New infection rate of bovine mammary quarters after application of a bismuth subnitrate-free internal teat sealant at dry-off

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Abstract

Internal teat sealants (ITS) are applied to prevent new intrammary infection during the dry period in dairy cattle. The common ITS products with confirmed efficacy contain the heavy metal salt bismuth subnitrate. The aim of this field study was to determine the efficacy of a novel bismuth subnitrate-free ITS prototype (BSFITS) in reducing new infections in comparison to untreated quarters (control). Therefore 50 cows from two organic German farms were treated with the BSFITS in a split-udder design. Cows included in the trial were free of clinical mastitis in the previous lactation and had a somatic cell count < 200,000 cells/mL in the last three dairy herd improvement tests. Quarter milk samples were collected at dry-off, within 5 to 12 d in milk (DIM) and 7 d later. The new infection rate (NIR) did not differ between treated and untreated quarters (12.6%). The predominant new infection causing agents were coryneforms and coagulase-negative staphylococci. The probability of developing NI during the dry period was higher in quarters infected with minor pathogens at dry-off. Within the first 100 DIM no case of clinical mastitis was reported in treated and untreated quarters. The present study could not determine a positive effect of the BSFITS in reducing the NIR in comparison to untreated quarters. Future studies with novel ITS should be performed on farms with a high incidence of IMI caused by environmental pathogens and further research about the relationship of causing agents and the sealing effect is recommended.

Key words: Teat sealant, bismuth subnitrate-free, new infection, dry period, minor pathogens

Introduction

The bovine mammary gland is markedly susceptible to new intramammary infection **(IMI)** during the dry period [1]. In a study by Bradley et al. [2] 98% of the infections present after calving were new IMI during the dry period.

However, the risk of developing IMI changes during the dry period, as the gland has the highest susceptibility to infections in the first weeks after dry-off and near parturition [3,4]. In the intervening time when the udder has become fully involuted and a natural keratin plug has formed, which acts as a physical barrier in each teat, the mammary gland is most resistant to new IMI [4]. Although timely closure of the teat canal by a keratin plug is an important defence mechanism, natural closure may not occur in a very high percentage of teats. About 50% of teats do not close within the first week of the dry period and 23.4% remain open after 6 weeks. Open teats are 1.8 times more likely to develop new IMI [4].

The application of an internal teat sealant **(ITS)** provides the opportunity to simulate the natural keratin plug. Teats are sealed by injecting a stable substance into the teat canal orifice at dry-off. Meaney [5] showed that the infusion of an ITS containing 25% w/w bismuth subnitrate provides a significant protection against new IMI during the dry period. A more viscous reformulation (65% w/w bismuth subnitrate) is reported to be more effective, especially in maintaining a plug at the teat sinus [6]. In the following years several studies proved the effect of ITS consisting of bismuth subnitrate in reducing the incidence of new IMI [7–9]. In a meta-analysis including 12 studies ITS reduced the risk of IMI by 73% compared with untreated cows [10]. ITS represent an antibiotic free alternative for dry cow treatment on organic farms [11]. However, bismuth subnitrate is a heavy metal salt and the use of ITS on organic farms is controversial for ecological and health reasons, as the calf often suckles the first milk after calving and may ingest teat sealer material (Notz, 2005, unpublished). Moreover, a link between residual ITS and the appearance of the black spot defect in cheese was found [12].

The aim of this study was to determine the efficacy of a bismuth subnitrate-free ITS **(BSFITS)** in preventing new IMI during the dry period.

Material and methods

Farms and animals: The field study was carried out between July and December 2014 on two organic dairy farms located in Lower Saxony, Germany. The farms kept 71 and 147 lactating German Holstein dairy cows. Average annual yields were 6500 and 6540 kg per cow with a mean fat content of 4.17 ± 0.33% and a mean protein content of 3.39 ± 0.12%. The mean intercalving interval was 382 ± 20 d. Bulk tank somatic cell count at the beginning of the study was 250,000 and 394,000 cells/ml, respectively. Both farms performed a twice-a-day milking routine and seasonal abrupt dry-off.

Only clinically healthy cows with four functional quarters and teats without any injuries were selected for the study. Trial animals had to have a somatic cell count of < 200,000 cells/mL in the three previous

Table 1. Bacteriological distribution at dry-off and post partum (p.p.) and new infections during the dry period in untreated and treated quarters.

Bacteriological result	untreated		treated	
	dry-off, n (%)	p.p., n (%)	dry-off, n (%)	p.p., n (%)
No growth	52 (26.0)	66 (33.2)	48 (24.0)	48 (24.1)
Staphylococcus aureus	4 (2.0)	0 (0)	8 (4.0)	2 (1.0)
Streptococcus uberis	0 (0)	1 (0.5)	0 (0)	0 (0)
Coagulase-negative staphylococci	12 (6.0)	10 (5.0)	16 (8.0)	14 (7.0)
Coryneforms	24 (12.0)	22 (11.1)	21 (10.5)	34 (17.1)
Mixed infection	8 (4.0)	0 (0)	6 (3.0)	0 (0)
Other	0 (0)	1 (0.5)	1 (0.5)	1 (0.5)
Total	100 (50.0)	100 (50.3)	100 (50.0)	99 (49.7)
New infections	25 (12.6*)		25 (12.6*)	

*Percentage of new infections in untreated and treated quarters.

dairy herd improvement tests and no case of clinical mastitis in the present lactation.

Treatment: To detect a difference in the new infection rate **(NIR)** between treated quarters (assuming a NIR of 5%) and an untreated control (NIR of 17%), 82 quarters per treatment group were required (α=0.05; β=0.2; one-sided test). The prototype K2041/1.23 of the tested BSFITS formulation contains titanium dioxide instead of bismuth subnitrate in an oily base. Titanium dioxide is a white pigment used in food without any restriction regarding the upper limit of the used quantity. The prototype was packed in long nozzle plastic syringes for infusion through the teat canal. Dry-off treatment was performed using a split-udder design. Trial animals were randomly allocated to two treatment schemes using an allocation list: Front right-side and rear left-side quarters treated or front left-side and rear right-side quarters treated. The opposite quarters were left untreated as control, respectively. At dry-off, all four teat ends were aseptically prepared. The BSFITS was applied by the first and last author using hygienic procedures. Then, all four teats were disinfected.

Sampling and laboratory procedures: Quarter foremilk samples were aseptically collected at three points in time for cyto-bacteriological analysis: **(DO)** At dry-off, **(C1)** within 5 to 12 d in milk **(DIM)** and **(C2)** 7 d later. The milk samples were transferred refrigerated to the microbiological laboratory at the University of Applied Sciences, Hannover. Sampling and culturing were performed according to the National Mastitis Council recommendations (NMC, 1999) as cited by the GVA [13].

Definitions: The two most numerous grown colony types on an esculin blood agar plate were differentiated. A sample was bacteriological positive if ≥ 500 colony forming units **(cfu)**/mL of the same bacterial species were cultured. The growth of contagious mastitis pathogens like Staphylococcus **(S.)** aureus, Group B and C streptococci and Trueperella pygonenes were assessed if ≥ 100 cfu/mL were cultured. IMI after calving was assessed if both samples C1 and C2 were positive. Quarters with only one bacteriologically positive sample or different bacterial species in C1 and C2 were termed "non-infected". Quarters were termed "newly infected" if they presented positive results for a pathogen that was not isolated at dry-off. Animals were examined by the farm staff for clinical mastitis until 100 DIM.

Statistical analysis: Data were collected and analysed using Excel 2010 (Microsoft Corporation) and SPSS (SPSS 22.0, Chicago, USA). The statistical unit was the udder quarter. The untreated opposite quarters served as a paired control. The dependent variable New Infection **(NI)** was analysed using a backward, stepwise mixed model logistic regression analysis. For the regression model, the linear predictor was given by

$$\text{Logit(NI)} = \text{Parity} + \text{Treatment} + \text{Herd(random)} + e$$

where "parity" (1 and 2+) and "treatment" (internal teat sealant, no treatment) were considered as fixed effects, "herd" was included as a random effect and "e" was a binomial error term. For all outcome variables, treatment was compared with the control group using significance tests with a 5% significance level (P value). The homogeneity of treatment groups was given due to the paired study design.

Results

A total number of n = 200 quarters in 50 cows was used for the analysis; in one quarter the bacteriological result and the occurrence of NI could not be assessed, as it was treated with antibiotics before C1. In total 100 quarters were treated. Immediately after the application and after calving, no reactions to the BSFITS or changes at the treated teats were detected. No findings of BSFITS material in the foremilk after calving were reported by the farm staff. Table 1 shows the bacteriological results. The NIR did not differ between treated and untreated quarters: 12.6% (25/99 after BSFITS and 25/100 after no treatment). Of the new infections in treated and untreated quarters, 72% were caused by coryneforms, 22% by coagulase-negative staphylococci **(CNS)** and 2% by coliforms, Streptococcus **(Sc.)** uberis and yeasts, respectively. The cure rate of quarters infected with coryneforms at dry-off was 60% (27/45) and differed between the untreated and the sealed group (79% and 38%, respectively; $P < 0.001$). The probability of developing NI was higher in quarters infected with coryneforms or minor pathogens at dry-off (odds ratio, 5.122; 95 % CI 1.919 to 13.670 and 3.495, 95 % CI 1.796 to 6.802 respectively; $P < 0.01$). Within the first 100 DIM no case of clinical mastitis was reported. The logistic regression proved that there was no association between treatment with the BSFITS and the occurrence of NI (odds ratio, 1.015; 95% CI, 0.524 to 1.966; Table 2). The other factors (parity, herd) were not significant.

Discussion

The dry period is an important time with respect to the udder health management and the increased rate of new IMI [4]. The application of an ITS is one possibility to support the udder health by preventing

Table 2. Final mixed logistic regression model used to assess the efficacy of the treatment with the bismuth subnitrate-free internal teat sealant in reducing new infections during the dry period.

Effect	Estimate	SE	t Value	P Value	Odds Ratio	95% CI
Intercept	1.133	0.585	1.937	0.054	3.106	0.980-9.846
Treatment, untreated vs. treated	0.015	0.335	0.044	0.965	1.015	0.524-1.966
Parity, 1 or 2+	- 0.302	0.351	- 0.861	0.390	0.739	0.370-1.477

NI. The common ITS products contain bismuth subnitrate. The present trial intended to determine the efficacy of a bismuth subnitrate-free prototype in reducing NI under field conditions.

All animals included in the study were free of clinical mastitis in the previous lactation. To ensure that cows were free of mastitis at dry-off, the previous three dairy herd improvement tests had to be < 200,000 cells/ml. Similar thresholds were used in earlier trials investigating ITS [7,8]. The influence of animal-related confounders was minimized by matching case quarters with control quarters within the same cow. This method was applied using a split-udder design [9,14]. In this design interdependence of quarters is not eliminated [15]. However, as the frequency of IMI at dry-off did not differ between treated and untreated quarters (P = 0.58), this issue appears negligible in this trial. Allocating animals to treatment schemes randomly avoided introducing a bias related to differences in infection status between quarter positions [14].

Generally, the studies dealing with ITS containing bismuth subnitrate found that sealed quarters have a significant lower NIR than untreated quarters. Several authors calculated NIR between 2.5 and 3.5% for sealing and between 10.5 and 32.0% for no treatment [5,6,8,9]. With the same NIR of 12.6% for sealed and untreated quarters in the present study, a positive effect of BSFITS could not be determined.

However, the pathogens causing new IMI in this trial differ from the reported ones. Minor pathogens (coryneforms and CNS) were the most prominent NI agents. In two studies Sc. uberis was the predominant pathogen causing NI and for infections caused by CNS no significant difference between the sealed and untreated group could be found [6,8]. At Huxley et al. [7] there was no significant difference between the treatment groups for all minor pathogens. As in the present trial the efficacy of BSFITS is calculated on the basis of new IMI caused mainly by minor pathogens, a comparison of the NIR with the other studies is limited. In the study by Krömker at al. [9] CNS besides environmental pathogens were the most prominent new infection agents and ITS reduced the incidence of CNS infections by 50%. In the current trial new IMI caused by CNS occurred 5 times in sealed quarters and 6 times in untreated quarters (P = 0.83). According to Oliver and Mitchell [1] CNS are the most frequently encountered isolates during the dry period. IMI caused by CNS increase during the dry period with the highest number at parturition, but the frequency in late and early lactation is similar. This suggests a high cure rate within the first days of the new lactation, which may lead to an underestimation of the sealing effect in preventing new infections during the dry period. For coryneforms, the same study found a decreasing number of infections during the dry period and again the percentage of positive samples during early lactation was similar to frequencies prior to dry-off. Therefore most infections caused by coryneforms probably occur shortly after the calving and sealing teats at dry-off may not offer a significant protection. In conclusion, the reduction in new infections does not take place independently of the type of pathogen [9] and the naturally occurring bacterial flora at trial farms may influence the assessment of sealing products.

The dry period cure rates for coryneforms are reported to be high with 47.6 to 63.9% for self-cure and the same or a little bit higher for ITS, but both below the level of antibiotic dry cow therapy (> 90%) [7,8]. The self-cure rate of 60.0% in the present study is within the reported range. However, in treated quarters the reduction of infections by coryneforms was lower. The application of the BSFITS may have an influence on the teat canal flora.

Several studies dealt with the effects of coryneforms and CNS on other udder pathogens and came to different conclusions. It has been demonstrated that quarters infected with minor pathogens are significantly less likely to become infected with major pathogens [16,17]. Others, however, have demonstrated that minor pathogens-positive quarters are significantly more likely to become infected with environmental streptococci and coliforms [8,18]. In the present study quarters infected with coryneforms or minor pathogens at dry-off had a higher probability to develop NI during the dry period.

To provide proper protection against new IMI, the teat sealant material has to stay in the teat for the whole dry period. X-ray imaging of teats sealed with a teat sealant containing bismuth subnitrate showed that the material retained in the lower teat sinus up to 100 days of the dry period [6]. In several studies ITS was found in the foremilk of treated cows after calving [6,8]. As no farmer in the present study reported on flecks of BSFITS in the foremilk, one reason for the inefficacy of the sealant may be, that the material did not retain in the teat. During the initial few days of the dry period the secretion volume increases [19] leading to an intramammary pressure (IMP), that impacts on the ITS. The study results suggest that the titanium dioxide containing prototype has "weaker" properties to withstand increasing IMP at the beginning and the end of the dry period.

Another reason for the inefficacy of the BSFITS may be that the structure of the sealant material changed in the sinus during the period. Meaney (1977) showed via post-mortem examination on two udders that the seal had broken and dispersed throughout the udder. If most of the seal broke in the teat and the pieces become a dispersion in milk, this may lead to a loss of effectivity in avoiding bacterial penetration.

In the present study the tested bismuth subnitrate-free prototype of an ITS did not show a positive effect in reducing the NIR mainly caused by minor pathogens in comparison to untreated quarters. In the development of ITS, materials should be used that show similar properties to bismuth subnitrate, which is the main component of the common teat seal products with confirmed efficacy. Future studies about novel teat seal products should be performed on farms with a higher incidence of new IMI caused by environmental pathogens. Furthermore, research about the relationship between NI causing agents (especially minor pathogens) and the sealing effect is recommended.

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Conflict of interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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