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Effect of dry period therapy on prevalence of mastitis in buffaloes in Haryana

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ABSTRACT

The study attempts to compute the effect of dry period therapy on occurrence of mastitis in buffaloes with the help of control (164 buffaloes) and treatment (330 buffaloes) groups at different stages as per disease diagnostic protocol. The data pertains to the year 2018–19. The study concludes overall prevalence of SCM at dry-off was 83.81% and at 5 days postpartum was 38.87% at animal level. In treatment group, prevalence of SCM was 89.70% and 28.48% at dry-off and 5 days postpartum respectively whereas 73.17% and 62.8% in control group. The most prevalent udder pathogens isolated from composite milk samples at dry off were coagulase-negative *Staphylococcus*, representing 41.29% of all recovered isolates followed by other gram positive, *Streptococcus* spp. Overall, 63.02% of buffaloes with IMI were cured during the dry period. Buffaloes receiving dry period therapy were 3.85 times more likely to cure.

Keywords: Bacteriological cure, Buffalo, Dry period, Mastitis, Prevalence, Therapy

Mastitis, an inflammatory reaction of the udder is characterized by physical and chemical changes in the milk and pathological changes in the glandular tissue. Mastitis-related economic losses include decreased milk yield, reduced reproductive performance, dairy animal replacement cost and increased treatment expenses (Moore *et al.* 1991, Seegers *et al.* 2003, Halasa *et al.* 2007). Bovine mastitis is associated with a wide spectrum of pathogenic agents but Staphylococci, Streptococci and coliform group are the most prevalent bacteria in bovine mastitis globally (Watts 1988, Bradley 2002). Dry period therapy is defined as the infusion of long acting intramammary antimicrobial formulation in all quarters of animal at dry off, is suggested by the National Mastitis Council (NMC 1999) as a part of mastitis control programme. Dry period therapy is aimed at, both eliminating existing intramammary infection (IMI) carried from previous lactation into dry period and preventing occurrence of new intramammary infections (NIMI) that are acquired during nonlactating period (Browning *et al*. 1990, Bradley and Green 2001). This approach has reduced milk production, decline in reproductive performance, and higher risk for death or culling (Dohoo and Wayne Martin 1984, Millan-Suazo *et al.* 1989, Barker *et al.* 1998, Schrick *et al.* 2001, Bradley *et al.* 2010).

In general, the dry period in buffalo is of about 153 days and 318 days lactation length with a production of about 2,335 kg per lactation (Singh and Pandey 2013). The IMI that are carried from the preceding lactation into the dry period and NIMI that emerge during the dry period can significantly contribute to the occurrence of mastitis in subsequent lactation (Green *et al*. 2007). Therefore, nonlactating period is important for understanding mastitis epidemiology and improving milk quality (Dingwell *et al*. 2003, Green *et al.* 2005). The NIMI acquired during dry period often remain subclinical, but flare up as a clinical mastitis in early lactation phase (first 3 months) or until the fifth month of the subsequent lactation (Green *et al.* 2002, Pantoja *et al*. 2009b, Blowey and Edmondson 2010). There is an evidence that 60% of clinical mastitis in early lactation period (first four weeks after calving) originate from new IMI developed during the dry period (Green *et al.* 2002). The identification of IMI requires the bacteriological culture (BC) of mastitis pathogens from milk samples, a gold standard for diagnosis of IMI. However, somatic cell count (SCC) an indicator of IMI has been used extensively as a screening test to monitor the udder health status in dairy herds (Dohoo and Leslie 1991, Ruegg 2003). In this backdrop, it becomes essential to evaluate the effect of dry period therapy in buffaloes with intramammary antibiotic Ceftiofur hydrochloride (500 mg) formulation on the prevalence of SCM, bacteriological cure postpartum and early lactation CM in subsequent lactation.

MATERIALS AND METHODS

The data pertains to the year 2018–19 with the participation of organized buffalo herds from Haryana and villages covered under Farmer FIRST Project (FFP). Microbiological identification is confirmed when isolation and identification of bacteria were carried out on the basis of standard procedures (Quinn *et al.* 1994). A loopful of each milk sample was streaked on 5% defibrinated blood

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agar and MacConkey's agar plates (Himedia) and incubated at 37°C for 24–48 h. Plates with the growth of three or more colony types were considered contaminated and discarded. Bacteriological cure is confirmed in the treated quarter when an udder pathogen present in pre-treatment milk sample before drying off from originally infected quarter was not present in any of the post-treatment milk samples (Oliver and Mitchell 1983). The criterion of the presence of new intramammary infection is when a quarter from which no udder pathogen was detected in pre-treatment milk sample before dry off but growth of a bacterium species was found in post-treatment milk sample.

To evaluate the effect of DPT on approaching dry-off, buffaloes (494) of participating farmers of villages covered under Farmer FIRST Project (FFP) were enrolled. The buffaloes approaching end of lactation at drying off were randomized to treatment group (T1, 330) and untreated control group (T2, 164). All the four quarters of treatment group were treated at day 0 immediately following the last milking at dry-off with IMI of Ceftiofur hydrochloride formulation. The buffaloes in control group did not receive treatment on the day of dry-off. Composite milk samples at animal level from 494 buffaloes were taken aseptically at day 0 immediately preceding drying off and after 5-day post-partum for somatic cell count (SCC) and bacteriological examination. Milk samples were kept in ice-box in laboratory. The California mastitis test (CMT) count was conducted for screening of subclinical mastitis. The CMT indicates presence of subclinical mastitis when somatic cells /ml is more than 200,000 (NMC 1999). The information was recorded after the intervention for evaluating occurrence of clinical mastitis post-calving.

RESULTS AND DISCUSSION

Prevalence of subclinical mastitis and udder pathogens at dry off: Based on the result of microbiological examination (Table 1), the overall prevalence of subclinical mastitis in buffalo (at animal level) was 83.81% at dry off as was 38.87% at 5 days postpartum. Most prevalent causative agent (on animal basis) at dry off was coagulase-negative *Staphylococcus,* representing 41.09% of all recovered isolates followed by other gram positive (17.21%), mixed infection (4.45%), *Escherichia coli* & *Streptococcus* spp (each 4.05%), other coliform (3.85%), *Staphylococcus aureus* and *Pseudomonas* spp (each 2.83%) and *Micrococcus* spp (2.63%). Gram-positive organisms, gram-negatives and mixed infections represented 80.92%, 13.77% and 5.31% of all udder pathogens isolated, respectively.

Table 1. Overall distribution of bacterial species and prevalence of subclinical mastitis at drying off and 5 days postpartum in buffaloes

Bacteriological diagnosis	Before drying off		5 days Postpartum	
	No.	Prevalence (%)	No.	Prevalence (%)
Staphylococcus aureus	14	2.83	10	2.02
CNS	203	41.09	79	15.99
Streptococcus	20	4.05	11	2.23
Micrococcus	13	2.63	5	1.01
Other gram positive	85	17.21	44	8.91
E. coli	20	4.05	10	2.02
Other coliforms	19	3.85	9	1.82
Other gram negative	4	0.81	10	2.02
Pseudomonas	14	2.83	9	1.82
Mixed	22	4.45	5	1.01
No growth	80	16.19	302	61.13
Total	494	100.00	494	100.00

Control group: Before drying off, 26.83% (44) of 164 composite milk samples from control group were culture negative and 73.17% (120) were culture positive depicting subclinical mastitis prevalence rate of 73.17% (Table 2). Of the 164 samples at dry off, 68 (41.46%) isolates belonged to coagulase negative Staphylococcus (CNS) spp. followed by 24 (14.63%) isolates of other gram-positive bacteria. At 5 days postpartum, 62.80% (103) of 164 milk samples were bacteriologically positive.

Table 2. Distribution of bacterila spices and prevalence of subclinical mastitis from composite sample at dry off and 5 days postpartum in control group

Bacteriological diagnosis	Before drying off		5 days Postpartum	
	No.	Prevalence (%)	No.	Prevalence (%)
Staphylococcus aureus	6	3.66	6	3.66
CNS	68	41.46	59	35.98
Streptococcus	5	3.05	3	1.83
Micrococcus	0	0.00	1	0.61
Other gram positive	24	14.63	19	11.59
E. coli	4	2.44	5	3.05
Other coliforms	4	2.44	3	1.83
Other gram negative	0	0.00	3	1.83
Pseudomonas	4	2.44	4	2.44
Mixed	5	3.05	0	0.00
No growth	44	26.83	61	37.20
Total	164	100.00	164	100.00

Treatment group: A total of 296 bacterial isolates were recovered from 330 composite milk samples of buffaloes of treatment group approaching dry off. Before drying off, 10.30% (34) of 330 composite milk samples from treatment group were bacteriologically negative and 89.70% (296) were bacteriologically positive (Table 3). At 5 days postpartum, 28.48% (94) of 330 milk samples were bacteriologically positive. Of the 330 samples at dry off, 135 (40.91%) isolates belonged to coagulase negative Staphylococcus (CNS) spp. followed by 61 (18.48%) isolates of other gram-positive bacteria. At 5 days postpartum, 6.97% (23) infections with other gram positive bacteria and coagulase negative staphylococci (CNS) each were observed.

Bacteriological cure rate: Overall, 63.28% of buffaloes with intramammary infections (IMI) were cured during the dry period. Of 115 composite udder milk samples before

Table 3. Distribution of bacterial species and prevalence of subclinical mastitis at drying off and 5 days postpartum in treatment group

Bacteriological diagnosis	Before drying off		5 days Postpartum	
	No.	Prevalence (%)	No.	Prevalence (%)
Staphylococcus aureus	7	2.12	4	1.21
CNS	135	40.91	23	6.97
Streptococcus	16	4.85	9	2.73
Micrococcus	13	3.94	4	1.21
Other gram positive	61	18.48	23	6.97
E. coli	17	5.15	5	1.52
Other coliforms	15	4.55	7	2.12
Other gram negative	4	1.21	6	1.82
Pseudomonas	10	3.03	5	1.52
Mixed	18	5.45	8	2.42
No growth	34	10.30	236	71.52
Total	330	100.00	330	100.00

drying off in the control group (excluding new IMI and no growth culture samples), 90 samples continued positive with the same mastitis bacteria postpartum, yielding a bacteriological cure rate of 21.74% (Table 4). Apparent bacteriological cure rate of IMI was 83.26% at animal level in the treatment group receiving dry period therapy (Table 5). Out of 239 composite udder milk samples before drying off from treatment group, 40 samples continued positive with the same mastitis bacteria postpartum. Buffaloes receiving ceftiofur dry period therapy were 3.83 times more likely to cure than control group. In the control group, where dry period therapy was not used, 78.26% of existing intramammary infections (115) carried from previous lactation into dry period were still present 5 days postpartum whereas only 16.74% were present after calving in the treatment group (239).

New intramammary infections and clinical mastitis postpartum: Overall, NIMI rate was 14.37% (494) 5 days postpartum and most new IMI were of CNS followed by other gram positive. Approximately, 7.93% of buffaloes developed NIMI during the dry period in control group compared to 17.58% NIMI postpartum in treatment group (data not shown). Early lactation clinical mastitis (CM) cases postpartum were lower for treatment group (2.73%) compared to control group (7.31%).

Overall prevalence of IMIs at animal level during dry off (83.81%) and 5 days postpartum (38.87%) in this study is higher than other studies (Oliver and Mitchell 1983, Pantoja *et al.* 2009a, Petzer *et al.* 2009). A possible reason for higher prevalence of SCM at dry off is the enrolment of buffaloes with mastitis history in previous lactation. The most prevalent causative agent (on animal basis) was CNS followed by other gram positive at the two sampling times which corroborates with previous reported studies on cow (Oliver and Mitchell 1983, Godden *et al.* 2003). The control group had higher rates of IMI postpartum, 62.80% in comparison to treatment group, 28.48% which is supported by studies conducted (Browning *et al.* 1990, Schukken *et al.* 1993, Berry and Hillerton 2002) in cow.

Overall, NIMI rate was 14.37% (494) during nonlactating period and predominantly caused by CNS (18.35%), other gram positive (16.90%), micrococcus (14.08%) and gram negative bacteria other than enterobacteriacea (12.68%). Buffaloes of treatment group

Table 4. The overall and pathogen specific bacteriological cure rate* from composite pre-treatment milk samples before drying off and postpartum excluding new IMI and NG samples

Bacteriological diagnosis	At drying off number	5 days Postpartum	Cured number	Cured % (5days)
Staphylococcus aureus	6	6	0	0.00
CNS	65	54	11	16.92
Streptococcus	5	2	3	60.00
Micrococcus	0	0	0	0.00
other Gram positive	24	17	7	29.17
E. coli	4	4	0	0.00
other coliforms	3	3	0	0.00
Other gram negative	0	0	0	0.00
Pseudomonas	4	4	0	0.00
Mixed	4	0	4	100.00
Total	115	90	25	21.74
Staphylococcus aureus	4	0	4	100.00
CNS	118	15	103	87.29
Streptococcus	9	0	9	100.00
Micrococcus	10	0	10	100.00
other Gram positive	52	13	39	75.00
E. coli	13	4	9	69.23
other coliforms	9	3	6	66.67
Other gram negative	4	0	4	100.00
Pseudomonas	8	2	6	75.00
Mixed	12	3	9	75.00
Total	239	40	199	83.26

developed more NIMI during the dry period compared to control group. This is dissimilar to previous studies on NIMI in 8–25% quarters during the dry period (Godden *et al.* 2003, Singh and Pandey, 2013). It needs to be emphasized that previous studies reported were quarter wise on cows whereas present study includes composite milk sample from buffalo at animal level.

There is nothing more disappointing than having an early lactation clinical mastitis when a buffalo achieves peak milk production. Dairy producers have impression that nutrition, housing and management should be minimized during dry period (resting phase) as buffalo is not milking. In fact, the dry period is a critical time in the lactation cycle which directly influences milk production, udder health and reproductive performance in the subsequent lactation. Bradley and Green (1998) highlighted that 91.6% of cows were spontaneously self-cured from dry period infection with the same pathogen during subsequent lactation and only 8.4% of quarters infected during dry period developed clinical mastitis. During the dry period, 8–12% of formerly healthy quarters establish IMI which can be detected postpartum if dry animal therapy is not given (Dingwell *et al.* 2003). This study shows that early lactation clinical mastitis (CM) cases are reduced in treatment group compared to control group which is further supported by the previous studies conducted (Gundelach *et al.* 2011, Berry and Hillerton 2002)

Apparent bacteriological cure rate of IMI for buffaloes that received intramammary 500 mg ceftiofur as dry period therapy compared with the untreated control group was 83.26% and 21.74% respectively. This is in agreement with previous report by Arruda *et al.* (2013) who reported 88% cure rate with ceftiofur as dry period therapy in cows. Results of current study demonstrate that administration of intramammary ceftiofur formulation at drying-off is an efficacious therapy in treating existing sub-clinical intramammary infections at the time of drying off and also prevent occurrence of clinical mastitis postpartum especially in early lactation period.

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