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 2,000 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.

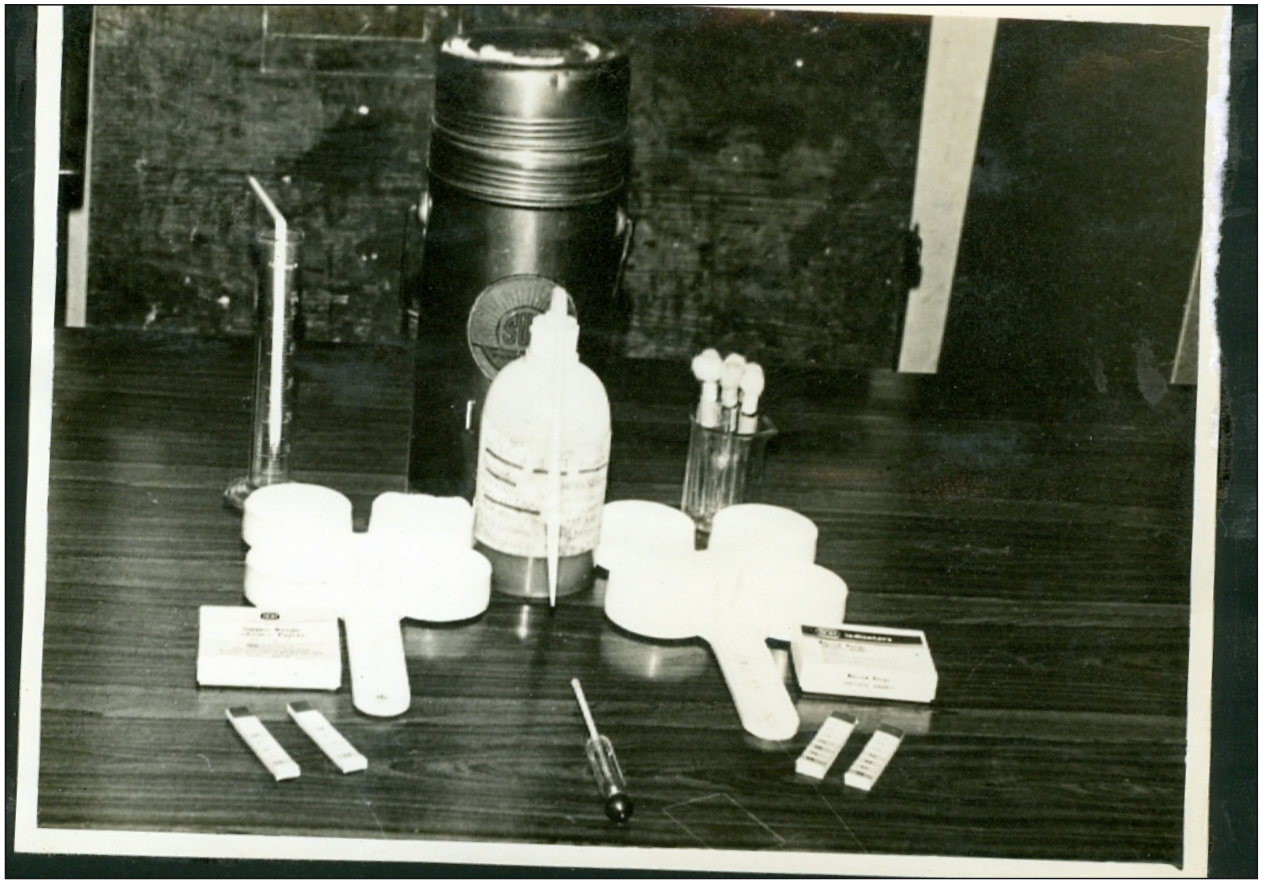

Fig-1- Showing Material required for MCMT and collection of milk samples.

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

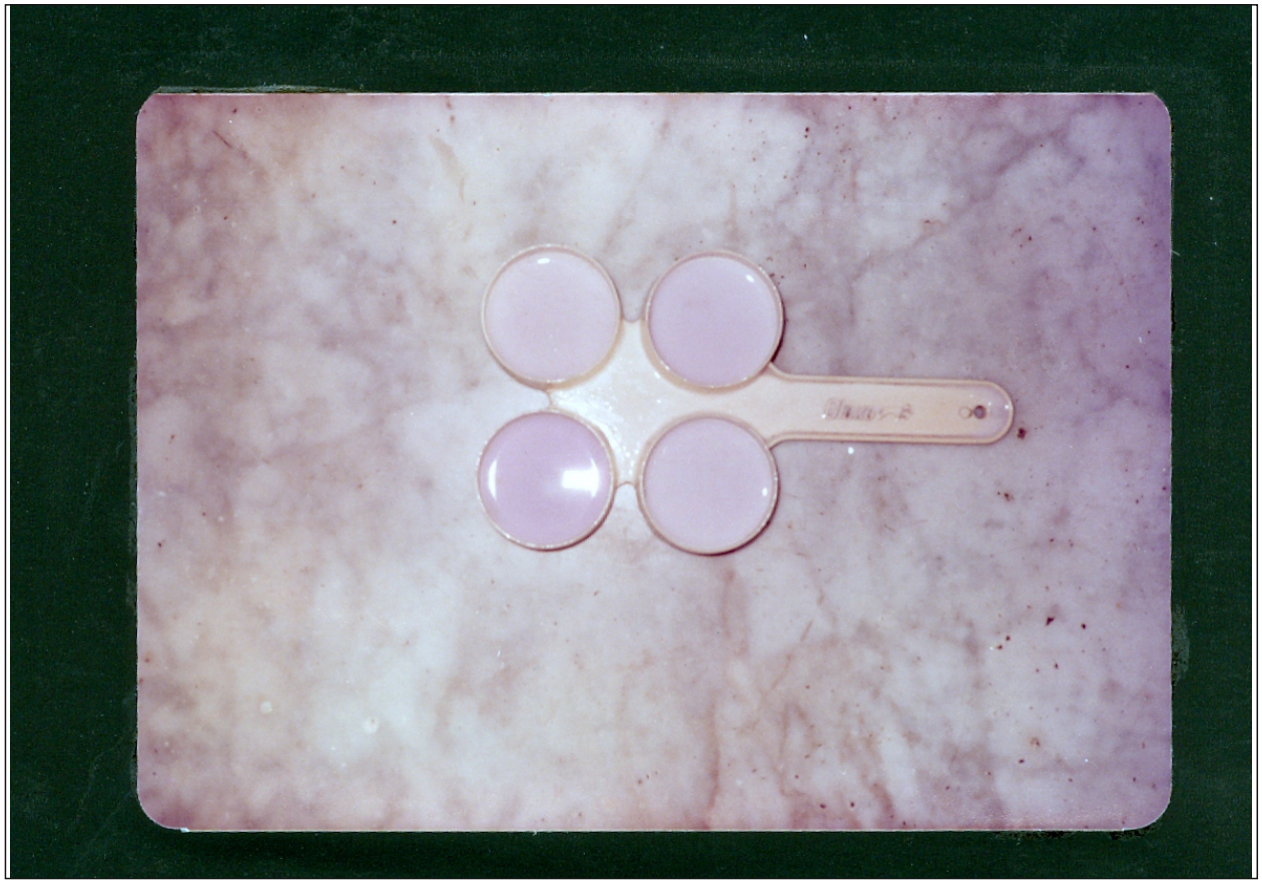

Fig-3- Showing a negative MCNT reaction

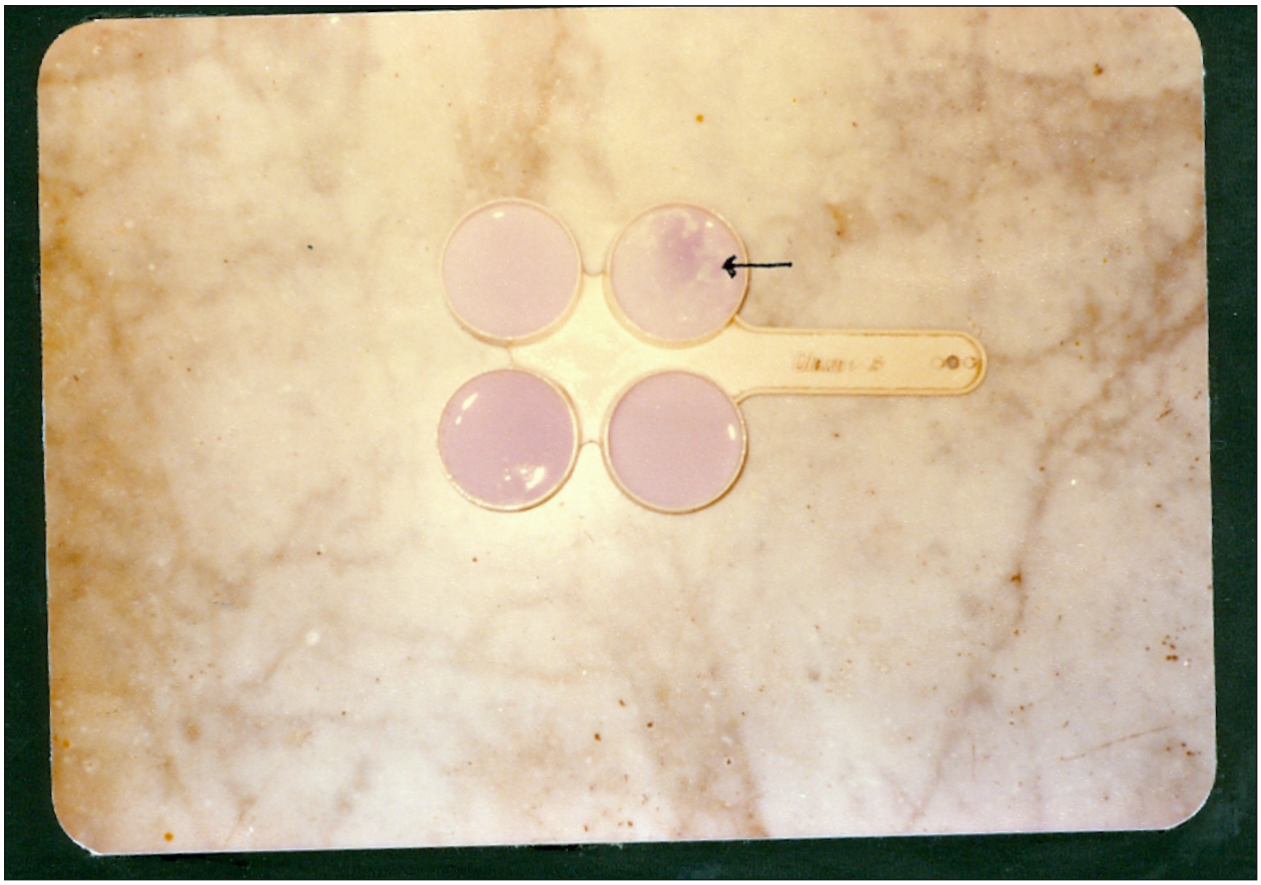

Fig -4- Showing a highly positive (++) MCMT, marked by an arrow.

Fig-5- Showing a method of cultural examination

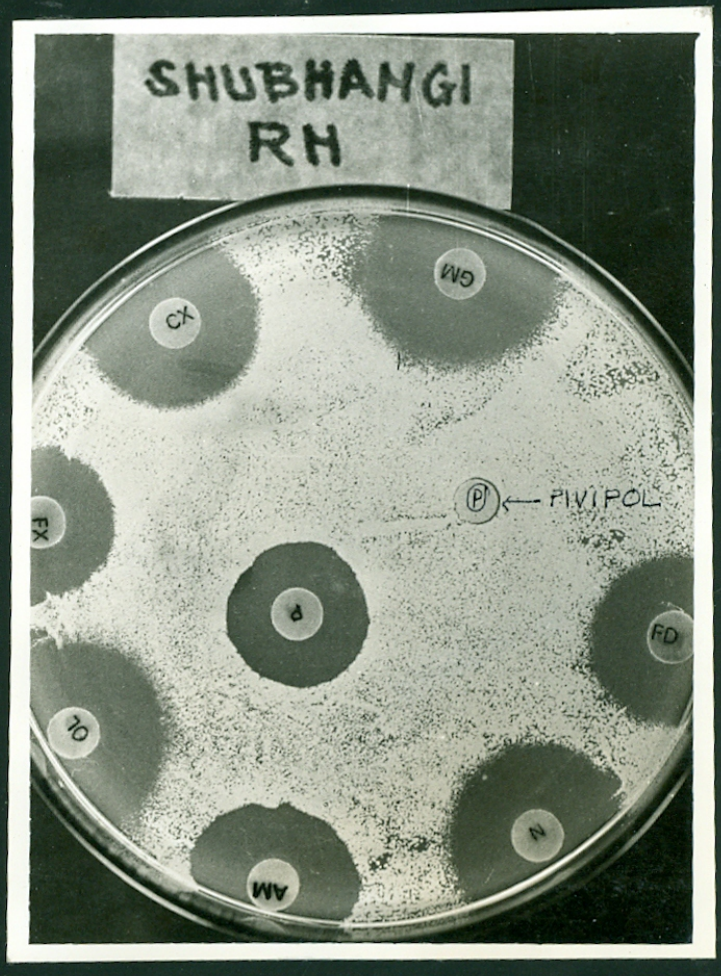

Fig-6-

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

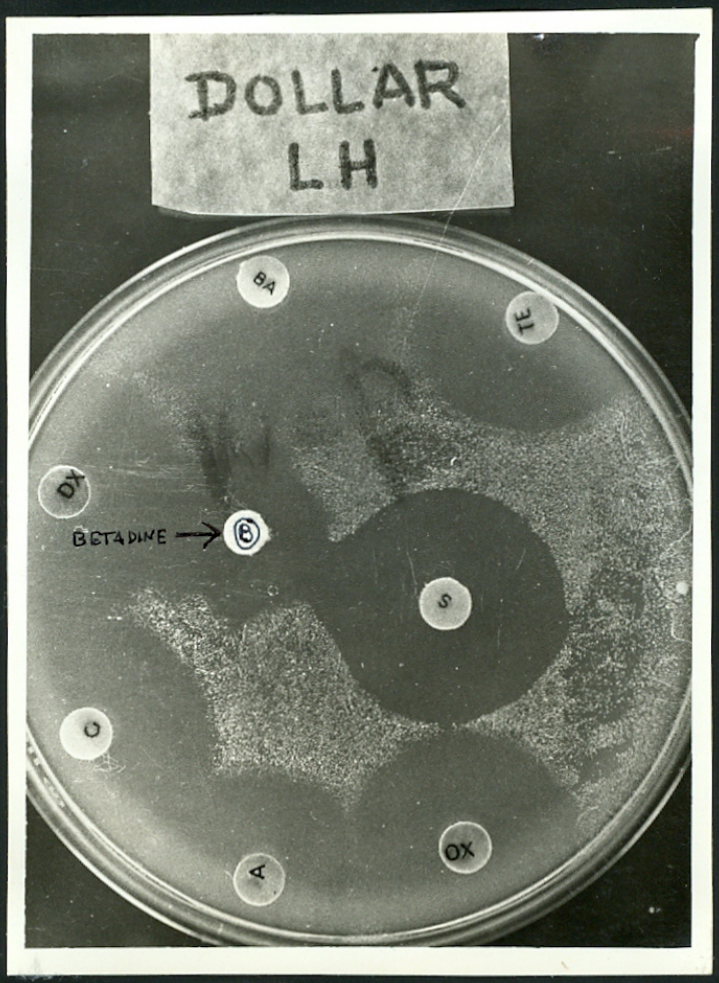

Fig-7-

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