

The effectiveness of an internal teat sealant in preventing new intra mammary infections in dairy cattle during dry period

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ABSTRACT

The aim of our study was to evaluate the effect of an internal teat sealant infused upon drying off on the incidence of new intra-mammary infections (IMI) during the dry period in dairy cattle. Due to the non-availability of the product on the Indian market, the product was prepared in a laboratory using bismuth subnitrate and liquid paraffin. A total of 64 quarters free from infection on culture were selected for the study at 60 days before the expected date of parturition. The quarters were randomly divided into two groups (group A and group B), with 32 quarters in each group. The group A quarters were infused with teat sealant at the time of drying off, and the quarters in group B were kept as the control and no treatment was provided to them. Milk samples from all the selected quarters were subjected to cultural examination at the time of drying off and 1-3 days post calving. The incidence of new IMI's between drying off and calving was significantly lower for group A quarters when compared to group B quarters (12.5% vs 34.4%). The incidence of new IMI's in group A and group B quarters was significantly lower for *Streptococcus uberis* (3.13% vs 18.75%) and *Streptococcus dysgalactiae* (3.13% vs 12.5%). The study concluded that infusion of teat sealant at drying off is helpful in lowering the incidence of new IMI's during the dry period.

Key words: dairy cattle; dry period; intra-mammary infections; teat sealant

Introduction

The epidemiology of intramammary bacterial infections is influenced by many risk factors: milk yield, udder hygiene, nutritional management, stage of lactation, and accordingly various therapeutic strategies have been devised to control the disease (COMPTON et al., 2007; DJURICIC et al., 2014; BENIĆ et al., 2018). The mammary gland is highly susceptible to new IMI's during the dry period (HOGAN et al., 1998; BRADLEY and GREEN,

2000; GREEN et al., 2002; KLECZKOWSKI et al., 2017). The practice of blanket dry cow antibiotic therapy treatment of all quarters with a long-acting antibiotic at dry off, has been successful in curing many existing subclinical infections, as well as offering short-term protection against new IMI's during the dry period (BROWNING et al., 1990; BRADLEY and GREEN, 2001). However, new IMI's may still occur if invading pathogens are not

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sensitive to the active ingredients or antibiotic is not continued at therapeutic levels throughout the entire dry period (SANFORD et al., 2006). The indiscriminate use of blanket dry cow therapy in farm animals is often associated with the development of antimicrobial resistance and dissemination (CHANG et al., 2008). These flaws of dry cow therapy were to some extent overcome by the introduction of internal teat sealants in the management of dry cows. Teat sealants are bismuth salt in a wax base that is infused into the teat canal at drying off (WOOLFORD et al., 1998). These form a barrier to block the entry of pathogenic micro-organisms into the mammary gland. The product, given its non-antibiotic nature, is preferred to conventional antibiotic dry cow therapies (HUXLEY et al., 2002; QUINN et al., 2004). Teat sealants have mostly been used in combination with antibiotic preparations (PETROVSKI et al., 2011; BATES and SALDIAS, 2018), and studies on the use of internal teat sealant in preventing IMI's during dry period are rare (HUXLEY et al., 2002; BONSAGLIA et al., 2017). The present study was planned to evaluate the effect of internal teat sealant prepared in a laboratory using bismuth subnitrate and liquid paraffin, without antibiotic preparation, in preventing new IMI's during the dry period.

Materials and methods

Ethical approval. The study was conducted keeping all ethical and animal welfare issues under consideration and was approved by the Institutional Animal Ethics Committee, registered by CPCSEA, under registration number P62/ac/04/cpcsea dated: 16/12/2004.

Selection of animals. The study was conducted at an organized dairy farm that manages a total of 900 crossbred dairy cattle of Frieswal breed (Cross of Holstein Friesian and Sahiwal breeds). The animals selected for study were stall fed and machine milked. Before recruitment, key cow details, including parity, estimated milk yield, treatment history and estimated calving date were collected from the farm records. A total of 25 animals were selected and 64 quarters that showed no infection upon cultural examination of milk at the time of drying were enrolled for the study. The quarters were randomly divided into two groups

(group A and group B), with 32 quarters in each treatment group. Group A was infused with teat sealant product at the time of drying off and group B quarters were not given any treatment and served as the control.

Preparation of Teat sealant product. Due to the non-availability of commercial teat sealant products on the Indian market, the product was prepared in the Central Laboratory of the Medicine Division, F.V.Sc & A.H., R.S.Pura, Jammu, India. The product was prepared using a commercial preparation of bismuth subnitrate (Bismuth Subnitrate by HIMEDIA Labs) and liquid paraffin (Paraffin Liquid by HIMEDIA Labs). The product was prepared in the form of paste, containing 65% of bismuth subnitrate. Sterilization of the paste was carried out by autoclaving the product at a temperature of 121 °C and 15 lb pressure for 15 minutes. We tested if the product would be retained in the teat canal by infusing two concentrations (0.5 g and 2 g) of teat sealant into the teats of dry animals and a radiograph was taken 60 days after infusion



Fig. 1. Image showing retained teat sealant (0.5 g) 60 days after infusion



Fig. 2. Image showing retained teat sealant (2 g) 60 days after infusion

(Fig. 1 and 2). The product could be seen in the radiograph even 60 days after infusion.

Product administration. After complete milking of the selected quarters, the quarters were washed with antiseptic solution and allowed to dry. The teat ends were then disinfected with 70% ethyl alcohol, and the teat sealant product was infused (4 grams in each teat) with the help of a sterilized teat siphon.

Sampling. Milk samples were collected from the quarters for cultural examination at the time of drying off and 1-3 days post calving to identify new IMI's that had gained entry during the dry period.

Bacteriology. Milk samples collected in sterile glass vials were streaked primarily onto ovine blood agar plates with a sterile platinum loop under a strict sterile environment. The inoculated plates were incubated at 37 °C for 24 hours. The causative organisms were identified initially by colony characteristics on blood agar, Gram staining and biochemical characteristics for the presence of catalase and cytochrome C oxidase. Further, the organisms grown on the blood agar plates were streaked onto selective media e.g. Mannitol salt agar (for *Staphylococcus spp.*), Edward's media (for Streptococci), MacConkey agar (for Coliforms). The CAMP test and Aesculin hydrolysis on Edwards media were used for species identification of *Streptococcus spp.* (QUINN et al., 2004).

Somatic cell count. Total somatic cell count was done as per the modified technique of leukocyte count described by SCHLAM et al., (1971). After mixing the milk sample thoroughly (cream etc. dispersed uniformly), 0.01 mL (10µL) of milk was withdrawn by micropipette and spread evenly on a clean, grease free glass slide in 1 cm² area. The smear was allowed to dry in the air at room temperature. Staining of the dried milk smear was done by placing the slides for 2 minute in a covered couplin jar with Newman's Lampert stain. The excess stain was drained off by keeping the slides vertically on absorbent paper and air dried. The slides were then rinsed with tap water, drained and dried rapidly in the air. The cells were calculated in a total of 30 fields on the marked area under an oil immersion microscope (100 x). The average number of cells per square cm area was calculated.

For counting of cells per ml of milk the average number of cells per field was multiplied by the microscopic factor (1,10,097) for the microscope used in the present study. The somatic cell count is usually represented as the somatic cell score (SCS), which is calculated as $SCS = \log_2 (SCC/100\ 000) + 3$.

Statistical analysis. Data analysis was done by simple t-test and chi square analysis and odds ratio, using SPSS (Statistical Package for Social Sciences Software version 16.0- SPSS Inc.)

Results

There were no significant differences observed between the treatment groups in any of the key indices such as parity, milk yield before drying off, dry period length and somatic cell score at drying

Table 1. Key indices of animals selected for the study (Mean± S.E.M.)

Parameters	Group A	Group B
Number of quarters	32	32
Parity at dry off	3 ± 2.13	2.88 ± 2.35
Milk yield before dry off (kg/day)	11.0 ± 3.02	10.08 ± 2.95
Dry period (days)	82.5 ± 18.7	85.0 ± 19.2
Linear score at drying off	3.32 ± 1.08	3.34 ± 1.10

Table 2. Incidence of new intramammary infections acquired during the dry period

Parameters	Group A		Group B	
	n	%	n	%
Total no. of quarters	32		32	
Quarters infected	4	12.5	11	34.38
Microorganisms isolated	n	%	n	%
<i>Staphylococcus aureus</i>	2	6.25	4	12.5
CNS	2	6.25	3	9.39
<i>Streptococcus dysgalactiae</i>	1	3.13	4	12.5
<i>Streptococcus uberis</i>	1	3.13	4	18.75
Total no. of microorganisms	6		11	

Table 3. Comparative mean of Somatic cell score for the two groups after calving

	Group A	Group B
Somatic cell score	2.81 ± 0.21 ^a	3.41 ± 0.19 ^b

off, indicating that the randomization procedure was successful (Table 1).

Incidence of new IMI's acquired during the drying period. The overall incidence of quarters infected with new IMI's from drying off and calving recorded for group A and group B was 12.5% and 34.38%, respectively. The incidence was significantly lower for group A quarters, treated with teat sealant compared to group B quarters, that did not receive any treatment ($P = 0.038$, $\chi^2 = 4.27$). The incidence of major and minor pathogens combined was significant between the two group of quarters ($P = 0.029$, $\chi^2 = 4.65$). The incidence of combined major and minor pathogens was found to be 12.5% for teat sealant treated quarters (group A) and 43.75% for control group quarters (group B) (Table 2).

The incidence of new intra mammary infections by individual micro-organisms for the teat sealant treated quarters (group A) and control group quarters (group B) was found to be: *Staphylococcus aureus* 6.25% vs 12.5%; *Coagulase negative staphylococci* (CNS) 6.25% vs 9.39; *Streptococcus uberis* 3.13 vs 18.75% and *Streptococcus dysgalactiae* 3.13 vs 12.5 (Table 2). The difference in incidence was non-significant for *Staphylococcus aureus* ($P = 0.336$, $\chi^2 = 0.736$), CNS ($P = 0.500$, $\chi^2 = 0.217$) and *Streptococcus dysgalactiae* ($P = 0.500$, $\chi^2 = 1.01$). However, the incidence of new IMI's was found to be significant for *Streptococcus uberis* ($P = 0.049$, $\chi^2 = 4.01$).

Somatic cell score after calving. The mean linear scores of group A and group B quarters were recorded as 2.81 ± 0.21 and 3.41 ± 0.19 , respectively. There was a significant difference between the mean linear scores of group A and group B quarters (Table 3).

Discussion

The present study demonstrates the efficacy of an internal bismuth subnitrate teat sealant in

protecting quarters against new IMI's during the dry period under Indian field conditions. The use of teat sealant and blanket dry cow therapy is common in European countries (WOOLFORD et al., 1998), however, in India the practice is rarely used. Due to the unavailability of any teat sealant preparation on the Indian market, the product was manually prepared in a laboratory. The ability of the product to be retained in the teat cistern during the dry period was confirmed by X-ray of the teat cistern. The study demonstrated that infusion of internal teat sealant in quarters at drying off significantly reduced the incidence of newly infected quarters during the dry period (group A vs group B: 12.50% vs 34.38%). The overall incidence of new IMI's by both major and minor pathogens was significantly lower for teat sealant treated quarters compared to quarters left untreated. However, between the individual organisms the effect was most marked in the case of *Streptococcus uberis*. Our findings are in concurrence with other studies. HUXLEY et al. (2002) reported that cows treated with teat sealant alone acquired significantly lower IMIs during the dry period. The effect was marked in the case of *Streptococcus uberis* and all *Enterobacteriaceae*. PETROVSKI et al. (2011) reported the protective effect of internal teat sealant combined with 0.5% chlorhexidine against experimentally induced infection by *Streptococcus uberis*. The effect on the incidence of IMI's due to *Streptococcus* spp. could be attributed to their environmental nature, and the organism was isolated earlier in mastitis cases in the Jammu region of India (BHAT et al., 2017).

The protective effect of teat sealant against IMI's has also been reported by other authors (BONSAGLIA et al., 2017; BATES and SALDIAS, 2018; BERRY and HILLERTON, 2002; BRADLEY et al., 2010).

The increased susceptibility to IMI's during the dry period has been attributed to the widening of the streak canal, due to the increase in intramammary pressure following the drying of the udder (GREEN et al., 2002). However, some authors have reported the complete absence of the keratin plug, which closes the teat canal, as being responsible for IMI's during the dry period (WILLIAMSON et al., 1995). The keratin plug is the protective

barrier of the mammary gland against IMI's during the dry period, and reports suggest that 50% and 5% of teats had incomplete keratin plugs present after 7 and 50 days of the dry period, respectively (WILLIAMSON et al.,1995). The teat sealant acts like a functional keratin plug, and prevents the introduction of micro-organisms into the mammary gland. It has been reported that more than 50% of new infections may persist into the next lactation if not eradicated by appropriate treatment (BERRY and HILLERTON, 2002). Likewise, infections acquired during the dry period can cause clinical mastitis during the following lactation (SMITH et al., 1985; BRADLEY and GREEN, 1999; CVETNIĆ et al., 2016).

Conclusion

The present study concluded that infusion of an internal teat sealant preparation of a bismuth subnitrate and liquid paraffin, at the time of drying, can help in reducing the incidence of new intramammary infections during the dry period. In view of these findings, the use of the internal teat sealant during the dry period can be considered as an additional management tool for control of mastitis.

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SAŽETAK

Cilj ovog istraživanja bio je procijeniti učinak zatvaranja sisnih kanala na incidenciju infekcija vimena (IMI) mliječnih krava za vrijeme suhostaja. Zbog nedostupnosti komercijalnih preparata na indijskom tržištu, proizvod za zatvaranje sisnih kanala je pripremljen u laboratoriju upotrebom bizmut-subnitrata i tekućeg parafina. Za istraživanje su odabrane 64 četvrti vimena u kojima bakteriološkom kulturom nije bilo utvrđene infekcije 60 dana prije očekivanog teljenja. Četvrti su nasumično podijeljene u dvije skupine (skupina A i skupina B) sa po 32 četvrti u svakoj skupini. U skupini A, tijekom suhostaja, kravama su zatvoreni sisni kanali, dok u kontrolnoj skupini B nije proveden nikakav tretman. Uzorci mlijeka iz svih skupina podvrgnuti su testiranju bakteriološkom kulturom u vrijeme suhostaja i 1 – 3 dana poslije teljenja. Incidencija intramamarnih infekcija između suhostaja i teljenja bila je znakovito niža u skupini A u usporedbi sa skupinom B (12,5 % prema 34,4 %). Incidencija novih intramamarnih infekcija u skupinama A i B bila je znakovito niža za *Streptococcus uberis* (3,13 % prema 18,75 %) i *Streptococcus dysgalactiae* (3,13 % prema 12,5 %). Rezultati istraživanja pokazuju da zatvaranje sisnih kanala u suhostaju pomaže u smanjivanju incidencije novih intramamarnih infekcija.

Cljučne riječi: mliječna goveda; suhostaj; intramamarne infekcije; zatvaranje sisnih kanala
