International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(4): 1394-1398

Received: 07-05-2018
Accepted: 10-06-2018

Mrinalee Devi
Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India

Jyoti B Dutta
Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India

Correspondence
Mrinalee Devi
Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India

Efficacy of vitamin e and selenium in dry cows in preventing udder infection after calving

Mrinalee Devi and Jyoti B Dutta

Abstract
Mastitis is one of the most devastating diseases of dairy cattle and most of the dairy farmers have to face this problem as a constant challenge. Despite intensive research and implementation of various mastitis control strategies over the last few decades, bovine mastitis remains insurmountable to the dairy holders. Effective management of mastitis is a major challenge to dairy farmers and modern veterinarians. Administration of vitamin, mineral and antimicrobials at the end of lactation plays potential role in preventing mastitis in dairy cows. As an alternative to antibiotic therapy use of micronutrients and antioxidants had encouraging results in prevention of bovine mastitis. Vitamin E and Se are essential nutrients having antioxidant properties share common biological activities. Deficiencies in either of these micronutrients have been related in increased incidence and severity of mastitis. A study was undertaken to asses the efficacy of vitamin E and selenium (parenteral supplementation) in dry cows in preventing udder infection after calving. Thirty cows (6 cows from organized and 24 from unorganized farms) were treated with E-CARE Se injection (Vetcare, Bangalore) on the day of drying off. Somatic cell Count was carried out before the trial and 7th day post-calving. Among the 113 quarters from 30 cows, the SCC declined to threshold limit after therapy in 93 quarters (82.30%; 20 quarters from organized and 73 quarters from unorganized farms). In the remaining 20 quarters (17.70%) the SCC was above the threshold limit. In the organized farm though the decrease of SCC was not significant after therapy, the mean SCC was within the threshold limit. On the other hand on the unorganized farms, the SCC was significantly reduced after therapy, but it did not fall within the threshold limit of <5, 00,000 cells/ml of milk.

Keywords: Mastitis, antioxidant, somatic cell count, vitamin e, selenium

Introduction
Mastitis is a parenchymal inflammation of the mammary gland, which is caused by microorganisms, usually bacteria that invade the udder, multiply and produce toxins which are harmful to the mammary gland [1]. It is characterized by physical, chemical and usually bacteriological changes in milk and pathological changes in glandular tissues [2]. Because of their anatomical position are subject to outside influences and are prone to both inflammation and non-inflammatory conditions [3]. Prevalence of mastitis varies from country to country i.e. 53.30 % in Bangladesh [4], 47.0 % in Great Britain [5], 44.7 % in Iran [6] 19.14 % in Egypt [7] and 66.0 % in India [8].

Mastitis continues to be an economically vital disease all over the world [9]. It is a most important deadly disease of dairy animals and causes heavy economic losses to dairy industry due to reduced milk yield (up to 70%), milk discard after treatment (9%), cost of veterinary services (7%) and premature culling (14%) [10]. The first report on mastitis caused losses in India was about Rs. 52.9 crore annualy [11]. These losses increased tremendously i.e., it was about Rs. 6053.21 crore annually in the year 2001 [12]. Most recent report from India revealed a total economic loss of Rs. 7824/- in one month per cow. Apart from its economic importance it also carries public health significance [13] which greatly affected the export of milk and milk products in the developing international trade [14]. Moreover, presence of antibiotic residues in the milk is undesirable due to its public health concern. Mastitis control mainly focused on the use of chemical disinfection, antiseptic teat dipping and antibiotic therapy, but frequent use of antibiotics led to emergence of drug resistance in the mastitis pathogens [15]. This resulted in the failure of therapy through the conventional antibiotic treatment. The presence of pathogenic bacteria and antibiotic residues in milk of mastitic cows made it unsuitable for human consumption and disseminated several diseases like tuberculosis, brucellosis, scarlet fever, gastroenteritis, food poisoning, staphylococcal toxaemia etc.

Hence, the milk and its products from infected cows posed a serious threat to public's health. Consumers today wanted milk from healthy animals with no drug resistant bacteria and antibiotic residues in it [16].

Mastitis is a complex disease; there is no simple solution to control it. Well over 100 different microorganisms can cause mastitis. Even also no single vaccine is successful to control mastitis due to its multietiolgical nature of disease. However, antibiotics were introduced in mastitis therapy from 50 year back for the control of mastitis. But the problem in dairy animals remain as same as it was prior to antibiotic era. The antibiotic treatment may help in minimizing the losses but may lead drug resistance**.** Therefore, attention is being paid to find alternative approaches to control mastitis [17] which may improve the ability of dairy animal to fight off environmental pathogens is reactionary and implies that the cow needs to respond to an infection. These approaches are directed to enhance udder defense and antibacterial system in milk by using immune regulatory micronutrients (vitamin E and selenium), vaccines and cytokines.

Research on alternative anti-mastitic therapy had been carried out in order to replace the use of antibiotics. As an alternative to antibiotic therapy micronutrients and antioxidants had yielded encouraging results in prevention of bovine mastitis. Mammary gland infection was reduced upto 42 per cent and clinical mastitis by 32 per cent after supplementation with vitamin E and selenium [18].

The National Research Council [19] recommended supplementation of vitamin E @ dose rate of 150 IU/day for dry cows and 300 IU/day for lactating cows which requires greater dose than the NRC recommendation for defense against mastitis @ dose rate of 1000 IU/ day dry period and 500 IU supplemental vitamin E for lactating cows [20].

Vitamin E and selenium, two important antioxidants were extensively used in bovine mastitis therapy [21]. Vitamin E is the most important lipid soluble antioxidant and integral component of lipid membranes of the mammary cells and thus it protects the mammary cells from the attack of reactive oxygen species produced by the phagocytic cells for intra cellular bacterial kill [22].

Selenium is an antioxidant, having role in protection of body tissues from auto-oxidative damage due to oxygen radicals produced during phagocytosis and intracellular killing of organism. Singh [23] observed reinforcement of the immune response by increasing the release of leucocytes and efficiency of phagocytes in playing a significant role in SCM caused by *Staphylococcus aureus*, *Candida albicans* and *E coli*. Dietary supplementation of selenium induces neutrophil killing while vitamin E enhances intracellular killing of bacteria resulting in reduced incidence of mastitis, new infection at calving and severity and duration of CM.

Selenium is present in the cells throughout the body and is an integral component of enzyme glutathione peroxidase (GSH-px). High concentration of hydrogen peroxidase and super oxides are produced in the cell after destruction of bacteria which may also damage the PMN cell and mammary cells. Enzyme GSH-px converts hydrogen peroxidase into water and hydrogen super oxides into alcohol. Thus, it protects the mammary cells from the destructive action of the oxidative molecules [22]. The role of PMNs harming the many tissues by releasing reactive oxygen intermediates and proteolytic enzyme was studied recommending antioxidant and other protective compounds in mastitis control. That yields in alleviating damage to secretory cells and reducing subsequent milk loss [24].

Vitamin E and selenium as cellular antioxidants protect the body cells against cytotoxic effects of oxygen metabolites produced by neutrophills in response to intra-mammary bacterial infection [15]. Supplementation of dairy cows with vitamin E and selenium had potential benefits on enhancing the natural resistance to mastitis [25].

Intramammary infections (IMIs) found in early lactation can be the result of either IMI that do not resolve or new IMIs that develops during previous dry period. The importance of dry period in the dynamics of IMIs in dairy cattle was well established [26, 27]. Interest had been increased gradually from recent years in novel dry cow management strategies to prevent new IMIs such as administration of vitamin E and selenium [27], intramammary infusions with antimicrobials [28] and external and internal teat sealers [29]. Intramammary infections flare ups in dairy farms during some specific time periods even though the best dry cow management is practiced [30]. During dry period about 50.0–75.0 per cent of all bovine mastitis originated [31]. Hence, mastitis prevention strategies should be attempted during dry period [32, 33] for effective prevention and control.

Therefore, keeping the points in view towards the importance of non-antibiotic approach in controlling bovine subclinical mastitis, the present work was carried out with the objective to assess the efficacy of vitamin E and selenium (parenteral supplementation) in dry cows in preventing udder infection after calving.

Materials and Methods

1. Ethical Approval

The research work was duly permitted by the Institutional Animal Ethics Committee. All samples were collected as per standard procedure without harming or laying stress to the animals.

2. Source of Animals

The study was carried out during the period October, 2008 to June, 2009 on the Instructional Livestock Farm, College of Veterinary Science, Khanapara and on the 17 private dairy units located on the hillock nearby the Khanapara campus of Assam Agricultural University.

A total of 30 dry pregnant cows (irrespective of their parity from private dairy units henceforth referred to as unorganized farms) on the hillock nearby the Khanapara campus of Assam Agricultural University and the Instructional Livestock Farm, College of Veterinary Science Khanapara were included in the present study.

3. Milking Practice

Milking was done manually on all the farms by dry fist method. However, towards the end of the milking, strip milking was also observed to be carried out by the milkers. At the time of milking, teats were massaged with mustard oil.

In all the private dairy farms, milking was done by the owners by themselves. Milking was done twice daily on all the farms. In the morning milking started from 4.30 am to 5 am and in the afternoon milking was started from 3 pm onwards.

4. Detection of Subclinical Mastitis

A. Disinfection of Teats: Proper aseptic measures were adopted during collection of milk.

A piece of cotton soaked with 70 per cent alcohol large enough to permit scrubbing the teats was used for thorough cleaning of the teat ends. Individual piece was used for each teat for complete disinfection of the teats.

B. Collection of Milk Samples: Screw capped flat bottomed plastic milk vials of 20 ml capacity were used for collection of milk samples. These were sterilized by autoclaving at 15 lb pressure for 15 minutes. On the body of the vials respective teats were marked as RF (Right Fore), RH (Right Hind), LF (Left Fore) and LH (Left Hind) along with the cow numbers. About 20 ml of milk was collected after discarding the first three strippings from each quarter and cap was replaced tightly. The milk samples were brought to the laboratory as soon as possible for further processing.

C. Somatic Cell Count: The somatic cell count was carried out on all the milk samples as per the standard method to detect subclinical mastitis [34].
The stain used in this study was the Modified Newman-Lampert Stain.

Range of somatic cell count [35]:
< 5, 00,000 cell/ml of milk – normal
> 5, 00,000 cell/ml of milk – positive for mastitis

Results
Thirty cows (6 cows from organized and 24 from unorganized farms) were treated with E-CARE Se injection (Vetcare, Bangalore). Somatic cell Count was carried out before the trial and 7th day post-calving.
After therapy of the 24 quarters (6 cows) on the organized farm the therapy had reduced the SCC in 20 quarters (4cows) to >5,00,000 cells/ml of milk, but the SCC reduced in 4 quarters did not fall to below 5,00,000 cells/ml of milk. (Table 1).
Out of 89 quarters (24 cows) on the unorganized farm with SCC > 5,00,000 cells/ml of milk reduced to threshold limit in 73 quarters after therapy with E-CARE Se, but in 16 quarters the SCC did not decline to threshold limit (5,00,000 cells/ml of milk.).
A total of 93 quarters out of 113 quarter milk samples (82.30%) showed reduction in the SCC after therapy with E-CARE Se injection

Table 1: Somatic cell counts reduction after treatment

Group	No of quarters examine	SCC within normal range after treatment	Percentage	SCC within normal range after treatment	Percentage
Organized	24	20	83.33	4	16.67
Unorganized	89	71	79.78	18	20.22

The effect of therapy on the milk SCC was analyzed statistically and it is presented in the Table 2, 3, and 4.

Table 2: Somatic cell count of scm positive milk samples from different groups before and after treatment with e-care se

Groups	Somatic Cell Count (Lakhs/ml of milk)	
	Before treatment Mean ± SE	After treatment Mean ± SE
Organized	8.38 ^{a} ± 1.20 (24)	4.02 ^{a} ± 0.47 (24)
Unorganized	54.79 ^{a} ± 3.98 (89)	6.33 ^{b} ± 0.47 (24)
Control	70.79 ^{c} ± 10.33 (24)	68.29 ^{b} ± 8.98 (24)

Figures within the parentheses indicate number of observations and means bearing similar superscript in a column do not differ significantly.

Table 3: analysis of variance of somatic cell counts in milk from different groups of cows

Source	df	SS	MS	F
Between groups	2	99675.15	49836.59	3.17 (57.57)*
Between treatment period	1	73200.59	73200.59	4.64 (84.26)*
Interaction	2	31632.84	15816.42	18.26
Error	268	23201.05	865.72	

*p<0.05

There was a significant decrease in the mean SCC in the milk samples after therapy on unorganized farms, though it was not reduced to the threshold limit. Conversely, on the organized farm the mean SCC declined to the threshold limit in the milk samples after the therapy, though the decrease was not significant. In the control group there was no significant decrease in SCC and it remained much higher than the threshold limit (>5,00,000 cells/ml of milk).

Table 4: Critical difference of somatic cell count in milk samples collected before and after treatment with e-care se in different groups of subclinical positive cows

Groups of cows	Mean somatic cell count (lakhs/ml of milk)		
	Pre- treatment	Post- treatment	Differences
Organized	8.38 ^{a} ± 1.20 (24)	4.02 ^{a} ± 0.47 (24)	4.36 NS
Unorganized	54.79 ^{a} ± 3.98 (89)	6.33 ^{b} ± 0.47 (24)	48.46*
Control	70.79 ^{c} ± 10.33 (24)	68.29 ^{b} ± 8.98 (24)	2.50 NS

Figures within the parentheses indicate number of observations and means bearing similar superscript in a column do not differ significantly.

Discussion
Somatic cell count of milk samples collected from the organized and unorganized farms were shown in the Table 4.4.5. The mean SCC on the organized farm before therapy was 8.38 ± 1.20 lakhs cells/ml of milk which declined to 4.02 ± 0.47 lakhs cells/ml of milk after therapy indicating a positive response of the vitamin E and selenium injection (E-CARE Se) on the intrmammry defense mechanism reflected

by the decreased number of SCC. The present finding was corroborated by the reports of several workers [23, 36 – 44].
The mean SCC on the unorganized farm decreased significantly after therapy, but did not attain the threshold limit. The possible explanation for the present findings might be multifactorial including the managemental stress i.e. overcrowding, lack of hygienic practices at milking or nutritional stress which had negatively affected the udder defense mechanism in spite of the therapy with vitamin E and selenium.

Conclusion
The findings of the current investigation concluded that bovine subclinical mastitis were detected on the basis of the somatic cell count of the milk samples. The SCC of quarter milk samples had shown no significant reduction of the count on the organized farm after therapy with E-Care Se but the mean SCC remained within the threshold limit (<5,00,000 cells/ml of milk). On the unorganized farm, on the other hand there was a significant reduction of the mean SCC of quarter milk samples but the mean did not fall to the threshold limit of less than 5,00,000 cells/ml of milk. The somatic cell count of the milk samples of the unorganized farms might be fall to the threshold limit with some more subsequence doses of vitamin E and selenium injections.

Acknowledgment
The fund and infrastructure facility for the study was provided by College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati – 22, Assam. The authors acknowledge the Dean, Faculty of Veterinary Science, Director of Post-Graduate Studies, and Directorate of Research (Veterinary), Assam Agricultural University for providing necessary facilities to carry out the research work. They would also like to extend their sincere thanks to Farm manager and other staff members of the Instructional Livestock Farm, College of Veterinary Science Khanapara (ILF) and the owners of the 17 private dairy units located on the hillock nearby the Khanapara campus of Assam Agricultural University for their cooperation and help throughout the period of research work.

Competing Interests
The authors declare that they have no competing interests.

References
1. Sharma N, Gautam A, Upadhyay SR, Hussain K, Soodan JS and Gupta SK. Role of antioxidants in udder health: A review. Indian J Field Veterinarian, 2006; 2:73-76.
2. Radostits OM, Gay CC, Blood DC, Hinchkliff KW. A Text Book of Veterinary Medicine. 9th Edn, W.B. Saunders, New York, 2000, 563-618.
3. Tripathi BN. Diseases of mammary glands of goats and sheep. Vet. Bull. 2000; 70:1117-1142.
4. Rahman MM, Islam MR, Uddin MB, Aktaruzzaman M. Prevalence of subclinical mastitis in dairy cows reared in Sylhet district of Bangladesh. International Journal of Bio Research. 2010; 1:23-28.
5. Bradley AJ. Bovine mastitis: an evolving disease. Veterinary Journal. 2002; 164:116-128.
6. Hashemi M, Kafi M, Safdarian M. The prevalence of clinical and subclinical mastitis in dairy cows in the central region of Fars Province, South of Iran. Iran J Vet. Res. 2011; 12:236-241.
7. Abdel-Rady A, Sayed M. Epidemiological studies on subclinical mastitis in dairy cows in Assuite Governorate. Vet. World. 2009; 2:373-380.
8. Mubarack MH, Doss A, Vijayasanthi M. Study on prevalence of bovine mastitis on dairy cows in and around Coimbatore district, Tamilnadu, South India. Indian Journal of Drugs and Disease. 2012; 1:35-38.
9. Hyrettin C, Sema YG, Oktay K, Mehmet Osman K and. Omer K. Investigation of antioxidant enzymes and some biochemical parameters in ewes with gangrenous mastitis. Turk. J Vet. Anim. Sci. 2005; 29:303-308.
10. Bhikane AV. and Kawitkar SB. Hand book for Veterinary Clincian. Venkatesh Books. 2000. Udgir, India.
11. Dhanda MR, Sethi MS. Investigation of Mastitis in India. Icar Res. Series No. 35, 1962, New Delhi, India.
12. Dua K. Incidence, etiology and estimated economic losses due to mastitis in Punjab and in India-An update. Indian Dairyman. 2001; 53:41-48.
13. Vasavda G. Public Health aspects of raw goat milk. Dairy Goat J. 1988; 64:226:227.
14. Bansal BK and Randhwa SS. How mastitis effects the quality of milk and milk products. In Proceedings of the 4th Round Table Conference on Mastitis, 2003; 137-146.
15. Wadhwa D, Wadhwa DR and Sharma KS. Nutritional status and mastitis in dairy cows. 4th round table conference, 2003, 53-64.
16. Misra SS, Bhattacharya TK, Shaikh FD and Sharma A. Genetics of mastitis resistance in dairy animals. In Proceedings of the 4th Round Table Conference on Mastitis, 2003, 32-46.
17. Sharma N. Epidemiological investigation on sub clinical mastitis in dairy animals: Role of Vitamin E and Selenium supplementation on its control in cattle. M.V.Sc. Thesis, 2003. Submitted to I.G.K.V.V, Raipur, Chhattisgarh, India.
18. Smith KL. Animal Nutro Events, Roche Symp. Hoffmann-La Roche, Basle Switzerland, 1986.
19. NRC. Nutrient Requirement of Dairy Cattle. 6th Rev. Edn. National Academy Science, Washington, DC. USA, 1989.
20. Rao Nageswara SB and Singh N. Role of micronutrients in prevention of mastitis in dairy animals. In Proceedings of 3rd Round Table Conference on Mastitis: February. 2002; (3):78-84.
21. Nauriyal DS. Role of vitamin E in augmentation of mammary defense against intra mammary infection in dairy cows. In Proceedings of 3rd Round Table Conference on Mastitis: February, 2002, 66-77.
22. Singh DK. Soluble factors involved in Immunity to bovine mastitis. In Proceedings of 4th Round Table Conference on Mastitis: April, 2003, 12-18.
23. Bannalikar AS, Shah DH, Singh SK and Verma R. Immune-prophylaxis modulation and Nutrition in mastitis. In Proceedings of the 3rd Round Table Conference on Mastitis: February, 2002, 85-96.
24. Zhao X, Lacasse P. Mammary tissue damage during bovine mastitis: Causes and control. J Anim Sci. 2008; 86:57-65
25. Ndiweni N and Finch JM. Effects of *in vitro* supplementation with a-tocopherol and Selenium on bovine neutrophil functions for resistance to mastitis. Vet. Immun. Immunopath. 1996; 51:67-78.
26. Green MJ, Green LE, Medley GF, Schukken YH, Bradley AJ. Influence of dry period bacterial

intramammary infection on clinical mastitis in dairy cows. J Dairy Sci. 2002; 85:2589-99.
27. Bouwstra RJ, Nielen M, Stegeman JA, Dobbelaar P, Newbold JR, Jansen EHJM *et al.* Vitamin E supplementation during the dry period in dairy cattle. Part I: Adverse effect on incidence of mastitis postpartum in a double-blind randomized field trial. J Dairy Sci. 2010; 93:5684-95.
28. Bastan A, Cengiz M, Cengiz S, Polat B, Colak A, Akan M *et al*. Effects of precalving antibiotic treatment on mastitis and individual somatic cell count in heifers. J Anim Vet Adv. 2010; 9:1245-9.
29. Cengiz M, Bastan A. Effectiveness of dry cow therapy antibiotic treatment, internal teat sealant, and a-tocopherol against new intramammary infections in cows. Bull Vet Inst Pulawy. 2015; 59:71-8.
30. Pritee G, Upadhyay AK, Gangwar NK, Rajput MKS. Relationship of mineral and vitamin supplementation with mastitis. Vet World. 2008; 1:103-4.
31. Bradley AJ, Green MJ. An investigation of the impact of intramammary antibiotic dry cow therapy on clinical coliform mastitis. J Dairy Sci. 2001; 84:1632-9.
32. Sharma N, Maiti SK. Effect of dietary supplementation of vitamin E and selenium in subclinical mastitis in dairy cows. Indian J Vet Med. 2005; 25:76-9.
33. Sampimon OC, de Vliegher S, Barkema HW, Sol J, Lam TJGM. Effect of prepartum dry cow antibiotic treatment in dairy heifers on udder health and milk production. J Dairy Sci. 2009; 92:4395-403.
34. Schalm OW, Carrol EJ and Jain NC Bovine Mastitis. 1971. Lea and Febiger, Philadelphia.
35. International Dairy Federation J Vet. Med. Asso. 1981; 34:721.
36. Hogan JS, Weiss WP, Todhunter DA, Smith KL and Schoenberger PS. Bovine neutrophil responses to parenteral vitamin E. J Anim Sci. 1992; 75(2):399-405.
37. Hogan JS, Weiss, WP and Smith KL. Role of vitamin E and selenium in host defense against mastitis. J Dairy Sci. 1993; 76(9):2795-803.
38. Smith KL, Hogan, J.S and Weiss WP. Dietary vitamin E and selenium affect mastitis and milk quality. 2: J Anim Sci. 1997; 75(6):1659-1665.
39. Morgante M, Beghelli D, Pauselli M, Dall'ara P, Capuccella M and Ranucci S. Effect of administration of vitamin E and selenium during the dry period on mammary health and milk cell counts in dairy ewes. J Dairy Sci. 1999; 82(3):623- 631.
40. Paoschoal JJ, Zanetti MA and Cunha JA. Supplementation of selenium and vitamin E on milk somatic cell count of Holstein cows. R. Bras. Zootec. 2003; 32(6):2032-2039.
41. Lauzon K, Zhao X, Bouetard A, Delbecchi L, Paquette B and Lacasse Relationships among vitamin E, selenium and bovine neutrophils. J Dairy Sci. 2005; 88:4295-4303.
42. Goel P, Kumar A, Sharma A. Role of micro nutrient as immune regulator in farm animals with special reference to mastitis-A review, Intas Polyvet. 2008; 9(1):13-19.
43. Salman S, Khol-Parisini A, Schafft H, Lahrssen-Wiederholt M, Hulan HW, Dinse D *et al.* The role of dietary selenium in bovine mammary gland health and immune function. Anim Health Res Rev. 2009; 10(1):21-34. [doi:10.1017/S1466252308001588. Epub 2009 Feb 6].
44. Prakash V, Kumar A, Yadav MPS and Singh SP. Administration of different doses of vitamin E and selenium to control mastitis in cow, Asian J Animal Sci. 2010; 5(2):223-224.