EFFICACY OF HERBO-MINERAL FORMULATION (PRE-MAST) IN PREVENTION AND TREATMENT OF SUBCLINICAL MASTITIS IN CROSSBRED COWS

Introduction

Mastitis is an inflammation of parenchyma of mammary gland regardless of the cause. It is characterised by physical and chemical changes in milk and pathological change in glandular tissue. The most important changes in the milk include discolouration, presence of clots, flakes and the presence of large number of leucocytes. The mammary gland is hot swollen and painful in acute cases. As the condition advances, the affected gland gradually looses its productive capacity. Infection of mammary gland is usually caused by bacteria *viz. Staphylococcus spp*., *Streptococcus spp*., *Escherichia coli*, *Klebsiella spp*., *Pseudomonas, Proteus*, *Serratta spp.* and *Mycoplasma spp*. The inflammatory process comprises three phases, *viz.* invasion, infection and inflammation.

Mastitis is the most prevalent infectious disease of adult dairy cows having greatest economic importance. The economic losses are due to gross involvement of parenchymal tissues of udder followed by loss of either one or more quarters. The market value of the mastitic animal is significantly reduced even when only one quarter is damaged. Further, average decrease in milk yield due to subclinical mastitis and clinical mastitis, was found to be 17.5 per cent and 50 per cent, respectively (Singh *et al*., 1994). The estimated loss due to mastitis alone is to the tune of 15 % of its total production during each lactation. Financial losses occurred due to mastitis in India accounts to Rs. 6053.21 crore per year. Out of this loss 4365.32 crore has been attributed to sub clinical mastitis (Dua, 2001). Clinical and subclinical mastitis affections are also important from public health point of view. Although advances have been made in various diseases of dairy animal, mastitis continues to be a major threat to high yielders and in particular exotic crossbreds.

It is important to diagnose this disease in the subclinical stage. The California Mastitis Test (CMT) is commonly used cow side test for screening of herd for subclinical mastitis. The test is simple, easy to perform and very cheap. Somatic cell count (SCC) has been widely used as screening test to identify intramammary infections owing to its high sensitivity and specificity. The high incidence of mastitis along with raised SCC is observed during rainy season due to unhygienic environment, more calving, and animals in the stage of peak lactation. Maximum number of sub clinical mastitis (SCM) cases has been recorded in rainy season than that of other seasons.

Herbal medicines, having proven their efficacy by standards of both history and modern medicine, are now the subject of closer scrutiny by the researchers. The use of these herbs, however, took a back seat with the advent of antibiotics, but is once again viewed as potential agents to treat various ailments, giving a fresh push to the herbal medicine. These herbs are now also being considered quite effective in treatment of mastitis.

The present study therefore aims to evaluate therapeutic efficacy of herbo-mineral formulation (PRE-MAST powder) against SCM in crossbred cows.

Objectives

1. To record prevalence of sub clinical mastitis in crossbred cows.
2. To evaluate the efficacy of herbo-mineral preparation (PRE-MAST) in prevention of subclinical mastitis in crossbred cows during lactation.
3. To evaluate the efficacy of herbo- mineral preparation (PRE-MAST) in treatment of sub clinical mastitis in crossbred cows.
4. To determine the antibiogram of organisms isolated from clinical cases of subclinical mastitis.

Technical Programme

1. The present study was conducted on a dairy cow herd located in Udgir, Dist Latur having total 41 lactating Holstein Friesian (HF) cows during monsoon. Whole herd was screened for subclinical mastitis by performing CMT. History pertaining to physiological status of an individual animal, age, milk yield per day, lactation number and lactation stage was collected. Moreover colour, consistency, odour, taste of milk and clinical manifestation if any, was also recorded.
2. In order to study the prevention aspect of herbo-mineral formulation, cows with negative CMT were selected, divided in two groups (Treatment and control group) and administered herbal formulation powder as mentioned in the experimental design (Group A1 and A2)
3. Cows with positive CMT were selected and distributed among two groups (Treatment and Control group) as mentioned in the protocol (Group B1 and B2) to study efficacy of herbo-mineral formulation in treatment of subclinical mastitis.
4. Milk samples were collected before and after administration of herbo-mineral formulation powder (PRE-MAST). The sampling was done at the milking time in the evening hours

from all the four quarters of cow with hand milking, taking aseptic precautions. Individual samples were collected in sterile glass vials of 100 ml capacity. The samples were immediately subjected to the determination of somatic cell count, estimation of milk fat, microbiological investigations and for drug sensitivity against isolated organisms.

5. Somatic cell count was determined as per the method described by Schalm *et al.* (1971) except staining, which was done as per the method described by Newlander and Artherton (1964). To determine somatic cell count, clean, grease free micro-slide delineated 1 sq. cm area by a glass marker with piece of white cardboard. Thoroughly mixed 0.01 ml milk sample was drawn with help of micropipette and evenly spread on the marked area with the help of clean and sterilized bacteriological loop. The smear was stained by using Newman's Lampert stain. After complete drying of the stain at room temperature the slide was washed with water to remove excess stain. Counting under oil immersion lens was done in 30 fields and the average count was determined per field. Total SCC was calculated by multiplying the average count for one field with microscope factor. The somatic cell count was expressed as cells /ml of milk.
6. Fat content of milk was calculated using Gerber's method. In this method milk was treated with sulphuric acid of known specific gravity and small amount of amyl alcohol. The mixture was then centrifuged using a special type of Gerber tube. The volume of separated fat then read in a graduated part of tube at a fixed temperature.
7. Isolation of bacteria was done by streaking the samples on blood agar and incubating aerobically at 37^0 C, for 24 hours. (Cruickshank *et al.*, 1975). The isolates were tentatively identified by Gram's staining and on the basis of morphological characters. The *in vitro* antibacterial sensitivity pattern of these bacterial isolates was determined by a standard disc diffusion technique (Bauer *et al.*, 1966) using antibiotic discs for recording antibiogram of the isolates.
8. Therapeutic efficacy was determined on the basis of improvement in somatic cell count (Reduction in somatic cell count is a positive sign of improvement), milk yield, milk fat content and microbiological investigations (Reduction in bacterial load in the milk).
9. The data was analysed according to the methods described by Snedecor and Cochran (1994).

Table 1: Experimental design for evaluating efficacy of herbo-mineral formulation (pre-mast) in prevention of subclinical mastitis.

GROUP	PHYSIOLOGICAL STATUS	NO OF	DRUG & DOSE

NO.		ANIMALS	
A1(Control group 1)	Cows in lactation not exposed to subclinical mastitis.	10	No treatment
A2 (Treatment group 1)	Cows in lactation not exposed to subclinical mastitis.	10	PRE-MAST powder 60 g bid orally for 5 days.

Table 2: Experimental design for evaluating efficacy of herbo-mineral formulation (pre-mast) in treatment of subclinical mastitis.

GROUP NO.	PHYSIOLOGICAL STATUS	NO OF ANIMALS	DRUG & DOSE
B1(Control group 2)	Cows in lactation, exposed to subclinical mastitis.	10	No treatment
B2 (Treatment group 2)	Cows in lactation, exposed to subclinical mastitis.	10	PRE-MAST powder 60 g bid orally for 5 days.

Results and Discussion

Subclinical mastitis is the most serious type as the infected animal shows no obvious symptoms and secretes apparently normal milk for a long time, during which causative organisms spread infection in herd, so it is an important feature of the epidemiology of many forms of bovine mastitis (Bakken and Gudding, 1982).

The study was undertaken to evaluate the preventive aspect and therapeutic efficacy of herbo-mineral formulation (PRE-MAST powder) against subclinical mastitis in crossbred cows.

Prevalence:

In the present investigation sub clinical mastitis (SCM) was recorded in 23 HF crossbred cows among 41 cows screened indicating overall prevalence rate as 56.1 per cent. The incidence rates of sub clinical mastitis reported from different states of India by various workers were 17.33% (Singh *et al.*, 1994), 48.7% (Bansal *et al.*, 1995), 53.54% (Tiwari *et al.*, 2000), 56.76% (Chandra *et al.*, 1989), 25-78% (Paul *et al.*, 2000), 74.10% (Pachauri *et al.*, 2001), 94.54% (Shukla and Supekar, 1986). The high incidence observed in the study may be attributed to a) Monsoon season b) Stress of high milk yield c) Breed predilection and d) Non-implementation of strict hygienic measures for control of mastitis.

Quarter-wise highest prevalence of subclinical mastitis was recorded in right hind quarter (37.78%) followed by left hind quarter (26.08%), right forequarter (21.73%) and left forequarter (17.39%). The similar pattern of affection of quarters was earlier observed by Shastri (2001). The highest prevalence in hind quarter might be due to continuous soiling of hind teats during defecation and urination and subsequently ready environment for microorganisms to grow. Apart from that difference in the quarter infection rate could be due to genetic resistance, difference in teat length, teat shape, teat orifice morphology, breed and management of houses (Radostitis *et al.*, 2000).

Lactation stage-wise analysis indicated more prevalence in 4^{th} to 5^{th} month (70%) followed by 1^{st} to 3^{rd} month of lactation (30%). Similar observations were recorded by Rasool *et al.* (1985), Pal and Verma (1991) and Prasad *et al.* (2001).

The present experiment findings of season and breed also corroborates with the findings of Rady and Sayed (2009) who noticed higher prevalence of sub clinical mastitis in hot humid weather than in summer and winter while Holstein Friesian breed is most susceptible to mastitis than native breed.

California Mastitis Test:

The CMT was used for diagnosis of sub clinical mastitis as it is reliable, easy, rapid and cheap tool helping in diagnosis of mastitis. (Viani *et al*; 1990; Behera and Dwivedi, 1992; El- Balkemy *et al.;* 1997). It gives an indirect estimate of SCC because it is based upon a gelling reaction between the nucleic acid of the cells and a detergent reagent.

Milk yield and milk fat:

On clinical examination there was no visible abnormality in udder and milk. The average reduction in milk yield was 400 ml /day/cow. The milk fat percentage in the affected animal was 3.1 ± 0.6%. When value of milk Fat percentage of treatment group (3.1 ± 0.6%) compared with value of control group (3.5 ± 0.9%), there was an average 0.4% reduction in milk fat in affected animals. The change in the consistency of milk from normal to watery might have affected the average fat percentage (Singh *et al.*, 2006).

Microbiology:

Milk samples of all the affected quarters were found positive bacteriologically. The findings of the current study revealed predominance of *Staphylococcus, Streptococcus* and

Escherichia coli as the causative agents of bovine sub clinical mastitis. The different isolates recovered from the milk samples of affected cows were *Staphylococcus aureus* (70%), *Staphylococcus aureus* and *Streptococcus agalactiae* (10%), *Staphylococcus aureus* and *Escherichia coli* (5%), *Staphylococcus aureus* and *Bacillus cereus* (5%), *Bacillus cereus* (5%) and *Escherichia coli* (5%). This is in agreement to the earlier reports (Rady and Sayed, 2009; Bhalerao *et al;* 2000; Pednekar and Swarup, 1991; Singh and Baxi, 1982; Rahman *et al*; 1984).

Antibiogram:

Overall sensitivity of isolates showed maximum sensitivity to Gentamicin (93.75%) followed by Chloramphenicol (87.5%), Ciprofloxacin (75%), Enrofloxacin (68.75%), Ampicillin (31.25%), Ceftriaxone (25%), Oxytetracyclin (18.75%) and Streptomycin (6.25%). Similar sensitivity results were earlier reported by Dhakal *et al.* (2007), Kumar and Sharma (2002), Rao *et al.* (1989) and Sumathi *et al.* (2008).

Antibiogram of *Staphylococcus aureus* showed maximum sensitivity to Chloramphenicol (92.8%) and Gentamicin (92.8%) followed by Ciprofloxacin (71.4%), Enrofloxacin (64.3%) and Ampicillin (28.6%). Though most of the authors found proven efficacy of third generation cephalosporins in clinical mastitis, in present study sensitivity to cephalosporin limited to only three milk samples (21.4%). All the milk samples were resistant to Streptomycin (7.14% sensitivity) except one. Highest sensitivity of organism to Gentamicin in sub clinical mastitis was also earlier reported by Mathews *et al*. (1992). However some clinical and subclinical cases of mastitis caused by *Staphylococcus spp.* and *Streptococcus spp.* had been treated successfully with intramammary infusion of chloramphenicol.

Colonies of *Streptococcus* were sensitive to Enrofloxacin, Chloramphenicol, Gentamicin (100% each) and Ciprofloxacin (50%).

E.coli and *Bacillus cereus* were found sensitive to Enrofloxacin, Ciprofloxacin, Gentamicin (100% each) and Chloramphenicol (75%).

Somatic Cell Count:

Somatic cell count in milk is an indication of the presence of udder infection (Radostitis *et al.*, 2007). Determination of somatic cell count is reliable tool for diagnosis of subclinical mastitis.

In present study, there were no visible changes in udder tissue or gross abnormalities in milk secretion but there was complaint of reduction in milk yield and the samples revealed reasonably increased somatic cell count in the milk.

Somatic cell count of affected quarters was higher than threshold of $3.5x10^5$ cells/ ml of milk and were identified to be affected with sub- clinical mastitis (Radiostitis *et al.*, 2007). The mean somatic cell count of affected quarters ($6.01x10^5$ ± 4.62 cells / ml) was significantly higher than healthy quarters ($2.00x10^5$ ±1.94 cells /ml). In the present survey, the majority of affected cows revealed SCC between $2.00x10^5$ to $6.01x10^5$ cells / ml of milk. The increased count could be attributed to damage to alveolar tissue of mammary gland (Radostits *et al.*, 2007). The increase in SCC was found directly proportional to the severity of infection as also reported by Schalm *et al.* (1971).

Post treatment results:

After treatment milk yield and milk fat reached to near to normalcy. On 5^{th} day of treatment both the values were non significant. However it is interesting to note that the average milk yield increased from 9.3 ± 0.36 lit /day to 9.6 ± 0.3 lit/ day on 10^{th} day of treatment. The average milk fat level simultaneously increased from 3.1± 0.071% to 3.3 ± 0.074% at the end of experiment.

Mean somatic cell count of milk (Group B2) reduced from pre treatment $6.01X10^5$ ± 4.62 cells/ml to $3.21x10^5$ ± 1.93 cells / ml on 5^{th} day of treatment suggested 46.47 % improvement in somatic cell count. On continuation of treatment of PRE-MAST powder for next 5 days to the same animals the somatic cell count further decreased to $2.89x10^5$ ± 0.836 cells/ ml indicating overall improvement by 51.93 % at the end of experiement.

In present study, on administration of herbo-mineral oral therapy (Group B2) out of 10 bacteriologically positive milk samples 7 were found negative on 5^{th} day of treatment, giving cure rate of 70.00 %. Further five days treatment to the same animals revealed no evidence of bacterial colonies in 2 milk samples giving cure rate of 90 % (9 out of 10) on 10^{th} day of treatment.

The PRE-MAST powder is reported to contain herbal ingredients with antibacterial, anti-inflammatory, analgesic and immunostimulatory properties and thus might be effective

against not any specific organism but plethora of organisms, which are responsible for persistence of disease (Singh *et al.*, 2006).

Prevention aspect:

In order to assess the efficacy of PRE-MAST in prevention of sub clinical mastitis, cows in lactation but not exposed to subclinical mastitis were divided into two groups. (Group A1- Control group 1 and Group A2 - Treatment group 1). Treatment group (Group A1) was supplemented with PRE-MAST powder @ 60 gm BID orally for 5 days. On 5^{th} day of treatment there was no any deviation in milk yield, milk fat and somatic cell count in treatment group. All the values of control group and treatment group were at par. In spite of discontinuation of PRE-MAST powder on 5^{th} day, there was no evidence or signs of sub clinical mastitis on termination of experiment *i.e.* on 10^{th} day. The disease prevention in the current experiment might be due to the immunopotentiating activity of herbal drugs that might have enhanced the body's defence mechanism along with udder immunity, thereby keeping all sort of intramammary infections at bay (Singh *et al.*, 2006).

Summary and Conclusion

After treatment with PRE-MAST, milk yield and milk fat increased from 9.3 ± 0.36 lit /day to 9.6 ± 0.3 lit/ day and from 3.1± 0.071% to 3.3 ± 0.074% respectively in cows suffering from sub clinical mastitis. Affected animals were negative for subclinical mastitis at the end of the experiment. Somatic cell count markedly reduced from $6.01x10^5$ ± 4.62 cells / ml to $2.89x10^5$ ± 0.836 cells/ ml which was below the threshold line. Overall sensitivity of isolates from sub clinical mastitis in HF cows indicated maximum sensitivity to Gentamicin (93.75%) followed by Chloramphenicol (87.5%) and Ciprofloxacin (75%). The improvement in milk yield and milk fat and reduction in somatic cell count after the treatment indicated that PRE-MAST by potentiating the udder immunity, not only eliminates udder infection in sub clinical mastitis but also control the mastitis without any side effects. It also augments repair of mammary gland, firmness and normalize udder functioning with improved milk quality.