



EVALUATION STUDIES ON A TRI-SODIUM CITRATE BASED NOVEL FORMULATION FOR PREVENTION AND TREATMENT OF BOVINE MASTITIS

Ram Bahal Rai<sup>1</sup>, Tukaram Damodaran<sup>2</sup>, Kuldeep Dhama<sup>1</sup>, Sandip Chakraborty<sup>3</sup>,  
Balvir Singh<sup>1</sup>, Hamid Ali<sup>1</sup>, Sweta Rai<sup>1</sup> and Saminathan Mani<sup>1</sup>

<sup>1</sup>Division of Pathology, Indian Veterinary Research Institute, Izatnagar, Bareilly (U.P.)- 243122

<sup>2</sup>Central Soil Salinity Research Institute, Regional Research Station, Near Knshiram Smarak, Old Jail Road, Lucknow (U.P.) - 226005

<sup>3</sup>Animal Resources Development Department, Pt. Nehru Complex, Agartala, Pin – 799006, India

ARTICLE INFO

Article History:

Received 30<sup>th</sup> April, 2013  
Received in revised form  
14<sup>th</sup> May, 2013  
Accepted 28<sup>th</sup> June, 2013  
Published online 18<sup>th</sup> July, 2013

Key words:

Bovine mastitis,  
Dairy,  
Fibrosis,  
Tri-sodium citrate,  
Formulation,  
Kreb's cycle,  
Prevention,  
Treatment.

ABSTRACT

Mastitis is an inflammation of the mammary glands caused by several pathogens as well as circulating metabolites, injuries etc. and its prevention remains a challenge to the veterinarians. It is the second most economically significant disease standing next to foot and mouth disease (FMD). In mastitis, the standard treatment is antibiotic therapy (parental as well as intra- mammary infusions) apart from fomentation of udder accompanied by drainage of milk but the success rate is only reasonable. Between 1999-2012, the present long term study was conducted starting from Port Blair, Andaman & Nicobar Islands to Northern Plains of India. Animals from Military Dairy Farm, Experimental Dairy, owned by farmers in the villages and private un-organized dairies were included. The managemental conditions varied greatly and accordingly mastitis incidences also. Standard treatment, trisodium citrate and the present formulation have been evaluated considering that trisodium citrate helps in continuous milk production by making available citrate as substrate. It has been observed that clinical mastitis is predominant at 0-2 weeks of calving (67.3 %) while sub-clinical mastitis is predominant during 8-24 weeks period (96.6 %). The standard treatment given one day prior to expected day of calving did not have significant effect on prevention of onset of clinical mastitis during the first fortnight while tri-sodium citrate reduced the incidence to 6.9% against 29.2% in the control population during the period. The present formulation reduced the incidence to 1.3%. In the control untreated clinical mastitis cases, majority of cases developed fibrosis and restoration of milk was less than 10%. The animals who received standard treatment showed moderate recovery restoration of milk varied between 20-60% but 5.7% of the animals developed fibrosis. The animals treated with present formulation showed excellent recovery. The blood and flakes disappeared within 2-3 days, and the swelling subsided in 4-5 days. Milk recovery rate from the affected quarter (s) was between 60-80%. In case of animals affected with sub-clinical mastitis also the animals showed excellent recovery with the present formulation and flakes or blood disappeared by 2<sup>nd</sup>-3<sup>rd</sup> day and milk yield was restored. Thus it can be concluded that in comparison to standard antibiotic therapy, the present formulation is more efficacious and cost-effective as far as prevention and the recovery from clinical as well as sub-clinical mastitis is concerned. Its wide usage is recommended to lessen animal sufferings from this economically significant disease condition as well as enhancing milk production.

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INTRODUCTION

Mastitis is basically an inflammation of mammary glands resulting from varieties of causes including pathogens, circulating metabolites, injury etc. The annual loss due to bovine mastitis at present is estimated to the tune of Rs. 71651.5 millions/yrs (Sudhan and Sharma, 2010). During the last decade, mastitis stood just next to foot and mouth disease (FMD) as the most challenging disease in high yielding dairy animals but recent evidences suggest prevalence of mastitis to be the highest in high yielder cross bred dairy cows and categorized as first in terms of challenging diseases (Joshi and Gokhale, 2006; Sharma, 2008; Sailo and Chakraborty, 2012). The review of various reports evidenced that the average prevalence of mastitis in sixties to early nineties was not more than 30%. However, afterwards the prevalence increased to even more than 60%. Two decades ago, the average incidence of clinical mastitis in India was 1-10% with sub-

clinical mastitis ranging from 10-50% in cows and 5-20% in buffaloes. More than 100 recent studies spread over 32 states of India indicate that the overall prevalence of mastitis ranges from 25-97% with an average prevalence of 45% (Joshi and Gokhale, 2006; Nielsen, 2009; Kumar *et al.*, 2010; Sharma *et al.*, 2012). Mastitis remains a challenge to the veterinarians due to the involvement of multiple etiological agents (Vashney *et al.*, 2012). Involvement of a large number of pathogens have made the prevention of mastitis a challenge (Food and Agricultural Organization, 2005; Sudhakar and Khode, 2009; Kumar *et al.*, 2010). At the same time the condition as occurs in various forms (subclinical as well as clinical and acute) becomes a Pandora box for microbiologists (Musser *et al.*, 1998; De Vlieghe *et al.*, 2012). Since the etiology of mastitis is diverse and route of infection is both from circulating blood or entry through teat canal, prevention is problematic (Kemp *et al.*, 2008). Standard sanitation and shed hygiene play major role in controlling the menace, but practically in field conditions it is difficult. Many workers tried the antibiotic sensitivity pattern of the isolates, but it is not very effective (Sharma *et al.*, 2007a; Bogni *et al.*, 2011).

\*Corresponding author: Kuldeep Dhama

Division of Pathology, Indian Veterinary Research Institute, Izatnagar, Bareilly (U.P.) - 243122

The prevalence rate is very high in first fortnight of calving (particularly in first week) owing to metabolites released during parturition leading to inflammation of the udder. Secondly, due to higher let down of milk the entry of environmental pathogens through teat canal is very common and results in clinical mastitis (Rai *et al.*, 1998; Schepers *et al.*, 1998; Zhao and Lacasse, 2008). At later stages of lactation, incidence of sub-clinical mastitis is very high and the major causes are circulating pathogens or their toxins/ metabolites (Harmon, 1994; Khate and Yadav, 2010). In USA, in supplementation with sanitation and hygiene, vaccines against most prevalent pathogens have been introduced with reasonable success (Talbot and Lacasse, 2005). In mastitis, the standard treatment is antibiotic therapy (parental as well as intra-mammary infusions), fomentation of udder accompanied by drainage of milk (Sahay *et al.*, 2006; Hawari and Fawzi, 2008). The success rate is only reasonable and milk yield restoration in affected quarter is less than 65% (Owens *et al.*, 1997; Pyörala, 2009). There is no successful measure to prevent the occurrence of mastitis. In this scenario in the present study, an attempt has been made to develop an economical formulation which can prevent the onset of mastitis in the first fortnight of calving and treat both clinical and sub-clinical mastitis at later stages of the lactation.

## MATERIALS AND METHODS

The present long term study was conducted between 1999-2012, starting from Port Blair, Andaman & Nicobar Islands (having very high prevalence rate of clinical mastitis due to hot & humid climate) to Northern Plains of India. In the study, animals from Military Dairy Farm, Experimental Dairy, owned by farmers in the villages and private un-organized dairies were included. The managerial conditions varied greatly and accordingly the mastitis incidences too.

### Evaluation of standard treatment

The standard antibiotic treatment prescribed by Blood *et al.* (1983) was evaluated for prevention during first week/fortnight of calving and its efficacy was evaluated. Antibiotic sensitivity pattern in randomly selected cases was also done for selection of suitable antibiotics.

### Evaluation of tri-sodium citrate

Tri-sodium citrate (30g/ animal/day) was given orally for 5 days starting one day prior to the expected day of calving for prevention. In clinical and sub-clinical mastitis, it was fed for 5 days in supplementation of standard treatment.

### Evaluation of present formulation

The formulation [submitted for patent by ICAR (Application No. 1154/DEL/2013 dated 18.4.2013)] was given orally for 5 days starting one day prior to expected day of calving for assessing the efficacy in preventing the onset of mastitis in the first week/fortnight. To assess its therapeutic efficacy it was given by farmers/ owners from the first day of onset of clinical or sub-clinical (the day it was diagnosed) mastitis and continued for 5 days.

### Comparative evaluation of cost of treatment

Cost of treatment in standard treatment practice and with present formulation at existing market prices was calculated in Indian rupees (IR).

## RESULTS

The evaluation of standard treatment regime being practiced indicated that in 52.1% cases the restoration of milk yield was only 43.4% (30-50% range) followed by 56.1% (range 50-70%) restoration in 34.4% cases. In spite of all the attempts, 5.7% cases in the field developed fibrosis of the affected quarter. The results are given in the Table 1.

**Table 1. Outcome of antibiotic therapy regimen**

Recovery status in comparison with pre-mastitis stage (milk yield restored)	Percentage average (range)
Milk yield restored (30-50%)	52.1 (37-62)
Milk yield restored (50-70%)	30.4 (22-36)
Milk yield restored (10-30%)	11.8 (7-14)
Fibrosed	5.7 (2-10)

### Prevalence of mastitis

Out of 785 cases of clinical mastitis examined in the study, 67.3% cases occurred during 0-2 weeks of calving. In contrast, out of 655 cases of sub-clinical mastitis included in the study, 96.6% cases appeared during 8-24 weeks. The results are given in the Table 2.

**Table 2. Stage of lactation showing mastitis**

Stage of lactation	Percentage of total cases	Probable reason
<b>Clinical Mastitis</b>		
0-2 weeks	67.3	Infection /toxins/ metabolites
2-8 weeks	20.1	-do-
8-24 weeks	12.6	Mainly injury, insect bite, clinical infection etc.
<b>Sub-Clinical Mastitis</b>		
0-8 weeks	3.4	Infections, insect bite
8-24 weeks	96.6	-do-

### Evaluation studies on tri-sodium based formulation for prevention of mastitis

The standard treatment of antibiotic therapy given one day prior to expected day of calving did not show significant effect on prevention of onset of clinical mastitis during the first fortnight. However, tri-sodium citrate reduced the incidence to 6.9% against 29.2% in the control population during the period. The present formulation reduced the incidence to 1.3%. The results are given in the Table 3.

**Table 3. Evaluation of Tri-sodium citrate in prevention of clinical mastitis during first fortnight of calving**

Treatment (No. of animals)	Percent of mastitis
Tri-sodium citrate (1435)	6.9
Control (145)	29.2
Present formulation (385)	1.3

### Evaluation against clinical and sub-clinical mastitis

In the control untreated clinical mastitis cases, majority of cases developed fibrosis and restoration of milk was less than 10%. Another control group was characterized by inadequate treatment in field conditions. These affected animals received one injection of parental antibiotics and at least one time intra-mammary infusions, but further treatment was not continued. Out of 57 animals characterized in this group, 8 animals (14.04%) developed partial fibrosis of the affected quarter. The animals (273 nos.), who received standard treatment, showed moderate recovery and restoration of milk varied between 20-60%. However, full cure, manifested by complete recovery from visible inflammation, flakes less milk and restoration of milk was not fully achieved. The animals treated with present formulation (patent applied) showed excellent recovery. The blood and flakes disappeared within 2-3 days, swelling subsided in 4-5 days and milk recovery rate from the affected quarter (s) was between 60-80%. In sever cases where udder was highly distended, hard and painful, one injection of long acting antibiotic (Terramycin-LA, Pfizer) was found helpful in reducing the microbial load immediately. The results are presented in the Table 4.

### Grading;

- No improvement
- + Blood in milk became nil but flakes visible (mild healing)

++ No blood flakes occasional, swelling reduced significantly (moderate healing)

+++ No blood, no flakes, swelling disappeared (full recovery)

The trial in sub-clinical mastitis was conducted in 1372 animals. A total of 37 animals having sub-clinical mastitis were left untreated as control, 650 animals received standard antibiotic treatment either by us or local veterinarians. The recovery was moderate. In these cases, the serum/blood appearing in the milk disappeared, and the flakes in the milk were occasionally observed. However, 722 animals who received the present formulation showed excellent recovery. Flakes or blood disappeared by 2<sup>nd</sup>-3<sup>rd</sup> day and milk was resorted. Results are given in the Table 5.

**Table 4. Therapeutic evaluation of tri-sodium citrate based novel formulation in clinical mastitis**

Treatment regime	Recovery grading	Restoration of milk yield (%)
Control*- I (26)	-	<10***
Control**-II (57)	+ or -	10-30
Standard Treatment (273)	++	20-60
Present formulation (214)	+++	60-80

\* Represent the animals where farmers have applied fomentations and home remedies.

\*\* Besides fomentation, parental antibiotics (1-2 injections) were given by farmers.

\*\*\* Majority of cases lead to fibrosis in the udder while in control-II, 14% cases developed partial fibrosis.

**Table 5. Effect of tri-sodium citrate based novel formulation on sub-clinical mastitis**

Treatment (No.)	Recovery	Remark
Control (37)	-	Persisted
Standard Treatment (650)	++	Flakes occasional, no blood
Present Formulation (722)	+++	Full healing, no flakes, no blood, milk restored

### Evaluation of treatment/ prevention cost

A comparison was made on the cost incurred by the farmer/owner on each clinical case, Figures are presented in the Table 6, the cost of present formulation was much lower than the standard antibiotic treatment, even in the cases where the injection of long acting (LA) antibiotics was given.

**Table 6. Comparison of treatment/ prevention cost**

Treatment	Average cost (in IRs.)
1. Standard treatment 5 injections of antibiotics 5 days intra-mammary infusion	Rs. 550.00
2. Present formulation	Rs. 52.00
3. Formulation with LA antibiotic	Rs. 132.00

### DISCUSSIONS

Mastitis is basically inflammation of parenchyma of mammary glands. According to Awale *et al.* (2012) and Schroeder (2012), it accounts for 38% of total direct cost of the diseases and results in heavy economic losses due to reduced milk yield (up to 70%), milk discard after treatment (9%), cost of veterinary services (7%) and premature culling (14%). It is the major hindrance in expansion of dairy as a source of livelihood and poverty alleviation (Pyörala, 2003; Viguier *et al.*, 2009). Mastitis and infertility are 2 major problems that require satisfactory control measures (Varshney and Mukherjee, 2002). The incidence of clinical mastitis in high producing bovines is more just after calving and attributed to the metabolites released during parturition, besides microbes (Rai *et al.* 1998; Mubarack *et al.*, 2012). These are no universal microbe causing mastitis and complex etiology reduces either developing vaccination or effective control measures, though newer diagnostics and better control strategies are upcoming (Tenhagen *et al.*, 2006; Radostits, 2007; Deb *et al.*, 2013). Sharma *et al.* (2007b) opines that the therapy of mastitis aims to reduce the microbial load in the mammary glands not to sterilize it. Since for one liter of milk synthesis, it is estimated that 400 liter of blood passes through udder, thus practically it is difficult to sterilize for microbes. Furthermore, in various studies it has been established that in above

60% cases, pathogens enter the udder through teat canal (Waldner, 1999; Reyher *et al.*, 2012). Kutila (2005) has already evaluated role of lactoferrin through *in vitro* studies against several microbes. But the present study is a novel approach for the first time to develop a chemical based formulation which when administered orally can reduce the load of mastitis causing pathogens. The present formulation has been developed on a concept to provide substrate for continuous milk synthesis, reducing the microbial load and inflammation and supporting the tissue (epithelium) healing. Citrate deficiency adversely influences the secretory alveoli and optimum level of citrate is required for Krebs cycle (Pal *et al.*, 1995). It can thus be concluded that in comparison to standard antibiotics the present formulation (patent applied) is much efficacious and cost-

effective as far as prevention and recovery from clinical as well as sub-clinical mastitis is concerned, for the purpose of which its wide applicability and adaptability is recommended to alleviate animal sufferings from this economically important disease as well as boosting milk production.

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