# Efficacy of Organic and Inorganic Selenium in Treatment of Bovine Subclinical Mastitis

Sripad, K<sup>1</sup>., Upendra, H.A.<sup>2</sup> and Yathiraj, S.<sup>3</sup>

<sup>1</sup> Deputy Director, Institute of Animal Health & Veterinary Biologicals, Hebbal, Bangalore, <sup>2</sup> Professor, Department of TVCC, Veterinary College, Bangalore <sup>3</sup> Dean, Veterinary College, Bangalore

**Abstract:** Subclinical mastitis (SCM) could only be diagnosed by laboratory examination since it is bereft of any gross inflammatory changes. Treatment of SCM in dairy cows plays a major role in dairy industry. Researchers have tried antibiotics/antibacterials either alone or with supportive therapy. Among the supportive treatment adopted, Vitamin E and selenium tops the list. The present study is taken up to evaluate the efficacy of organic and inorganic selenium with/without Enrofloxacin, in the treatment of SCM. Twentyfour cows which were positive for SCM, randomly allocated into four treatment groups of six animals each - Group I(control), Group II(Enrofloxacin) Group III(Enrofloxacin+inorganic selenium) and Group IV (Enrofloxacin+organic selenium) were used for the study. Response to treatment group revealed significant decrease ( $P \le 0.05$ ) from zero day. As compared to all the three treatment groups, mean SCC was lowest and also prolonged upto 90<sup>th</sup> day PT (less than 5 Lakh/ml), in the group treated with organic selenium. This indicated that oral organic selenium along with Enrofloxacin, is more efficacious and beneficial in the treatment of SCM in dairy cows. **Keywords:** Bovinesubclinicalmastitis, Efficacy, Inorganic, Organic, selenium.

# I. Introduction

Mastitis, an inflammation of the mammary gland, remains as a serious problem in dairy industry since several decades and more so is the SCM. Occurrence of mastitis depends on the interaction of host, agent and environmental factors. Improvisation of dairy industry in terms of quality and quantity of milk produced is threatened by mastitis, which continues to be a cause of significant economic loss to the dairy industry not only in India, but also globally.

Clinical mastitis represents only the 'tip of the iceberg' and it is the significance of the sub-clinical mastitis that is frequently underestimated. SCM is considered economically the most important infection / disease in dairy herds, affecting 20 per cent to 50 per cent of cows in given herds [1,2]. Although the loss due to SCM is difficult to quantify, most experts agree that it costs the average dairy farmer more than the clinical mastitis does [3]. As there are no gross inflammatory changes either in the cow or udder or the milk, SCM could only be diagnosed by laboratory examination,

Treatment of SCM in dairy cows plays a major role in dairy industry. Researchers have tried antibiotics or antibacterials either alone or in combination and further with supportive or additional chemotherapeutic agents (anti inflammatory drugs, vitamins, etc.,) or chemicals. In addition to the treatment of mastitis with antibiotic/antibacterial, in order to hasten recovery, to trigger immune response or to ensure 100 per cent normalcy, supportive therapy have been advocated by veterinary practitioners. Among the supportive treatments adopted in the treatment of mastitis, Vitamin E and selenium tops the list. Over a period of time, researchers have undermined the information regarding activity, metabolism and beneficial effects of organic and inorganic selenium. Keeping this in mind, the present study is taken up to evaluate and compare the efficacy of organic and inorganic selenium along with a single chemotherapeutic agent (Enrofloxacin) for the treatment of SCM in dairy cows.

# 2.1. Source of animals :

# II. Material and Methods

Twentyfour cows which were detected positive for SCM and randomly allocated into four groups namely Group I, Group II, Group III and Group IV, with each group comprising of six animals, formed the source of animals for the study.

#### 2.2. Therapeutic agents used in the study :

Enrofloxacin – Floxidin <sup>R</sup> - obtained from M/s.Intervet India Pvt. Ltd., Pune Inorganic selenium - procured from M/s. Vet Care Pvt. Ltd., Bangalore Organic selenium - procured from Indian Herbs Pvt. Ltd., Bangalore

### 2.3. Treatment Regimens :

The following regimens of treatment was followed for different groups -

Group I: Control Group

**Group II:** Enrofloxacin @ 5 mg/Kg body weight Intramsucular s.i.d for five days.

**Group III**: Enrofloxacin @ 5 mg/Kg body weight Intramsucular s.i.d for five days and inorganic selenium @ 6 mg / animal / day orally for five days.

**Group IV**: Enrofloxacin @ 5 mg/Kg body weight Intramsucular s.i.d for five days and organic selenium @ 0.3 mg / animal / day orally for five days.

#### 2.4.Laboratory Analysis :

Response to the treatment using SCC, was studied by collecting milk samples from all the twentyfour cows belonging to four groups namely Group I, Group II, Group III and Group IV, on zero, 4<sup>th</sup>, 8<sup>th</sup>, 15<sup>th</sup>, 30<sup>th</sup>, 45<sup>th</sup>, 60<sup>th</sup>, 75<sup>th</sup>, 90<sup>th</sup>, 105<sup>th</sup> and 120<sup>th</sup> day PT.

### 2.5.Somatic Cell Count(SCC) :

Somatic Cell Count was estimated using Nucleocounter (Chemo Metec, Denmark) following the instructions given by the manufacturer.

### 2.6.Statistical Analysis

The data generated in the study were statistically analyzed by Student's 't' test and one way ANOVA at  $P \le 0.05$ , by using statistical software, to arrive at the conclusion.

# III. Results

The mean SCC of each group on different days of PT are given in Table 1. On observation the mean SCC within Group 1(control group) ranged between 7.63 Lakh/ml to 21.17 Lakh/ml with lot of variations and no definite pattern. In Group II (Enrofloxacin alone), PT mean SCC decreased from a base value of 14.35 to a lowest value of 2.45 Lakh/ml on 8<sup>th</sup> day PT, later increased without any definite pattern and remained in the range between 2.45 Lakh/ml to 6.67 Lakh/ml. In Group III (Enrofloxacin with inorganic selenium) PT mean SCC decreased from a base value of 16.46 Lakh/ml to a lowest value of 2.22 Lakh/ml on 8<sup>th</sup> and 15<sup>th</sup> day PT, later increased without any definite pattern and remained in the range between 2.22 Lakh/ml to 6.24 Lakh/ml. In Group IV (Enrofloxacin with organic selenium), there was a definite drop in the PT mean SCC from a base value of 14.92 Lakh/ml (zero day) and the lowest mean SCC of 1.62 Lakh/ml was recorded on 15<sup>th</sup> day PT, then it increased gradually along with the subsequent collections and reached a maximum of 6.83 Lakh/ml on 120<sup>th</sup> day.

Statistical comparison of mean SCC on different days of PT within each treatment group revealed significant decrease ( $P \le 0.05$ ) from the base value viz., zero day, in all the three treatment groups, whereas the control group mean SCC did not reveal any significant difference ( $P \ge 0.05$ ). This indicated that all the three treatments when compared to the control group, resulted in significant reduction ( $P \le 0.05$ ) of mean SCC from 4<sup>th</sup> day PT till the end of the experiment i.e., 120 days.

On comparison of mean SCC between the groups, there was no statistically significant difference on Zero day ( $P \ge 0.05$ ). However, the mean SCC of Group II (Enrofloxacin), Group III (Enrofloxacin + inorgamic selenium) and Group IV (Enrofloxacin + orgamic selenium) differed significantly ( $P \le 0.05$ ) from Group I (control) on 4<sup>th</sup>, 8<sup>th</sup>, 15<sup>th</sup>, 30<sup>th</sup> and 45<sup>th</sup> day PT and were statistically lower in Group II (Enrofloxacin), Group III (Enrofloxacin + inorgamic selenium) and Group IV (Enrofloxacin + organic selenium). At the same time there was no statistically significant difference ( $P \ge 0.05$ ) between Group II (Enrofloxacin), Group III (Enrofloxacin + inorganic selenium) and Group IV (Enrofloxacin + organic selenium), during this period. On 60<sup>th</sup> day PT mean SCC in Group IV was significantly lower ( $P \le 0.05$ ) than Group II (Enrofloxacin) and Group III (Enrofloxacin + inorgamic selenium). No statistically significant difference ( $P \ge 0.05$ ) was noticed in the mean SCC of control and different treatment groups on 75<sup>th</sup>, 90<sup>th</sup>, 105<sup>th</sup> and 120<sup>th</sup> day PT.

When SCC of more than 5 Lakh/ml was considered positive for SCM [4], it was observed that Enrofloxacin alone resulted in SCC less than 5 Lakh/ml up to  $30^{th}$  day PT, Enrofloxacin + inorganic selenium resulted in SCC lower than 5 Lakh/ml up to  $60^{th}$  day PT, whereas Enrofloxacin + organic selenium treatment resulted in SCC lower than 5 Lakh/ml up to  $90^{th}$  day PT. This indicated that therapeutic effect of Enrofloxacin + organic selenium was highest followed by Enrofloxacin + inorganic selenium and Enrofloxacin alone in descending order. Oral organic selenium as an additional therapeutic agent resulted in a prolonged reduction of SCC as compared to antibacterial therapy alone.

### IV. Discussion

In the group that was treated with Enrofloxacin (Group II), Enrofloxacin + inorganic selenium (Group III) and Enrofloxacin + organic selenium (Group IV), mean SCC reduced significantly as compared to the control group (Group I). When mean SCC of Group II, Group III and Group IV was compared, there was no significant difference in mean SCC up to 45<sup>th</sup> day PT. The lowest mean SCC observed was 2.45, 2.22 and 1.62 Lakh/ml respectively in Enrofloxacin, Enrofloxacin + inorganic selenium and Enrofloxacin + organic selenium treated group.

In Group II (Enrofloxacin), Group III (Enrofloxacin + inorganic selenium) and Group IV (Enrofloxacin + organic selenium), the mean SCC reduced below 5 Lakh/ml as early as 4<sup>th</sup> day PT. Further reduction in mean SCC was noticed for an extended period in Enrofloxacin + organic selenium treated group (Group IV) followed by Enrofloxacin + inorganic selenium (Group III) and Enrofloxacin (Group II) treated group in the descending order. The mean SCC was significantly reduced (below 5 lakhs/ml) up to  $30^{th}$ ,  $60^{th}$  and  $90^{th}$  days PT in Enrofloxacin (Group II), Enrofloxacin + inorganic selenium (Group III) and Enrofloxacin + organic selenium (Group IV) groups respectively. The mean SCC was significantly lower in Group IV (Enrofloxacin + organic selenium) as compared to other two treatment groups on  $60^{th}$  day PT.

It was noticed that the therapeutic efficacy of Enrofloxacin along with organic selenium (Group IV) was more prolonged as compared with the group treated only with Enrofloxacin (Group II) or group treated with Enrofloxacin + inorganic selenium (Group III).

The results of the present study indicated that treatment with Enrofloxacin (Group II), Enrofloxacin + inorganic selenium (Group III) and Enrofloxacin + organic selenium (Group IV) resulted in therapeutic recovery in cases of SCM in dairy cows. It was noticed that therapeutic efficacy was better when selenium (inorganic or organic) was combined with Enrofloxacin. Further, organic selenium with Enrofloxacin exhibited comparatively much better therapeutic recovery than the other two treatment groups.

The observation of reduced mean SCC following treatment during lactation period is in agreement with the observation made by earlier workers namely [5,6,7].

Treatment of SCM with Enrofloxacin alone resulted in significant reduction in mean SCC. This indicates Enrofloxacin can be an antibacterial of choice in the treatment of SCM in dairy cows. This observation is in agreement with the findings of [8,9,10,11,12,13,14, 15]

When organic selenium and inorganic selenium were administered orally along with parental Enrofloxacin, these two treatment regimens resulted in lowered mean and their effect was noticed for prolonged period as compared to group treated with Enrofloxacin alone (Group II). This indicates that oral administration of selenium (organic selenium or inorganic selenium) is beneficial in treatment of SCM in dairy cows.

The beneficial therapeutic effect of inorganic selenium observed in the present study derives support from the findings of [16] who has reported that inorganic selenium is beneficial in treatment of SCM. However, [17] failed to observe the beneficial effects of Vitamin E and selenium in decreasing SCC. The beneficial effects of administration of inorganic selenium in treatment of SCM in dairy cows could be attributed to the antioxidant property, increased glutathione peroxidise activity, increased conversion of  $T_4$  to active  $T_3$  form of Thyroxine as indicated by [18].

Further, administration of organic selenium resulted in reduction of mean SCC for a prolonged period up to 90<sup>th</sup> day PT as compared to 60<sup>th</sup> day PT in cows treated with inorganic selenium, indicating organic selenium has better therapeutic effect in the treatment of SCM in dairy cows as compared to inorganic selenium. Organic selenium supplementation along with antibacterial resulted in reduction in SCC [19]. Organic selenium, as compared to inorganic selenium is reported to have better bioavailability [20]. Further organic selenium improves the bactericidal activity of neutrophils [21,22] and has a role in enhancing the resistance of mammary gland to infection [23]. Organic selenium also plays a role in boosting the immune response of the body thus improving general health status of animals [24, 25]. In addition, organic selenium induces self cure of SCM and decreases the prevalence of SCM by recruiting phagocytes to the infected compartment of the udder and induces an unspecified antibactericidal activity in milk lactoserum [26]. (Ali Vehmas et al., 1997). Organic selenium also restricts the growth of mastitis causing pathogens, lowers SCC in milk and results in high milk quality [18]. In view of these properties, organic selenium as a supportive therapy seems to be beneficial in the treatment of SCM in dairy cows.

# V. Conclusion

Based on the results of the study, we can conclude that Enrofloxacin + organic selenium, Enrofloxacin + inorganic selenium and Enrofloxacin alone, can be the order of preference or choice for treatment of SCM in dairy cows. Enrofloxacin with organic selenium is the treatment of choice for treatment of SCM among dairy cows.

#### References

- Wilson, D.J., Gonzalez, R.N. and Das, H.H., (1997) Bovine Mastitis Pathogens in New York and effects on Somatic cell count and milk production. J. Dairy Sci., 80: 2592 – 2598.
- [2]. Pitkala, A., Haveri, M., Pyorala, S., Myllys, V. and Honkanen, B. T., (2004) Bovine mastitis in Finland 2001- prevalence, distribution of bacteria, and antimicrobial resistance. J. Dairy Sci., 87: 2433-41.
- [3]. Hegde, R., (2011) Ph.D. Thesis "Rapid identification of bacterial pathogens causing subclinical bovine mastitis with special reference to Staphylococcus aureus, E.coli and predominant streptococcal species by molecular methods" submitted to Karnataka Veterianry Animal and Fisheries Sciences University, Bidar.
- [4]. Narayana, T. and Iya, K.K., (1954). Studies on bovine mastitis.Incidence of mastitis in cows and buffaloes. Ind. J. Dairy. Sci., 6: 169-179.
- [5]. Storper, M., Ziv, G. and Saran, A., (1981) Evaluation of several milk sampling methods for the diagnosis of Staphylococcus aureus and Streptococcus agalactiae mastitis. Refuah Vet., 38:149–153
- [6]. Ziv, G. and Storper. M., )1985) Intramuscular treatment of subclinical staphylococcal mastitis in lactating cows with Penicillin G, Methicillin and their esters. J. Vet. Pharmacol. Ther., 8:276 - 283.
- [7]. Owens, W.E., Ray, C.H., Watts, J.L. and Yancey, R.J., (1997) Comparison of success of antibiotic therapy during lactation and results of antimicrobial susceptibility tests for bovine mastitis. J. Dairy. Sci., 80: 313-317.
- [8]. Sing, S.V., (2000) Studies on udder health profiles with special reference to acute phase proteins and supplementation therapy. M.V.Sc. thesis submitted to G.B.Pant Univ. Agri. and Tech., Pantnagar.
- [9]. Kader, M.A., Samad, M.A., Saha, S. and Taleb, M.A., (2002) Prevalence and aetiology of SCM with antibiotic sensitivity to isolated organisms among milch cows in Bangladesh. Ind. J. Dairy. Sci., 55(4): 218-223.
- [10]. Ramprabhu, R. and Rajeswar, J.J., (2006) Comparative efficacy of different indirect tests in the diagnosis of SCM in dairy cows. Indian Vet. J., 83(8): 903-904.
- [11]. Saluja, P.S., Gupta, S.L., Kapur, M.P. and Sharma, A., (2005) Antibiogram of bacterial isolates of bovine intramammary origin. Indian Vet. J., 82(3): 323-324.
- [12]. Awasthi, P. and Upadhyay, A.K., (2006) Screening of SCM using cow side test and California mastitis test. Indian Vet. J., 83(3): 275-276.
- [13]. Sahoo, S.S., Sahoo, N. and Parida, G.S., (2009) Antibiogram of bacterial isolates from bovine SCM. Indian Vet. J., 86(12): 1298-1299.
- [14]. Suresh, R.V., Srinivasan, S.R., Gowri, B. and Krishna, M.M., (2010) Clinical efficacy of long acting enrofloxacin in bovine SCM a report of six cases. INTAS Polivet., 11(1): 31-33.
- [15]. Rajeev, N.K., Isloor, S., Das, P.K. and Rathnamma, D., (2010) A dendrogram based analysis of antibiogram of S.aurues and E.coli isolates from bovine mastitis. Indian. Vet. J., 87(2): 107-110.
- [16]. Chetan Kumar., (2009) Clinical and Microbiological Studies on Bovine Mastitis. MVSc Thesis submitted to Karnataka Veterinary Animal and Fisheries Sciences University, Bidar.
- [17]. Paschoal, J. J., Zanetti, M.A. and Cunha, J.A., (2006) Milk somatic cell count of selenium and vitamin E supplemented cows. Braz. J. of Vet. Res. and Anim Sci., 43(6): 717-722.
- [18]. Mahan, D.C., (2001) Selenium Metabolism in animals : What role does selenium yeast have?. Alltech's 17<sup>th</sup> Annual Symposium, pp.257 -266.
- [19]. Jan, S., Tien, H., Bjorn, Akesson and Jacob, H. N., (2003) Dietary supplementation with organic selenium (Sel-Plex<sup>®</sup>) alters oxidation in raw and pasteurized milk. Alltech's 21<sup>st</sup> Annual Symposium, pp.249-257.
- [20]. Pehrson, B.G., [1993] Selenium in nutrition with special reference to the biopotency of organic and inorganic selenium compounds. In : Proc. Alltech's 9<sup>th</sup> Annual Symposium. T.P. Lyons(Ed). Altech technical publications, Nicholasville. pp 171.
- [21]. NRC., (1989) Nutrient requirements of Dairy Cattle, 6<sup>th</sup> revised edn., National Academy Press. Washington, DC, pp 268.
- [22]. Hogan, J.S., Smith, K.L., Weiss, W.P., Todhunter, D.A. and Shockey, W.L., (1990) Relationships among vitamin E, selenium and bovine blood neutrophils. J. Dairy Sci., 73: 2372.
- [23]. Weiss, W.P., Morgan, K.L., Smith. and Hobler., (1990) Relationship among selenium, vitamin E and mammary gland health in commercial dairy herds. J. Dairy Sci., 73: 381.
- [24]. Finley, J.W., Ip, C., Lisk, D.J., Davis, K.J., Hintze. and Whanger, P.D., (2001) Cancer protective properties of high selenium broccoli. J. agric. Food Chem., 49: 2679-2683.
- [25]. Klein, E.A., Thompson, S.M., Lippman, P.J., Goodman, D., Albanes, P.R., Taylor. and Coltman., (2003) The selenium and vitamin E cancer prevention trial. Urolog.Semin. Orig. Invest., 21: 59-65.
- [26]. Ali-Vehmas, T., Vikerpuur, M., Fang, W. and Sandholm, M., 1997. Giving selenium supplements to dairy cows strengthens the inflammatory response to intramammary infection and induces a growth-suppressing effect on mastitis pathogens in whey. J. Vet. Med., 44(9-10): 559-571.

Tuble Trateur = 51 of 500 m different freuthent groups				
PT days of collection	Group I	Group II	Group III	Group IV
Zero	<sup>x</sup> 15.44±2.90 <sup>a</sup>	x 14.35±2.69 <sup>a</sup>	<sup>x</sup> 16.46±3.67 <sup>a</sup>	<sup>x</sup> 14.92±3.54 <sup>a</sup>
4	<sup>x</sup> 13.33±1.94 <sup>a</sup>	<sup>y</sup> 2.92±0.48 <sup>b</sup>	<sup>y</sup> 2.52±0.32 <sup>b</sup>	<sup>y</sup> 2.32±0.27 <sup>b</sup>
8	<sup>x</sup> 14.53±2.54 <sup>a</sup>	<sup>y</sup> 2.45±0.39 <sup>b</sup>	<sup>y</sup> 2.22±0.25 <sup>b</sup>	<sup>y</sup> 1.82±0.28 <sup>b</sup>
15	x 15.09±2.54 a	<sup>y</sup> 2.92±0.53 <sup>b</sup>	<sup>y</sup> 2.22±0.24 <sup>b</sup>	<sup>y</sup> 1.62±0.23 <sup>b</sup>
30	<sup>x</sup> 14.33±2.77 <sup>a</sup>	<sup>y</sup> 4.25±0.61 <sup>b</sup>	<sup>y</sup> 2.90±0.31 <sup>b</sup>	<sup>y</sup> 2.06±0.20 <sup>b</sup>
45	<sup>x</sup> 20.27±8.27 <sup>a</sup>	<sup>y</sup> 5.44±0.33 <sup>b</sup>	<sup>y</sup> 4.44±0.32 <sup>b</sup>	<sup>y</sup> 2.09±0.20 <sup>b</sup>
60	<sup>x</sup> 10.02±2.95 <sup>a</sup>	<sup>y</sup> 5.10±0.22 <sup>ab</sup>	<sup>y</sup> 4.88±0.46 <sup>ab</sup>	<sup>y</sup> 3.28±0.38 <sup>b</sup>
75	<sup>x</sup> 7.63±3.82 <sup>a</sup>	<sup>y</sup> 5.17±0.27 <sup>a</sup>	<sup>y</sup> 5.20±0.19 <sup>a</sup>	<sup>y</sup> 4.01±0.42 <sup>a</sup>
90	<sup>x</sup> 19.45±12.07 <sup>a</sup>	<sup>y</sup> 5.51±0.40 <sup>a</sup>	<sup>y</sup> 5.34±0.16 <sup>a</sup>	<sup>y</sup> 4.68±0.34 <sup>a</sup>
105	<sup>x</sup> 21.17±14.14 <sup>a</sup>	<sup>y</sup> 4.51±0.49 <sup>a</sup>	<sup>y</sup> 4.84±0.39 <sup>a</sup>	<sup>y</sup> 5.30±0.26 <sup>a</sup>
120	<sup>x</sup> 19.56±9.96 <sup>a</sup>	<sup>y</sup> 6.67±0.44 <sup>a</sup>	<sup>y</sup> 6.24±0.37 <sup>a</sup>	<sup>y</sup> 6.83±0.20 <sup>a</sup>

Table 1. Mean ± SE of SCC in different treatment groups



Fig. 1. Mean SCC in different treatment groups (Enrofloxacin with / without selenium)