



Effect of administration of vitamin E, selenium and antimicrobial therapy on incidence of mastitis, productive and reproductive performances in dairy cows



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Abstract Administration of vitamin, mineral and antimicrobials at the end of lactation plays potential role in preventing mastitis in dairy cows. A total of 255 cows dividing into five groups (A = 50, B = 50, C = 50, D = 50 and E = 55) at their late gestation period were selected to explore the effect of vitamin E, selenium and antimicrobial therapy on mastitis incidence, their productive and reproductive performances. Each cow of group A received α -tocopherol + sodium selenite orally daily for last 30 days before calving, while each cow of group B, C and D was treated with α -tocopherol + sodium selenite intramuscular injection, procaine penicillin + neomycin sulfate, and dicloxacillin sodium intramammary infusions at Day 30–20 before calving, respectively. Group E cows served as untreated control. California mastitis test (CMT) revealed that 70.0%, 76.0%, 84.0% and 100.0% cows in group A, B, C and D, respectively were free from new intramammary infections (IMIs) during early lactation period. The bacteriological results showed highest IMIs rates (76.3%) in group E and lowest IMIs (2.0%) in group D ($P < 0.05$). *Staphylococcus aureus* and *Escherichia coli* were the most predominant udder pathogens in all groups except group D (only CNS). The mean IMIs incidence (20.5%) was significantly lower in treated cows ($P < 0.001$). The treated cows had higher cure rates (81.9%) than control cows (23.1%) and overall efficacy of treatments on cure rates was 71.8% for all mastitis pathogens ($P < 0.0001$). The productive and reproductive performances of the treated cows at postpartum and early lactation period remained always higher than their non-treated counterparts ($P < 0.001$). A number of cow and herd related factors were identified to be significantly associated with mastitis ($P < 0.001$). The results concluded that antimicrobial therapy had more beneficial effect in preventing mastitis over

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vitamin–mineral administration and intramammary infusion with dicloxacillin sodium remained as the best effective preventive strategies for mastitis in dairy cows.

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1. Introduction

Mastitis is one of the most devastating diseases of dairy cattle particularly for the backyard farmers in developing countries, and currently many dairy farmers have to face this problem as a constant challenge [1]. Despite intensive research and implementation of various mastitis control strategies over the last few decades, bovine mastitis has yet not disappeared, remains insurmountable to the dairy holders [2].

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 is well established [3,4]. Recently, there has been increased interest in novel dry cow management strategies to prevent new IMIs such as administration of vitamin E and selenium [4], intramammary infusions with antimicrobials [5], external and internal teat sealers [6]. Despite dry cow udder health management programs, identifying infected cows in the immediate postpartum period should be an important procedure for the control of mastitis [7]. Dairy farms without an effective mastitis control program may witness up to 40.0% morbidity with IMI on an average of two quarters [8]. Conversely, there have been continuous changes in the predominating etiological agents of mastitis, greater understanding of the host responses to IMI and treatment protocols like vitamin–mineral and antimicrobial therapy [9]. There are time periods when IMI flare ups occur in dairy farms even though the best dry cow management practices are over there [1]. In another study, [10] reported that 50.0–75.0% of all bovine mastitis originated during dry period. Hence, mastitis prevention strategies should be attempted during dry period [11,12].

Administration of vitamin E–selenium to cows during dry period increases the immune response of mammary quarters [1,4] and could significantly reduce new mammary infections at calving, while their deficiencies could be associated with increased incidence of IMI [13,14]. Supplementation or injection of α -tocopherol and selenium were reported to be supportive for mammary gland immune system [4,6]. Antimicrobial agents are extensively over the last 40 years to control clinical mastitis in dairy animals and quite effective in reducing IMI at individual quarter level [5]. The intramammary infusion of antibiotics either alone or in combination with teat sealers is one of the most effective measures to prevent new IMI and cure of existing infections [15] and thus, has been recommended worldwide for many years [16]. However in Bangladesh, clinical mastitis in dairy animals is traditionally treated with only systemic antibiotics, and thus, intramammary infusions at dry period are yet to be scheduled. Besides different treatment regimens during dry period, various factors like management aspects (housing condition of the dry cow, farm hygiene, teat dipping, hand washing) and individual animal features (breed, parity, body condition, teat condition, integrity of the teat canal, milk yield, mastitis

category) also affect udder health during and after dry period [16,17].

Therefore, this study was designed to explore the effect of administration of vitamin E–selenium and antimicrobial therapy on mastitis incidence in dairy cows, their productive and reproductive performances. Interest was also focused on identifying the factors affecting mastitis incidence, cure rates and productive and reproductive traits of cows.

2. Materials and methods

2.1. Study area and farm management

The study was conducted in 4 districts of Bangladesh *viz.* Chittagong, Gazipur, Mymensingh and Sirajganj during the period from July 2013 to June 2014. The geographic position of the study area is Latitude: 20°45′–26°40′ N, Longitude: 88°05′–92°40′ E. The average annual rainfall was of 3,450 mm. The day temperature ranges from 7 to 20 °C in the cool months and in the other months it varies between 23 and 32 °C. Most of the dairy farms were smallholders farms. The cows gave birth randomly throughout year (no particular control breeding), were milked once daily with their calves used for stimulating milk let-down. Calves survived on residual milk after the hand milking. Control weaning was not practiced. The cows were fed on rice straw, cut-and-carry grasses and milling by-products as concentrate (crashed rice and/or sometimes mustard oil cake) with limited grazing on roadside and community land.

2.2. Dairy farm selection and data collection

A total of 255 crossbred cows from different smallholders farms at their late gestation period with previous history of either subclinical or clinical mastitis were selected for the present study from Chittagong, Sirajgonj, Mymensingh and Gazipur districts of Bangladesh. The body condition score of the cows varied from 2.0 to 4.0 (1.0–5.0 scale). The cows were 2.5–10.0 years old and their parity ranged from 1 to 5. Most of the cows were milked manually and their average milk production ranged from 2 L to 18 L daily. The cows were divided into five groups (Group A = 50, B = 50, C = 50, D = 50 and E = 55). Cows belonged to group A, B, C and D received different preventive treatments against mastitis, while group E cows served as untreated control. Data from all selected cows regarding their age, breed, body condition score, parity, lactation stage, milk yield, pre and post-treatment mastitis incidence etc were collected using an interviewer administered questionnaire. All the treatment procedures and data collection were done either by researcher or by trained veterinarians.

2.3. Productive and reproductive parameters

Data regarding productive and reproductive performances of all experimental cows were collected during early lactation

and postpartum (up to 120 days after calving) from the selected smallholding dairy farms. The parameters were colostrums production (lit; first five milking), milk yield (lit; first 90 days of lactation), new IMI rate, calving to conception interval, estrus detection rate (during 120 days before calving), conception rate and service per conception [14].

2.4. Treatment protocols

Each cow of group A received 0.5 g alpha-tocopherol acetate + 0.25 mg sodium selenite (E-Sel[®] Powder, Square Pharmaceuticals Limited, Bangladesh) orally daily for last 30 days before calving [1]. The group B cows were treated with two intramuscular injections of 1 g alpha-tocopherol acetate + 5 mg sodium selenite (Inj. E-Vet Plus[®] Solution, The ACME Laboratories Limited, Bangladesh) per cow on Day 30 and 20, respectively before calving [14]. Each quarter of group C cows was infused with 50 mg procaine penicillin + 300 mg neomycin sulfate (Super Mastikort[®], Intervet International, Netherlands) after complete milking starting from Day 30–20 (four consecutive infusions) prior to calving [18] and the infused syringes were withdrawn 24 h after infusion. The quarters of group D cows were infused with 7.5 g dicloxacillin sodium (Dicloxa[®], Komipharm International Limited, South Korea) per quarter once only starting from Day 30–20 prior to calving [12] and all the infused syringes were withdrawn 48 h after infusion. The group E cows did not receive any therapy and served as control for other treated groups.

2.5. Milk sampling and California mastitis test (CMT)

Five milk samples were collected from each mammary quarter of the experimented cows at their pre and post calving period. The first milk sampling was done on the day of initiation of treatment from both treated and control cows and another four milk sampling were done on Day 0, 30, 60 and 90 before calving. The milk samples were collected and preserved following the method as described in our earlier study [19]. In brief, after proper sanitization of teat orifice with 70% ethyl alcohol, 20–30 mL of milk samples from all four quarters viz. Left Fore (LF), Left Hind (LH), Right Fore (RF) and Right Hind (RH) were collected aseptically following squirting first few streams in sterile screw capped Falcon tube (50 mL). A portion of the squirted milk samples were screened by CMT and rest of the milk samples were then kept in ice box and carried to the laboratory, where the milk samples were kept at 4–8 °C in refrigerator for further laboratory investigation. After complete milking, clinical examination of udder and teats was performed.

The California mastitis tests for all milk samples were performed as cow side diagnostic test following the procedure described by [19] along with manufacturer's instruction (CMT[®], Original Schalm reagent, ThechniVet, USA). In brief, about 10 mL of milk sample was squirted in each cup of mastitis paddle and an equal volume (10 mL) of CMT reagent was added to each cup. The reactions were developed within 10 s in positive samples and scoring was done according to 0 to +3 scoring scale. CMT positive cows (score \geq +1) were defined as having at least one positive quarter for mastitis [19].

2.6. Bacteriological culture

Bacteriological examination of the collected milk samples were performed according to [20]. In brief, a 10- μ L aliquot from each milk sample thawed at ambient temperature was spread separately on MacConkey and blood agar plates containing 5.0% of defibrinated sheep blood. The plates were incubated at 37 °C for 24 and 48 h, respectively. Colonies were classified according to catalase and coagulase test reactions, Gram staining, morphology and degree of haemolysis in order to determine IMI, contamination, and mixed infections [21].

2.7. Evaluation of culture results

The cultural results were evaluated according to the procedure described by [6]. Briefly, a sample was considered contaminated when 3 or more dissimilar colony types were isolated. A mammary quarter was considered infected which had one-three different pure colonies of bacterial species. A mammary quarter was characterized as having a new infection if the same pathogen was isolated from 3 of 4 samples collected during early lactation that was not present in prepartum samples. A mammary quarter remained infected if the same organism which was present prepartum isolated during early lactation. Major pathogens, such as *Staphylococcus aureus* (*Staph. aureus*), *Streptococcus agalactiae* (*Strep. agalactiae*), *Streptococcus uberis* (*Strep. uberis*), *Streptococcus dysgalactiae* (*Strep. dysgalactiae*) and *Escherichia coli* (*E. coli*) were considered as the causative agents for the mixed infection. Sampling was repeated if the culture results were negative or contaminated, to avoid false negative and false positive results, both before drying off and after calving. The quarters were considered as uninfected when the culture results were negative in both samplings. The quarters were considered as infected when the results of the second sampling were positive. Coagulase negative *Staphylococcus* (CNS), *Bacillus* sp. and *Corynebacterium bovis* (*Cor. bovis*) were considered as the minor pathogen. When the quarters, which were uninfected at drying off, became infected after calving, it was evaluated as new infection. A mammary quarter was considered cured if the pathogen present before calving was not isolated in any of the sample obtained during early lactation.

2.8. Data analysis

The data generated from this experiment were entered in Microsoft Excel (2007) worksheet, organized and processed for further descriptive analyses by using SPSS (version 16.5). Frequency tables on cure by treatment by groups were prepared, and the Cochran–Mantel–Haenszel χ^2 test within groups as strata was used to analyze the statistical significance of prepartum treatment on mastitis cure. The treatment efficacy, by individual group and for all groups combined were computed using the formula: $100 \times (1 - \text{cure risk ratio})$, where cure risk ratio = percentage of controlled quarters cured/percentage of treated quarters cured. The difference in new IMIs between treatment and control mammary quarters was evaluated using χ^2 test. Difference = IMI in treated as compared with control cows for 90 days postpartum = $100 \times [(\text{No. of IMI in treated cows} - \text{No. of IMI in control cows})/\text{No. of IMI in control cows}]$. Independent variables having an effect

with a P value < 0.05 were included in logistic regression models using the SPSS procedure and the odds ratio (OR) and 95% confidence interval (CI) were calculated for each variable in this model.

3. Results

3.1. Effect on intramammary infections (IMIs) and California mastitis test (CMT)

In our current investigation, intramuscular administration of vitamin E–selenium was found more effective in reducing new IMI than their oral administration. According to CMT scores in group A, 35 cows out of 50 (70.0%) had no new IMI in their postcalving period while 76.0%, 84.0% and 100.0% cows in group B, C and D, respectively were free from new IMI during early lactation period. The mean post treatment CMT scores were higher in control cows than the treated cows. The highest CMT scores were found in group E (2.90 ± 0.10) while the group D cows had the lowest CMT scores (1.04 ± 0.02) and thus, the proportion of cows with new IMIs was highest (75.4%) in this control group (Table 1).

3.2. Effect on bacteriology and new IMI rates in cows

According to cultural isolation and identification, the overall new IMIs rates were 38.0%, 30.0%, 18.0%, 2.0% and 76.3% in group A, B, C, D and E, respectively. Among the major pathogens, *S. aureus* and *E. coli* were the most predominant udder pathogen in group A, B, C and E. However, the coagulase-negative staphylococci (CNS) were the leading minor pathogens in all groups (Table 2). The bacteriological results of this study revealed highest ($P < 0.05$) new IMI rates (76.3%) in untreated control group (E). Moreover, none of the major pathogens were detected in group D and only one cow was found positive for minor pathogen (CNS) in this group. Thus, minimum new IMI rates (2.0%) were detected in this control group ($P < 0.05$) (Table 2).

3.3. Effect on cure rate for all pathogens causing mastitis

In this study, treated mammary quarters had higher cure rates (81.9%) than control quarters (23.1%) for all pathogens associated with mastitis. The efficacy of treatment on cure rate varied within different treatment regimens and highest cure rates

Table 1 Efficacy of vitamin E–selenium and antimicrobial therapy on postpartum intramammary infections (IMIs) and California mastitis test (CMT) scores (Mean \pm SEM).

Groups	Total cows	Post treatment status of cows		Pre-treatment CMT score	Post-treatment CMT score
		No IMIs (%)	IMIs (%)		
Group A	50	35 (70.0)	15 (30.0)	2.94 ± 0.12	1.52 ± 0.11^b
Group B	50	38 (76.0)	12 (24.0)	2.98 ± 0.12	1.32 ± 0.08^b
Group C	50	42 (84.0)	8 (16.0)	2.90 ± 0.12	1.12 ± 0.07^a
Group D	50	50 (100.0)	0 (0.0)	2.68 ± 0.13	1.04 ± 0.02^a
Group E	55	14 (25.4)	41 (74.5)	2.48 ± 0.13	2.90 ± 0.10^{ab}

Figures in the parenthesis indicate percentage of animals.

^{a,b} Differ significantly ($P < 0.001$).

Table 2 Bacteriology and new intramammary infections (IMIs) rate in cows during after calving period in response to different preventive approaches.

Bacteriology	Groups ¹				
	Group A (n = 50)	Group B (n = 50)	Group C (n = 50)	Group D (n = 50)	Group E (n = 55)
Culture-negative	31	35	41	50	13
Culture-positive	19 ^a	15 ^a	9 ^{ab}	1 ^b	42 ^c
<i>Major pathogens</i>					
<i>Staph. aureus</i>	11	10	6	0	28
<i>Strep. agalactiae</i>	2	0	0	0	2
<i>Strep. uberis</i>	1	0	0	0	1
<i>Strep. dysgalactiae</i>	0	1	0	0	1
<i>E. coli</i>	1	0	0	0	1
<i>Minor pathogens</i>					
CNS	4	3	2	1	7
<i>Bacillus</i> sp.	0	0	1	0	1
<i>Cor. bovis</i>	0	1	0	0	1
New infection rate (%)	38.0	30.0	18.0	2.0	76.3

Different superscripts (a,b,c) represent statistical difference at $P < 0.05$ level among the groups.

¹ A = vitamin E–selenium powder, B = vitamin E–selenium injection, C = penicillin + neomycin sulfate intramammary infusion, D = dicloxacillin sodium infusion, E = untreated control.

(85.0%) was found in group D cows followed by group C (73.6%), B (64.1%) and A (53.7%). The overall efficacy of treatments on cure rates was 71.8% against all pathogens causing mastitis (Table 3) and was statistically significant ($P < 0.0001$). At the start of the study, 32.0% (64/200) treated cows had IMI, as opposed to 50.9% (28/55) in controls. Individually, reduction in cows with new IMI was found in four groups out of five. On an average, there was a 65.6% reduction of IMI in treated cows and dicloxacin sodium was found as the most effective dry cow agent in decreasing mean IMI incidence (Table 4).

3.4. Effect on early lactation and postpartum reproductive performances of dairy cows

The treated cows produced more colostrums than their non-treated counterparts (2.27 ± 1.07 L vs 1.26 ± 0.54 L) and overall colostrums production was 41.5% higher in treated cows for an average of first five milking. The mean milk yield of first 90 days lactation was significantly ($P < 0.001$) higher (39.2%) in treated cows than the controls (8.02 ± 1.92 L vs 5.12 ± 1.31 L). Overall mastitis incidences were also lower in treated groups (22.5%) than the control group (45.1%). Furthermore, the treatment regimens had significant ($P < 0.001$) effect on postpartum reproductive performances and the calving to conception interval reduced greatly in treated cows than the controls (116 ± 1.33 vs 138 ± 3.01 days). Postpartum estrus detection (44.1%) and conception (40.8%) rates were also higher in treated cows than their control counterpart. However, the number of services per conception did not vary

significantly (1.37 ± 1.06 vs 1.22 ± 0.92) between the treated and control groups (Table 5).

In our present investigation, crossbred Friesian cows were mostly ($P < 0.001$) susceptible to mastitis causing pathogens (Table 6). Older cows having parity ≥ 4 had a higher risk of new IMIs during dry and early lactation period ($P < 0.005$). Udder quarters from cows having a BCS of 3.0–3.5 before drying off had almost twofold higher risk of developing new IMI than those with a BCS < 3.0 and > 3.5 . High yielding cows (> 10 L/day) and their rear quarters (LR, RR) were more prone to new IMI ($P < 0.001$). Furthermore, absence of regular teat dipping practice before and after milking, and cows always stalled in tie barns had higher incidence of IMI ($P < 0.005$). Although, most of the cows were suffering from non-specific clinical mastitis (mixed infections) ($P < 0.0001$) at dry period but the categories of mastitis had no significant effect during early lactation period.

4. Discussion

Bovine mammary quarters are markedly susceptible to IMIs at dry off period and the infections, which started in the dry period, are thought to be responsible for new IMIs in next lactation [22]. Our present findings revealed that intramuscular injections of α -tocopherol and sodium selenite at late gestation period was found more effective in reducing overall CMT scores and new IMIs, and thereby increasing cure rate than their oral administrations. One of the reasons for this difference could be the variation in formulation i.e. super-finely and equally ground, which increases bioavailability and has

Table 3 Intramammary infections (IMIs) in cows pre and post calving period: efficacy of treatment on cure rate for all pathogens causing mastitis.

Groups	Infected quarters		Cured quarter		Efficacy ¹ (%)	95% CI	P-value (χ^2)
	Treated	Control	Treated	Control			
Group A	46	38	34	13	53.7	35.0, 71.2	< 0.0096
Group B	62	54	48	15	64.1	15.7, 79.0	< 0.0157
Group C	79	44	66	10	73.6	17.2, 82.0	< 0.0001
Group D	112	63	96	8	85.0	53.6, 92.1	< 0.0001
Total	298	199	244	46	71.8	48.1, 67.9	< 0.0001

For the total overall comparisons of the efficacy and P-value was compiled using the Mantel–Haenszel statistic χ^2 test because no significant heterogeneity of effects between groups existed.

¹ Efficacy = $100 \times (1 - \text{cure risk ratio})$, where cure risk ratio = percentage of controlled quarters cured/percentage of treated quarters cured.

Table 4 The number of cows among groups with intramammary infections (IMIs) pre and post-calving period.

Groups	No. of cows enrolled		No. of cows with IMI prepartum		No. of cows with IMI postpartum		Difference ¹ (%)
	Treated	Control	Treated	Control	Treated	Control	
Group A	50	10	11	7	9	8	62.5
Group B	50	15	19	9	7	11	54.5
Group C	50	12	13	7	1	11	-36.4
Group D	50	18	21	5	0	2	*
Total	200	55	64	28	17	32	65.6

¹ Difference = IMI in treated as compared with control cows for 90 days postpartum = $100 \times [(\text{No. of IMI in treated cows} - \text{No. of IMI in control cows}) / \text{No. of IMI in control cows}]$.

* Undefined because relative risk could not be computed when no infections were present in mammary quarters of either control or treated cows.

Table 5 Effect of vitamin E–selenium and antimicrobial therapy on early lactation and postpartum reproductive performances of dairy cows (Mean \pm SEM).

Parameters	Control group	Treated groups
Colostrums production (lit; first five milking)	1.26 \pm 0.54 ^a	2.27 \pm 1.07 (41.5%) ^b
Milk production (lit; first 90 days of lactation)	5.12 \pm 1.31 ^a	8.02 \pm 1.92 (39.2%) ^b
Incidence of intramammary infections (%)	45.1 ^a	20.5 ^b
Calving to conception interval (Days open)	138 ^a	116 ^b
Estrus detection rate (% cows showed estrus during study period; 120 days of calving)	43.0 ^a	77.0 (44.1%) ^b
Conception rate (%)	31.0 ^a	52.4 (40.8%) ^b
Services per conception	1.22 \pm 0.92 ^a	1.37 \pm 1.06 ^a

Means with different letters (a & b) within a row differ significantly from each other ($P < 0.001$).

Table 6 Odds ratios (OR) and confidence intervals (CI) of factors having significant effect on IMI during the dry period and from calving to 90 days of postpartum (logistic regression model).

Independent variables	Categories	Incidence of IMI during dry off period			Incidence of IMI up to 90 days of postpartum		
		OR	95% CI	P -value (χ^2)	OR	95% CI	P -value (χ^2)
<i>Breeds</i>							
	Local zebu	0.539 ^a	52.0, 82.1	0.001	0.593 ^a	0.96, 17.2	0.005
	Crossbred Friesian	1.000 ^b			1.000 ^b		
	Sahiwal cross	0.723 ^a	21.7, 72.0		0.654 ^a	29.0, 53.5	
	Red Chittagong	0.601 ^a	33.2, 88.5		0.566 ^a	14.2, 66.1	
<i>Parity</i>							
	1–2	0.213 ^a	21.0, 66.1	0.005	0.533 ^a	10.2, 41.2	0.007
	3–4	0.352 ^a	39.2, 56.3		0.672 ^a	39.1, 40.1	
	≥ 4	1.003 ^b			1.003 ^b		
<i>Body condition score (BCS)</i>							
	≤ 2.5	0.672 ^a	1.5, 27.3	0.0003	No significant effect		
	3.0–3.5	1.912 ^{a,b}	31.2, 61.3				
	> 3.5	1.000 ^b					
<i>Milk yield (L/day)</i>							
	2–5	0.793 ^a	21.0, 38.2	0.001	0.954 ^a	14.0, 14.3	0.003
	6–10	1.000 ^a			1.000 ^a		
	11–15	1.811 ^{a,b}	3.6, 59.0		1.681 ^{a,b}	22.5, 48.1	
	> 15	1.308 ^b	1.7, 42.5		1.320 ^b	11.2, 51.0	
<i>Udder quarter localization</i>							
	Left rear (LR)	1.209 ^a	12.9, 30.2	0.0001	1.213 ^a	7.1, 52.7	0.002
	Right rear (RR)	1.625 ^{a,b}	7.0, 56.4		2.109 ^{a,b}	0.78, 36.3	
	Left front (LF)	0.807 ^b	0.97, 37.6		0.714 ^b	5.0, 19.6	
	Right front (RF)	1.000 ^a			1.000 ^a		
<i>Regular practice of teat dipping before and after milking (with antiseptic solutions)</i>							
	Yes	0.319 ^a	52.0, 82.1	0.001	0.213 ^a	0.87, 11.2	0.005
	No	1.000 ^b			1.102 ^b	0.54, 36.7	
<i>Dry cow housing</i>							
	Free-stall	0.638 ^a	52.0, 82.1	0.002	0.013 ^a	27.0, 76.1	0.049
	In tie-stall barns	1.001 ^b			0.901 ^b		
<i>Udder quarter mastitis category</i>							
	Subclinical and latent infection	1.105 ^a	0.69, 15.9	0.0001	No significant effect		
	Unspecific clinical mastitis	2.507 ^b	1.47, 38.9				

The OR describes the risk for udder quarters of developing CM.

^{a,b} ORs with different letter superscripts are statistically significantly different.

positive effects on the inhibitory concentration in udder tissues to kill intracellular bacteria by neutrophils [1,6]. Supplementation of vitamin E and selenium to cows during dry period had significantly lessen the new IMIs at calving compared with

cows not fed or injected with vitamin E and/or selenium [4,13]. Out of four treated groups, only the fourth (D) cows had lowest CMT scores (1.04 ± 0.02) and thus, were free from new IMIs.

The overall CMT scores and proportion of cows with new IMIs were highest (75.4%) in control group. CMT score point decreased significantly in all treated groups suggesting the beneficial effect of vitamin, mineral and antimicrobial therapy on the incidence of IMIs, as compare to the non-treated counter group. These results are also supported by the findings of [1,6].

Etiological identification showed that *S. aureus* and *E. coli* were the most predominant udder pathogen in four groups viz. A, B, C and E, and the coagulase-negative *Staphylococci* (CNS) were the leading minor pathogens in all groups. Intramammary infusion with dicloxacillin was found more effective than penicillin and neomycin (2.0% vs 18.0%; new IMIs) because it was effective to kill penicillin resistant intracellular *Staphylococci* and thus, there was no incidence of new IMIs with major pathogens in this group. However, according to bacteriology highest ($P < 0.05$) new IMIs rate (76.3%) was found in untreated control group. These findings are in support with several previous studies where the prophylactic effect of intramammary antibiotic therapy has been reported [5,21]. Recently, [6,16] reported that there was a decrease in *Staphylococcal* and *Streptococcal* infections in early lactation period following treatments with different antimicrobials. On the other hand, CNS was the most frequently isolated minor pathogens and responsible for higher infection rate in both treated and control groups in this study. However, [6,15] also reported high new CNS infection rate at early lactation period even after vitamin E and antimicrobial therapy. The overall mastitis incidence was also lower in treated groups (22.5%) than the control group (45.1%) which are in agreement with several earlier findings [1,14].

The cure rate among the treated and untreated mammary quarters varied significantly in our present study. The efficacy of treatments on cure rate also varied within different groups and always remained higher for antimicrobial groups. The average cure rate against all mastitis pathogens were higher (81.9%) in treated groups than the control (23.1%). Highest cure rate (85.0%) and efficacy on cure rate (71.8%) was found in cows receiving intramammary infusion with dicloxacillin sodium. This result coincides with the observation of [23,24] who reported 84.0–97.0% cure rate in animals receiving intramammary infusion with sodium cloxacillin. However, the highest cure rate of dicloxacillin could be due to their micronized formulations and high levels of uniform diffusion within all areas of the udder tissues. Recently, [16] also reported that cloxacillin formulation could be associated with a higher likelihood of cure compared with cefquinome or benethamine benzylpenicillin-penethamate hydriodide-framycetin sulfate. Although at the beginning of the study only 32.0% cows belonged to the treated groups had IMIs but higher proportion of cows (50.9%) were found with IMIs in control group E. In our current study, dry cow therapies were effective in reducing new IMIs approximately by 66.0% and eliminated more existing infections than by spontaneous cure or without any treatment. In a previous study, [21] reported overall 51.9% reduction of IMIs in heifers receiving treatment with different antimicrobials. However, whatever the pathogen involved in udder infections, the reduction of IMI in all cows was found individually [25].

In our current investigation, the treated cows resulted in more production of colostrums (41.5%) and milk (39.2%) as compared to the control cows. Moreover, all the scheduled

dry cow treatments had significant effect on postpartum reproductive performances. The calving to conception interval was reduced greatly in treated cows than the untreated controls (116 days vs 138 days). Although, higher estrus detection (44.1%) and conception (40.8%) rate were found in treated cows but there were no significant differences in number of services per conception between the treated and control cows. Administration of vitamin E, selenium and antimicrobials during late gestation period of dairy cows has beneficial effect on mean mastitis incidence, colostrums and milk production and postpartum fertility [1,14]. Since dairy cattle are most susceptible to mastitis during late gestation period and immediately after calving, proper therapy with vitamin E, selenium and antimicrobial agents could help them to get through this transition period smoothly leading to increased milk production and postpartum fertility [12,21].

In our present study, a number of cow and farm management related factors were found to be associated with the incidence of mastitis, productive and reproductive performances of dairy cows during dry and early lactation period. Crossbred Friesian cows with higher parity (≥ 4), desired body condition (BCS > 3.0) and high milk yield (> 10 L/day) were more defenseless to udder infections during the dry and early lactation period supporting the findings by other researchers [16,17]. The rear quarters of the high yielding cows had higher risk of new IMIs compared to other quarters which could be due to the poor hygienic conditions for this udder area. This result is in accordance with many previous findings [16] but opposes to our previous findings [19]. Anatomical conformation and localization of the mammary quarters could be the possible reasons for increased risk of IMIs [26]. Farm management practices like absence of regular practice of teat dipping and cows stalled in tie barns had significant role in the occurrence of IMIs before and after dry off period. Most of the cows were suffering from non-specific clinical mastitis during dry period, however, categories of mastitis did not have any significant effect during early lactation period and this is supported by the findings of [16,26].

5. Conclusion

The findings of our current investigation concluded that antimicrobial therapy at late gestation period of cows has more beneficial effect over vitamin–mineral administration in preventing dairy animal mastitis. However, among the antimicrobials, intramammary infusion with dicloxacillin sodium appears to be the best effective therapy in reducing the incidence of new IMIs in dairy cows and thereby, increasing their overall productive and reproductive traits in postpartum and early lactation period.

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