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Comparative efficacy of dry cow therapy and internal teat sealant on intramammary infection during subsequent lactation

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Abstract
In the present study cows were screened 75th day before expected date of calving by using MCMT, somatic cell count and pH. Then positive cows for subclinical mastitis were randomly divided into four groups, each comprising of 8 cows. MCMT, SCC and pH evaluation were performed in milk samples of all the 32 cattle under therapy on day 0 pre-treatment (at drying off) and 7, 45 and 90 post treatment (after calving). The result revealed significant decrease in SCC and MCMT grading in all the treatment groups that reached towards normal after treatment. On the basis of MCMT grading and drastic reduction in SCC on 7th, 45th and 90th day post treatment in animals of group T3 (Ceftiofur hydrochloride and Internal Teat sealant) revealed highest recovery followed by T1 (Ceftiofur hydrochloride) and T2 (Teat sealant (Bismuth subnitrate) alone.

Keywords: Dry cow therapy, teat sealant, intramammary infection, bismuth subnitrate, Ceftiofur hydrochloride, SCC, CMT

Introduction
The lactation therapy and dry cow therapy are the major ways to treat the cases of mastitis. However, in lactation therapy the use of antibiotics is criticized due to the concern of antibiotic residues in milk. Therefore, during dry period the initiation of therapy for control of mastitis is exceptionally profitable. The chances of getting new intramammary infections are high during the dry period. It is the most important period of dairy cow's lactation cycle. During this phase the mammary gland of the animal undergoes a series of changes that influence the cow's resistance to bacterial infection. The length of the dry period can also affect the udder health. Dry cow therapy has been one of the key step in mastitis control program. The aim of dry cow therapy is eliminating the present infection and prevention of new infection. Public concern is to reduce the use of antibiotic to prevent intra mammary infection because of development of microbial resistance and production of organic milk. An internal teat seal is the alternate management tool, which does not have antimicrobial properties. It is an inert non antibiotic substance forming an immediate physical barrier when infused in to quarter at the time of dry off. It is insoluble in milk with no antimicrobial properties with not any residue in milk or food safety risk (Lim *et al.* 2000) [9]. Thus, there is a need to find out suitable therapeutic agents to treat and control the mastitis in cattle. Looking for the above fact the following study was conducted to evaluate the comparative efficacy of dry cow therapy and internal teat sealant on intramammary infection during subsequent lactation.
With an increasing interest in organic production, there is also interest in a reduction in the use of antibiotics as prophylactics (Berry and Hillerton, 2002) [2]. Normally, the cow will develop a natural keratin plug in the teat canal at dry off, but research has shown that about 50% of all cows do not form a keratin plug within two weeks after drying off and some cows never develop this plug. This leaves the teat canal open for infection (Hutjens and Aalseth, 2005) [7]. External teat sealant is a measure that can be used to prevent new IMI during the dry period without use of antibiotics. It physically prevents entry of pathogenic bacteria into the teat canal. Internal teat sealant an alternative of external teat made from bismuth subnitrate in a paraffin base has been infused into the quarter at the time of drying off, forming an immediate

physical barrier in the distal portion of the teat cistern to prevent bacteria from ascending through the teat canal (Godden *et al.*, 2003) [5].

Material and Methods

Cows were screened 75th day before expected date of calving by using MCMT, somatic cell count and ultrasonography. Cows positive for subclinical mastitis (SCM) were selected for therapeutic regimen. The infected cows were randomly divided into four groups, each comprising of 8 cows for this study (table 01). However, eight apparently healthy cows were taken as healthy control. Intramammary administration of ceftiofur hydrochloride was done by partial insertion method. Before dry cow therapy sterilization of each teat was done using 70% alcohols.

Table 01: Therapies regimen

Group	Treatment
T1	Ceftiofur hydrochloride @ 500mg per teat
T2	Internal Teat sealant*
T3	Ceftiofur hydrochloride @ 500mg + Internal Teat sealant*
T4	Infected control
T5	Healthy control

*Teat sealant was used again after 30 days.

Procedure for preparation and application of teat sealant

Teat sealant was prepared by mixing bismuth sub nitrate (60g) in glycerin or liquid paraffin (100ml). Thereafter, add 1-2 drop methylene blue and sterilized it by autoclave at 120 °C 15 lbs for 20 minutes and cool at room temperature. Then use the teat sealant by dip the half length of teat for 1 minute. Repeat the procedure after 30 days.

Response to therapy

The results of the curative therapy was judged by retesting the milk samples after completion of the therapy on the basis of the MCMT and somatic cell count at 7th, 45th and 90th day post calving.

Result and Discussion

Therapeutic study

The present study was consisted of four groups of 8 apparently healthy animals (32 field cases of SCM) and 8 apparently healthy cows as healthy control. The investigation included epidemiological study, clinical examination of animal, udder and milk. The "T1" group was treated with inj Ceftiofur hydrochloride @ 500mg per teat. Group "T2" was treated with Internal Teat sealant alone repeated after 30day. Group "T3" was treated with Ceftiofur hydrochloride @ 500mg + Internal Teat sealant. Group "T4" was infected control. Group "T5" was kept as healthy control. The data obtained were statistically analyzed and presented.

Somatic cell count (SCC) (10^3 cells/ml) in response to different therapies

Somatic cell count (SCC) of the all 32 cow under therapeutic studies was recorded on at the day of drying off, day 0 (pre-treatment) and after calving 7th, 45th and 90th day (post treatment) and compare with the healthy cattle. The results revealed that SCC on day 0 (pre-treatment) was significantly higher in all treatment groups than healthy control group i.e. cattle of treatment groups T1, T2, T3 and T4 showed mean SCC 1180.12 ± 4.18 x 10^3 cells/ml, 1115.62 ± 22.52 x 10^3 cells/ml, 1150.87 ± 11.98 x 10^3 cells/ml, 1101.37 ± 16.05 x 10^3 cells/ml, respectively and 278.12 ± 23.25 x 10^3 cells/ml in cows of healthy control group (group T5). After treatment, the mean SCC value on day 7 in cattle of treatment group T1, T2, T3 and T4 were reduced to 234.37 ± 6.90 x 10^3 cells/ml, 285.25 ± 15.50 x 10^3 cells/ml, 230.12 ± 6.80 x 10^3 cells/ml and 559.37 ± 21.84 x 10^3 cells/ml, respectively. On day 45 post treatment, the mean SCC value of treatment groups T1, T2, T3 and T4 were 218.12 ± 6.19 x 10^3 cells/ml, 283.25 ± 14.23 x 10^3 cells/ml, 195.12 ± 8.02 x 10^3 cells/ml and 514.62.00 ± 14.23 x 10^3 cells/ml, respectively. On day 90 post treatment, the mean SCC value of treatment groups T1, T2, T3 and T4 were 202.50 ± 6.01 x 10^3 cells/ml, 290.25 ± 16.24 x 10^3 cells/ml, 195.12 ± 12.07 x 10^3 cells/ml and 514.62 ± 17.70 x 10^3 cells/ml, respectively.

A significant decrease in the SCC was noticed in all treatment groups on days 7 post treatment. However, the mean SCC on day 7, 45 and 90 post treatment in treatment groupsT3 and T1 were reduced significantly and similar to healthy group T5. A significant reduction in T3 group was observed on day 7, 45 and 90 post treatment followed by T1 and T2. The detailed variation in SCC in different treatment groups at different intervals are outlined in table 02.

Table 02: Mean somatic cell count (SCC) (10^3 cells/ml) in different treatment groups at different intervals

S. No	Group	Pre-treatment (at drying off)	Post-treatment (after calving)		
		Day 0	Day 7	Day 45	Day 90
1.	T1	1180.12^{bD} ± 04.18	234.37^{aC} ± 06.90	218.12^{aB} ± 06.19	202.50^{aA} ± 06.01
2.	T2	1115.62^{bB} ± 22.52	285.25^{bA} ± 15.50	283.25^{bA} ± 14.23	290.25^{bA} ± 16.24
3.	T3	1150.87^{bC} ± 11.98	230.12^{aB} ± 06.80	195.12^{aB} ± 08.02	195.12^{aA} ± 12.07
4.	T4	1101.37^{bB} ± 16.05	559.37^{cA} ± 21.84	514.62^{cA} ± 14.23	514.62^{cA} ± 17.70
5.	T5	278.12^{a} ± 23.25	223.12^{a} ± 01.87	204.87^{a} ± 01.69	197.00^{a} ± 03.11

Values with different superscript within the treatment group (abc: $p<0.05$) and between the group differ significantly (ABC: $p< 0.05$)

Milk pH

Milk pH of all the cattle under therapeutic trial were recorded on days 0 pre-treatment, 7, 45 and 90th day post treatment and compared with healthy cattle.

The results revealed that milk pH of cattle on day 0 pre treatment was significantly higher in all the treatment groups than the healthy control group i.e. animals of treatment groups T1, T2, T3 and T4 showed mean pH of 6.6 ±0.02, 6.67 ±0.02, 6.68 ±0.02, 6.47 ±0.02, respectively and 6.28 ±0.02, in cattle of healthy control group (group T5). After being treated, the mean pH on day 7 in cattle of treatment groups T1, T2, T3 and T4 were 6.51 ±0.05, 6.45 ±0.02, 6.23 ±0.027 and 6.38 ±0.02, respectively. On day 45 post treatment, the mean pH in treatment groups T1, T2, T3 and T4 were 6.48 ±0.06, 6.47 ±0.02, 6.21 ±0.065, and 6.40 ±0.04, respectively. On day 90 post treatment, the mean pH in treatment groups T1, T2, T3 and T4 were 6.50 ±0.05, 6.40 ±0.02, 6.18 ±0.04 and 6.37 ±0.02, respectively (Table 03).

A significant declining trend in the pH was noticed in all treatment groups on days 7 post treatment(after calving) as compare to day 0 pretreatment (at drying off). However, the mean pH of day 45 and 90 post treatment in cattle of

treatment groupsT2, T3 and T5 were showed the significant changes. The detailed variation in mean pH value in different treatment groups at different intervals (table 03). Similar finding was reported by Maheshwari *et al.* (2016) [10] observed significantly higher pH (7.30 ± 0.09) in cattle afflicted with SCM. However, Kandeel *et al.* (2019) [8] who reported that milk pH did not provide a clinically useful cow side screening method for detecting mastitis.

Table 03: Mean milk pH in different treatment groups at different intervals

S. No.	Group	Pre-treatment (at drying off)	Post-treatment (after calving)		
		Day 0	Day 7	Day 45	Day 90
1.	T1	6.60^{C} ±0.02	6.40^{A} ±0.05	6.48^{C} ±0.06	6.50^{C} ±0.05
2.	T2	6.67^{bC} ±0.02	6.45^{aB} ±0.02	6.47^{aC} ±0.02	6.40^{aC} ±0.02
3.	T3	6.68^{bC} ±0.02	6.23^{aA} ±0.02	6.21^{aA} ±0.06	6.18^{aA} ±0.04
4.	T4	6.47^{B} ±0.02	6.38^{B} ±0.02	6.40^{B} ±0.04	6.37^{B} ±0.02
5.	T5	6.28^{bA} ±0.02	6.20^{aA} ±0.02	6.28^{bB} ±0.01	6.20^{aA} ±0.02

Values with different superscript within the treatment group (ABC: $p< 0.05$) and between the group differ significantly (abc: $p< 0.05$)

MCMT Grading

The MCMT grading of the treatment group T1 and T3 were found to be negative on day 7th, 45th and 90th post treatment which were quite similar to the control group T5. However, in T2 group graded as trace on day 7th, 45th and 90th post treatment.

Comparative therapeutic efficacy

MCMT, SCC and pH evaluation were performed in milk samples of all the 32 cattle under different therapeutic regimen on day 0 pre-treatment (at drying off). Evaluation of different therapies was done by performing MCMT and SCC of milk sample from all the afflicted cattle on days 7, 45 and 90 post treatment (after calving). Comparative therapeutic efficacy of different treatment groups (table 04). No clinical case of mastitis were detected in any group during the dry period

Group T1: Amongst the eight cattle treated using Inj. Ceftiofur hydrochloride @ 500mg per teat, based on MCMT and SCC cure was noticed as 75 per cent (6 out of 8 cattle) and 72.27 per cent (8 out of 11 quarters) animal wise and quarter wise, respectively.

Group T2: In treatment group "T2" the Internal Teat sealant (Bismuth subnitrate) was used, based on MCMT and SCC the cure rate was observed as 37.5 per cent (3 out of 8 cattle) and 46.15 per cent (6 out of 13 quarters) animal wise and quarter wise, respectively.

Group T3: When SCM cattle treated with Inj. Ceftiofur hydrochloride @ 500mg + Internal Teat sealant in group "T3" then, based on MCMT and SCC cure rate was noticed as 87.55 per cent (7 out of 8 animals) and 83.33 per cent (10 out of 12 quarters) animal wise and quarter wise, respectively.

Group T4: Unhealthy control showed cure rate as 12.5 per cent (1 out of 8 animals) and 25 per cent (3 out of 12 quarters) animal wise and quarter wise, respectively.

Table 04: Comparative therapeutic efficacy on the basis of SCC, pH and MCMT

S. No.	Treatment group	Animal cure rate in per cent	Quarter cure rate in per cent
1.	T2	37.50	46.15
2.	T1	75.00	70.00
3.	T3	87.50	80.00
4.	T4	12.50	25.00

The response of therapeutic study revealed significant decrease in SCC and MCMT grading in all the treatment groups that reached towards normal after treatment. On the basis of MCMT grading and drastic reduction in SCC on 7th, 45th and 90th day post treatment in animals of group T3 revealed highest recovery followed by T1 and T2. Thus in view of these findings it is concluded that combination of Ceftiofur hydrochloride and Internal Teat sealant was found most efficacious followed by Ceftiofur hydrochloride and Teat sealant (Bismuth subnitrate) alone.

Similar findings have been reported by Godden *et al.* (2003) [5] and Sanford *et al.* (2006) [14] who have observed for a period of 60 days. Whereas, Baillargeon and LeBlane (2010) [1], Berry and Hillerton (2002) [2] and Cook *et al. (*2005) [4] observed for 105 day post calving. Ruchiman *et al.* (2010) [13] and Rabiee and Lean (2013) [12], who observed that dry cow therapy in the presence of teat sealants is an efficacious management tool for curing the IMI, over the non lactating period and reducing the SCC at the time of calving. Golder *et al.* (2016) [6] reported the positive effect of the long action antibiotic and the teat sealant act as a physical barrier of keratin plug during the dry period. Cizmeci *et al.* (2019) [3] reported that intramammary antibiotic + teat sealant provided the highest treatment success in recovery rate were 40.7% and 39.3% in the mammary lobe treated with the only internal teat sealant i.e. bismuth subnitrate. These findings are similar to T2 group. It was found to be more successful option when used in cow SCC below 200000. Its combination with parenteral antibiotic at dry off in infected quarter has enhanced the effectiveness of treatment. Nickerson *et al.* (2020) [11] reported that long acting antibiotic with teat sealant, gave 85.7% cure rate in IMM and reduced the risk of new intramammary infection after calving in lactating cows. He suggested that cure rate in teat sealant group is due to increase in the SCC in mammary secretion as a greater influx of neutrophils in response to foreign material, which was able to clear the infection. However, Baillargeon and LeBlane (2010) [1] found no significant reduction between antibiotic dry cow therapy and teat sealant and antibiotic combined group.

Conclusion

The results suggested that combination of internal teat sealant with long acting antibiotic at dry off significantly reduced the incidence of subclinical mastitis in early lactation of cattle.

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