

Original Research Article

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Comparison of Post Milking Teat Disinfection Alone With Pre and Post Milking Teat Disinfection in the Prevention of Bovine Mastitis

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ABSTRACT

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A split herd design experiment was undertaken on an organized dairy farm at Village Chimna Kalan near Jagraon, Ludhiana, Punjab (India) to test the benefit of pre-milking in addition to post-milking teat disinfection on new mastitis levels. A Lactic acid based germicidal pre-dip ('B4-MTM' from Hester biosciences Ltd, Ahmadabad) was applied manually using foaming cups, over a complete lactation. Post milking teat disinfection was done using Povidone iodine based germicidal post milking dip (Povidone Iodine: Glycerine 4:1). The study involved three groups with 30 cows in experimental design. Group 1 (n = 6): Cows with no teat disinfection (control); Group 2 (n = 12): Cows with post milking teat disinfection only and Group 3 (n = 12): Cows with pre and post milking teat disinfections. The quarter foremilk samples were analyzed for microbial culture, California Mastitis Test (CMT) score and somatic cell count (SCC). The CCM samples were evaluated for Electrical Conductivity (EC), pH, SCC and CMT. The prevention of new IMI was also assessed during the study period. There was a significant reduction in the values of Log₁₀SCC in the group 2 i.e. post milking teat disinfection while the changes in CMT, EC, and pH were non-significant. There was increase in prevalence of specific SCM from 33.3% to 62.5% in control group. In post milking teat disinfection there was significant reduction in specific SCM prevalence (46.15%). And in pre + post milking teat disinfection also there was significant reduction in prevalence of specific SCM vis. 33.33%. However, the difference between reduction by post milking and pre + post milking teat disinfection was found to be non-significant. The prevention of new IMI was also assessed and it was observed that there was significant reduction in post milking and pre + post milking teat disinfection as compared to control group. But the difference between post and pre + post milking teat disinfection was not significant.

Introduction

Intra-mammary infection (IMI) may be caused by contagious or environmental pathogens. Contagious pathogens lodge and grow on and in the cow's mammary gland. Environmental pathogens reside in cow's environment. Most udder pathogens spread from cow to cow during the milking process. Establishment of an IMI requires penetration of the mastitogens through the teat canal. Nickerson (2001) has observed a direct relationship between the number and types of bacteria on

the teat skin and the incidence and type of mastitis. Hence, reduction in IMI will be needed for controlling instances of mastitis.

Dairy farmers can put into practice several practices to successfully manage mastitis in their herd. For good udder health National Mastitis Council lays emphasis on ten point plan, which includes i) maintaining a clean, dry, comfortable environment, ii) proper milking procedures, iii) proper use and maintenance of milking equipments, iv) good record keeping, v) appropriate management of

clinical mastitis during lactation, vi) effective dry cow treatment, vii) maintenance of biosecurity for contagious pathogens and viii) culling of chronically infected cows, ix) regular monitoring of udder health status and x) periodic review of mastitis control program. Hygiene and udder preparation measures before and after milking have been used widely and considered useful in controlling environmental and contagious mastitis.

An open teat sphincter during milking provides the perfect opportunity for mastitis pathogens to invade, proper milking routines however will, make a cow less prone to these pathogens. For prevention of mastitis, farmers rely mainly on post milking teat dipping, as it reduces new IMI, clinical mastitis and reduces mastitis due to contagious organism and has variable effect on environmental pathogens (Bansal *et al.*, 1995 and Williamson and Hulbert, 2013). Due to its low cost, effectiveness and ease of application for controlling mastitis, post-milking teat dipping has been endorsed by dairy advisors and implemented by dairy producers globally (Oliver *et al.*, 1990).

Now with introduction of pre-milking teat disinfection farmers are being advised to use pre-milking teat disinfectants thus, reducing the microbial counts on teats before milking is important in prevention of mastitis. Pre-milking teat disinfection was developed as economic and effective method to control environmental pathogens by reducing bacterial load on teat skin before milking, thus reducing their penetration into teat canal (Nickerson 2001). Thus, prevents spread of microorganisms and incidence of new IMI. Pre milking teat dipping has been evaluated (Singh, 2015). But there is a need of assessing the advantage of pre and post milking teat dipping over post milking teat dipping. The present study aimed at comparison of post milking teat disinfection alone with pre and

post milking teat disinfection in the prevention of bovine mastitis.

Materials and Methods

A split herd design experiment was undertaken on an organized dairy farm at Village Chimna Kalan near Jagraon, Ludhiana, Punjab (India) to test the benefit of pre-milking in addition to post-milking teat disinfection on new mastitis levels. The herd had strength of 42 lactating cows of which 8 were in late lactation, 3 had recurrence of mastitis and 1 cow had two blind quarters, so they were excluded from the study resulting in 30 cows in experimental design.

The animals were kept under a semi-intensive system of rearing with both concrete and sandy floor and ear tagging was done for identification. Feed was provided according to the seasonal availability of fodder supplemented with commercially available feed supplements and water was provided *ad libitum*. The cows were bucket machine milked twice daily, i.e. morning 5:00 am and evening 5:00 pm.

The mean herd parity was 2.6. A Lactic acid based germicidal pre-dip ('B4-MTM', from Hester biosciences Ltd, Ahmadabad) was applied manually using foaming cups, over a complete lactation. Post milking teat disinfection was done using Povidone iodine based germicidal post milking dip (Povidone Iodine: Glycerine = 4:1). The experiment was conducted in 3 different types of groups by using the split herd design. The animals were marked by using ropes of three different colors (Red, Green and Yellow), which were tied around the neck of animals for identification of the test group to which they belonged.

Group 1 (n = 6): Cows with no teat disinfection (control)

Group 2 (n = 12): Cows with post milking teat disinfection only

Group 3 (n = 12): Cows with pre and post milking teat disinfections

Good pre milking udder preparation included washing of teats and base of udder with a minimum amount of water, if necessary (in case udder/ teats were apparently clean the pre-dip was applied directly without washing) and dried thoroughly with single service paper towel. The teats were fore stripped and then immersed in pre milking germicidal product, with a contact time of at least 30 seconds. In case of animals in only post milking teat disinfection group pre milking preparation was done in routine manner without use of pre milking product and after completion of milking post milking teat dipping was done with disinfection solution. Teats were dried thoroughly with individual paper towels. The cows in control groups were prepared thoroughly with individual paper towels. The cows in control group were prepared for milking in a routine manner using water. The trial was run for 6 months. Application of the teat disinfectant occurred after cup removal as part of the normal milking routine and was undertaken by the herd owner or laborer. While there was no supervision of the disinfectant application by research staff, the milking operators were instructed on how to achieve complete coverage of the teat. To verify correct application of post milking teat disinfectant, teats were rinsed prior to milking to determine concentration of iodine on teat skin

The farm was visited during evening hours and samples were collected just before machine milking. Two types of milk samples were collected i.e. Quarter foremilk (QFM) and Cow Composite milk (CCM) sample. Before collection of milk sample, proper cleanliness and dryness of teats was ensured.

The teat orifice was scrubbed with a cotton wool wetted with 70% alcohol (spirit). First few streaks of milk were discarded and the individual quarter samples (about 15 ml) were collected in sterilized labelled test tubes and approximately 40 ml of Composite Milk in sterile containers. The milk samples were then packed in ice and transferred immediately (within 1-2 hours of completion of sampling) to the mastitis lab in Department of Veterinary Medicine. Sampling was done before conduct of trial, and then repeatedly at monthly interval up to 6 months.

Analytical Procedures

The QFM samples were analyzed for microbial culture (National Mastitis Council, Brown *et al.*, 1969), California Mastitis Test (CMT) score (Pandit and Mehta, 1969) and somatic cell count (SCC) using automatic cell counter (Delta Instruments, Netherland). The CCM samples were evaluated for Electrical Conductivity (EC) (Mettler Toledo), pH (Mettler Toledo), SCC and CMT.

Defining of quarter health status

The health status of individual quarters was assessed and defined on the basis of bacteriology and SCC of quarter foremilk samples using IDF criteria as described below:

Diagnosis of new IMI

The establishment of new IMI in eligible quarters was considered in two phases: (i) during first three months post-initiation of trial, and (ii) during last three months. A new IMI quarter was said to be established when the same bacterial species was isolated from at least two out of three samples taken at monthly intervals. An individual quarter was eligible for only one new IMI per bacterial species during the trial. A quarter might become free of infection at 3 months post-

initiation of trial but the quarter was not eligible for another new IMI by the same pathogen.

Efficacy evaluation

Data on efficacy of pre-milking udder preparation was analysed in two ways: First, the mean percentage reduction in rate of new IMI achieved among treated cows was compared with the rate among control. Differences between the percentage of quarters becoming infected treatment and control groups, for a given period, were tested with the standard normal approximation. National Mastitis Council protocol described by Nickerson *et al.*, (2004).

Data were analyzed using SPSS package (IBM, SPSS version 25, 2017). The statistical significance of changes in occurrence of mastitis in pre and post-treatment phases between two groups was compared using Chi-square distribution. The effect of treatment on milk quality was evaluated by comparing data from treatment and control groups applying Student's t-test. The SCC values were converted to Log₁₀ to achieve normality.

Results and Discussion

Perusal of Table 1 revealed that there was a significant ($P < 0.05$) reduction in the values of Log₁₀SCC in the group II i.e. post milking teat disinfection while the changes in CMT, EC, and pH were non-significant on comparing before and after treatment values.

However, in group III i.e. pre + post milking teat disinfection, no significant changes in any of the milk parameters after trial were noted. A significant increase was observed in EC of milk after the trial in control group. There was non-significant increase in CMT score, and Log₁₀ SCC, whereas milk pH reduced non significantly in this group.

In case of control group there was increase in prevalence of specific SCM from 33.3% to 62.5%. In post milking teat disinfection there was significant reduction in specific SCM prevalence (46.15%). Similarly, in pre + post milking teat disinfection group, there was significant reduction in prevalence of specific SCM vis. 33.33% (Table 2). However, the difference in reduction of SCM by post milking and pre + post milking teat disinfection was found to be non-significant.

New IMI developed by *Corynebacterium* spp. in control group was high and in other two groups new IMI due to *Corynebacterium* spp. was considerably less. Other major pathogens which developed new IMI included Coagulase positive Staphylococci, Coagulase negative Staphylococci and *Streptococcus* spp.

The prevention of new IMI was also assessed and it was observed that there was significant reduction in new IMI in both post milking and pre + post milking teat disinfection groups as compared to control group (Table 3). But the difference between post and pre + post milking teat disinfection was found to be non-significant.

In the present study it appeared that the pre + post milking udder preparation was having some effect but it was non-significant as compared to post-milking disinfection only. In this study there was significant reduction in new IMI in pre + post-milking group as compared with control group but it could be due to post-milking disinfection as the difference was non-significant when compared with only post-milking group.

Also there was no significant change in the milk parameters in case of pre + post milking group when compared with post milking group which reduced the SCC significantly. So there is no significant advantage of pre-milking teat disinfection.

Defining of quarter health status

| Milk SCC (cells/ml) | Microbial pathogen | |
|------------------------|----------------------|-------------------|
| | Not detected | Detected |
| ≤ 400 000 | Healthy | Latent infection |
| > 400 000 | Nonspecific mastitis | Specific mastitis |

Table.1 Descriptive statistics for various variables in control and treatment groups over the period of lactation

| Parameter | Before Treatment | | | After treatment | | |
|-----------------------------|-------------------------------|-----------------------------|------------------|-------------------------------|-----------------------------|------------------|
| | C | G I | G II | C | G I | G II |
| CMT | 0.666 ± 0.307 | 0.708 ± 0.249 | 0.41 ± 0.19 | 1.16 ± 0.6 | 0.79 ± 0.28 | 0.83 ± 0.24 |
| EC (mS/cm) | 5.438 ± 0.339 ^a | 5.224 ± 0.162 | 5.620 ± 0.130 | 6.201 ± 0.268 ^a | 5.74 ± 0.194 | 5.957 ± 0.206 |
| pH | 6.60 ± 0.057 | 6.51 ± 0.046 | 6.40 ± 0.05 | 6.45 ± 0.14 | 6.51 ± 0.03 | 6.56 ± 0.06 |
| Log₁₀ SCC | 4.95 ± 0.131 | 5.74 ± 0.18 ^a | 5.47 ± 0.13 | 5.5 ± 0.31 | 5.23 ± 0.15 ^a | 5.44 ± 0.15 |

C = Control, G I = Post-milking only, G II = Pre + Post milking teat dipping
Values in rows with same superscript differ significantly (P<0.05)

Table.2 Effect of pre-milking and post milking teat disinfection on prevalence of specific SCM

| Group | Prevalence of specific SCM | | | |
|----------------|----------------------------|-----------------|----------|--------------|
| | Before treatment | After treatment | % change | Significance |
| Control | 33.33 % (8/24) | 62.50 % (15/24) | | |
| I | 27.08 % (13/48) | 14.58 % (7/48) | 46.15 | 17.313 |
| II | 14.58 % (7/48) | 8.33% (4/48) | 33.33 | 15.461 |

**Percent reduction in occurrence of specific subclinical mastitis = $\{(A-B)/A\} \times 100$

Where, A = Prevalence of mastitis in the herd under study at start of trial; and B = Prevalence of mastitis in the herd under study at end of trial

Table.3 Effect of pre-milking and post milking teat disinfection in prevention of new IMI

| Group | New intramammary infections (IMI) in eligible quarters | | | |
|----------------|--|-------|-------------|------------------------|
| | No. | % | % reduction | Significance of result |
| Control | 8/16 | 50 | | |
| I | 6/35 | 17.14 | 65.72 | $\chi^2 = 5.952$ |
| II | 9/41 | 21.95 | 56.10 | $\chi^2 = 4.326$ |

Quarters free from infection at start of trial: 53 in treatment group; 65 in control group

The teat disinfection is applied to control IMI and enhance milk quality (Oliver *et al.*, 2001). Various researchers have shown that pre milking teat disinfection is beneficial (Rasmussen *et al.*, 1991, Oliver *et al.*, 1993, Magnusson *et al.*, 2006 and Hillerton *et al.*, 2007). However, other workers have shown its limited effect (Grindal and Bramley 1989, Singh 2015 and Rowe *et al.*, 2018). The present study included assessing the advantage of pre and post milking teat disinfection over post milking teat disinfection alone. In our study, a lactic acid based product was used as pre dip and povidone iodine as post dip. Lactic acid based components have been studied as teat disinfectants (both pre and post dip) having wide spectrum of antibacterial activity against most of the mastitis pathogens (Boddie and Nickerson 1992 and Hillerton *et al.*, 2007).

We observed a reduction in SCC of post milking treatment group as compared to control group. Similarly, Singh (2015) also observed a significant ($P < 0.05$) decrease in SCC values post treatment using pre and post milking teat dip. The decline in SCC values could be attributed to prevention of IMI.

Williamson and Hulbert (2013) also observed similar results after the use of post-milking teat dipping which resulted in a lower incidence of new IMI, clinical mastitis and lower individual cow SCC than no post-milking teat dipping. No additional benefits of pre-milking disinfection compared with post milking teat disinfection alone were found. They opined that there is possibility of steady increase in the amount of cross-contamination between cows, and a higher incidence of new IMI, if Post milking teat disinfection is discontinued in a whole herd situation.

Our study reported higher percentage of new IMI by *Corynebacterium* spp. in control

group in comparison to other treated groups. This indicates the significance of teat dipping after the milking as evidenced by the results. *Corynebacterium* spp. are considered highly contagious and have been suggested as an indicator of inadequate teat disinfection efficacy. Herd in which the teat disinfection practice was known to be of a high standard, only 5% of disinfected quarters developed new IMI due to *Corynebacterium* spp compared with 53% of quarters across the commercial herds (Pankey, 1989). These data indicate that the overall efficacy of teat disinfection among the commercial herds may have been compromised by the time demands of the commercial operations; however, the reductions in new IMI achieved suggest that it was still effective in reducing the transfer of contagious pathogens.

However, Williamson and Hulbert (2013) observed that the disinfectant application reduced the rate of new IMI by around 60% for major pathogens, with the greatest impacts evident for *Staph. aureus* (56% reduction) and *Strep. uberis* (59% reduction). This is an expected result for *Staph. aureus* that is a contagious pathogen, but is contrary to common perception that post-milking teat disinfection does not effectively control environmental pathogens such as *Strep. uberis* (Radostits *et al.*, 1994).

The reduction in prevalence of SCM and new IMI in the present study substantiated the beneficial use of teat dipping, however, the variation between pre and post teat dip was not significant. Williamson and Hulbert (2013) also concluded that disinfecting teats prior to teat cup attachment did not improve control of early-season environmental mastitis in New Zealand herds when used in conjunction with post milking teat disinfection. Though the use of pre-milking teat disinfection alone was effective in

reducing bacterial load. Gleeson *et al.*, (2018) reported that the routine application of pre-milking teat disinfectant in pasture-grazed herds is unlikely to be of benefit because he found that pre-milking teat disinfection did not reduce the SCC and new IMI rates over the trial length, which was for complete lactation.

In conclusion, the results of the present study carried out under natural exposure conditions indicate that post milking teat disinfection was effective in curtailing new IMI and SCC. The results also indicate that the addition of pre milking teat disinfection provided no additional benefit. However, to conclude this, a large sample size study is required.

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