVIPOL _____	18.	BETADINE _____

FINAL RESULT: _____

Antibiotic shortforms :- Pl. see Appendix No.2

V. T R E A T M E N T :-

(i) PIVIPOL/BETADINE OR TILOX/GENTAVET

(ii) ANY OTHER _____

(iii) DOSE _____

(IV) DURATION OF TREATMENT _____

(v) IIND MCMT RESULT _____

(vi) RECOVERED/NOT _____

(vii) REMARKS _____

_____

APPENDIX - 2.

ZONE-SIZE INTERPRETATION CHART

Reset from University of Washington Hospital Practice, Feb., 1970 quoted by BRYANT (1981)

NO.	ANTIBIOTIC DISC	SYMBOL	CONTENT mcg.	ZONES IN mm.		
				R.	I.	S.
1.	AMPICILLIN	AM	10	11 or less.	12-13	14 or more.
				20	21-28	29 *
2.	CHLORAMPHENICOL	C	30	12	13-17	18
3.	CHLORTETRACYCLINE	A	30	14	15-18	19
4.	CLOXACILLIN	CL/CX	1	9	10-13	14
5.	DOXYCYCLINE	DX	30	14	15-18	19
6.	FURAZOLIDONE@	FX	100	-	-	-
7.	GENTAMICIN	G/GM	10	-	-	13
8.	NEOMYCIN	N	30	12	13-16	17
9.	NITROFURANTOIN	F/FD	300	14	15-16	17
10.	NYSTATIN@	NY	100 units.	-	-	-
11.	OLEANDOMYCIN	OL	15	11	12-16	17
12.	OXYTETRACYCLINE	OX	30	14	15-18	19
13.	PENICILLIN G	P	10 units.	11	12-21	22°
				20	21-28	29°°
14.	CO-TRIMOXAZOLE	BA	25	14	15-18	19
15.	STREPTOMYCIN	S	10	11	12-14	15
16.	TETRACYCLINE	TE	30	14	15-18	19
17.	PIVIPOL@			-	-	-
18.	BETADINE@			-	-	-

R = Resistant. I = Intermediate. S = Sensitive.

* Gram-negative organisms and enterococci.

** Staphylococci and highly penicillin sensitive organisms.

o Other organisms.

oo Staphylococci.

@ All these antibiotics, tentative standards were fixed according to Bailey and Scott (1966) and Kapur et al (1978

DIAMETER OF ZONE	INTERPRETATION
Greater than 15 mm	Susceptible.
10 - 15 mm.	Intermediate.
Less than 10 mm.	Resistant.

PREPARATION OF PIVIPOL/BETADINE DISCS :-

A Whatman Filter Paper No.42, diameter 15 cms. and weighing 1.2218 gms. 5 mm discs were punched with a punching machine. 10 discs weighed 0.0182 gms. 50 discs were soaked in 0.2 ml. of Pivipol and Betadine solution. The discs were dried in incubator at 37°c for 1/2 hour and stored in lebelled amber coloured bottles in refrigerator at 4 to 8°c.

A P P E N D I X - 3

No.	Name of drug	Content	Dose	Duration of treatment
1.	PIVIPOL Solution (1%)	Each 5 ml. contains Povidone Iodine -- USP -- 50 mg.	10 to 20 ml. per quarter per day. Upto 40 ml. per quarter per day.	1 day to 5 days. (Chronic Mastitis) 2 to 3 days.
2.	BETADINE Veterinary Solution. (5%)	5% Povidone Iodine--USP	10 ml. per quarter per day.	1 day to 5 days.
3.	TILOX vet. Ointment	75 mg. Ampicillin as Ampicillin Sodium. + 200 mg. Cloxacillin as Cloxacillin Sodium. B.P. (Vet) in 7 gm. of base.	1 tube per quarter per day	3 days.
4.	MASTALONE For mastitis Ointment.	Oxytetracycline HCL I.P. - 200 mg. Oleandomycin (as phosphate) 100 mg. Neomycin Sulphate I.P. equivalent to 100 mg. of Neomycin base. Prednisolone 5 mg. in 10 ml. base.	1 tube per quarter per day.	3 days.

No.	Name of drug	Content	Dose	Duration of treatment
5.	PENDISTRIN-SH Veterinary Ointment.	Procaine Penicillin G 1,00,000 i.u. Streptomycin Sulphate 100 mg. Sulfamerazine 500 mg. Hydrocortisone 20 mg. in 6 ml.	1 tube per quarter b.i.d.	3 days.
6.	ALBERCILIN Vet. (Inj.)	Sterile Ampicillin Sodium I.P. 250 mg. or 1 gm. vial.	500 mg. per quarter b.i.d.	3 days.
7.	LYKACETIN-S (Inj.)	Chloramphenicol Sodium succinate sterile - U.S.P. 1 gm. vial.	300 mg. per quarter per day.	4 days.
8.	TERRAMYCIN (Inj.)	Oxytetracycline HCL. 50 mg. per ml. (vials 10ml, 30ml, 100 ml.)	250 mg. per quarter o.i.d.	3 days.

No.	Name of drug	Content	Dose	Duration of treatment
9.	GENTAVET (Inj.)	Gentamicin Sulphate I.P. equivalent to 50 mg. (50,000 i.u.) of gentamicin per ml. (vial 2 ml.)	50 mg. per quarter per day.	4 days.
10.	ANTRIMA (Tab.)	Each tablet contains: Trimethoprim I.P. 80 mg. Sulphadiazine I.P. 400 mg.	2 tabs. per quarter b.i.d.	3 days.

N.B. Dosages from 6 to 10 preparations were diluted/dissolved in 10 ml distilled water.

IN MASTITIS

What conventional approach cannot achieve

"The failure to completely eliminate infection with conventional intramammary preparations is a contributing factor to the increasing importance of Staphylococci as udder pathogens".
—Mercer et. al;, JAVMA, 169:1104

Rx Tilox

... the modern approach can.

TILOX*

Intramammary Suspension

for mastitis in lactating animals

Cures mastitis in 48 hours

Restores milk production

TILOX* —*for unsurpassed clinical results*

FORMULA:
Each 7 gm (single dose) contains:

Ampicillin (as Ampicillin Sodium, I.P.)	75 mg
Cloxacillin (as Cloxacillin Sodium, I.P.)	200 mg
Base	q.s.

INDICATIONS:
TILOX Intramammary Suspension is indicated for the treatment of bovine mastitis. It is particularly recommended where the causative organisms have not been identified and where Penicillin-resistant Staphylococci, Streptococci and E. Coli are known or suspected to be involved.

DOSAGE:
One tube per affected quarter; to be repeated every twelve hourly for three times or as advised by the veterinarian.

ADMINISTRATION
After milking, clean and disinfect the teat. Insert the nozzle into the teat and apply gentle and continuous pressure until all the suspension is expressed. The treated quarter(s) may be milked out at the next normal milking time.

Remove the inner plastic plug prior to use.

WARNING:
Milk from treated animals must not be consumed until 60 hrs after last treatment. Withholding times are based on milking being carried out at the time on each day.

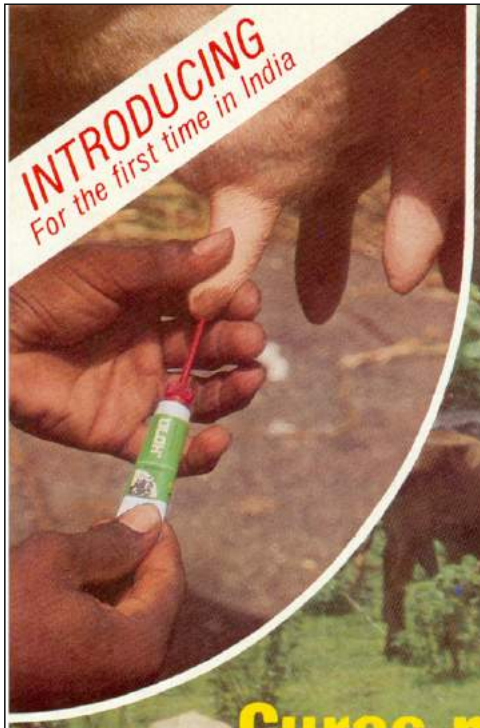
STORAGE:
Store in a cool, dry place.

AVAILABILITY:
TILOX Intramammary Suspension is available in single dose collapsible disposible tube with applicator, containing 75 mg ampicillin and 200 mg cloxacillin as sodium salts.

* Trademark

WOCKHARDT

Made in India by
Wockhardt Veterinary Pvt. Ltd.
Dr. Annie Besant Road
Bombay-400 018.



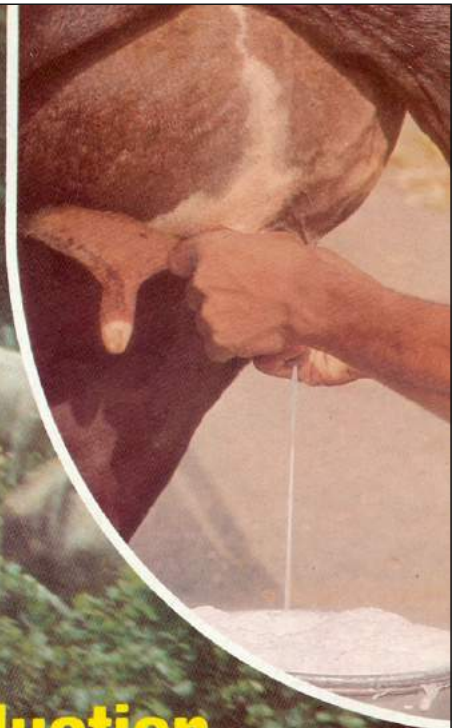
... the moden approach

New

TILOX*

Intramammar Suspension

(Ampicillin-Na-75 mg- Cloxacillin-Na-200 mg)



Cures mastitis in 48 hours Restores milk production

TILOX* acts synergestically

"The activity of the combination of Ampicillin+Cloxacillin against Streptococci was mainly due to ampicillin. Against Penicillin-resistant Staphylococci, the activity of the combination was mainly due to the activity of Cloxacillin. Against the other micro organisms, both compounds acted synergetically."

Os. et. al. (1977) Vet. Bull. 47:56

TILOX* cures mastitis

It was observed that, the chief aetiological agent was staphylococci followed by the streptococci. The microorganisms were found highly sensitive to cloxacillin, ampicillin while they were highly resistant to tetracyclines, streptopenicillins and nitrofurans. This concludes that routine antibiotic preparations available in the market are of little use as they contain streptopenicillin, tetracycline and nitrofurans.

Ksheersagar, (1984) M.V. Sc. Thesis, Konkan Krishi Vidyapeeth, Dapoli, (M.S.).

TILOX* restores milk production faster

"The recommended intervals between intramammary infusion of ampicillin + cloxacillin has been reduced from 24 to 12 hours. The expected saving in milk lost during the conventional three infusion treatment period is about the same as the cost of antibiotic."

—Minister, P. (1980), Dairy Farmer, 27:34-35, 39.

TILOX* offers unique 8 benefits

—Broadspectrum, synergestic, bactericidal

—Also effective against penicillin-resistant Staphylococci, Streptococci & E. Coli.

—Convenient 3 tubes treatment with only 12 hours interval

—High active concentration in udder during the whole treatment period

—High cure rate coupled with economy

—Prevents loss of teat

—Non-irritant in the udder tissues

—No recorded resistance to the combination and even cloxacillin alone.

—Brander and Pugh, 4th Edn, (1982).

TILOX*—for unsurpssed clinical results