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Efficacy of an internal teat sealant alone or in combination with an intramammary antibiotic during the dry period treatment in dairy cows

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Summary

In this study, the aim was to determine the effectiveness of internal teat sealant applied at the dry-off in preventing the formation of subclinical *mastitis* and to investigate whether this product could be a non-antibiotic alternative to total dry cow treatment. A total of 195 quarters from 52 Holstein and Holstein Crossbreed dairy cows, that did not have clinical mammary infection and were expected to calve within 60 days were used in the study. The quarters were divided into 3 treatment groups as follows: Group 1 (ANTB; n = 64): dry period intramammary antibiotic suspension (Ubrostar®); Group 2 (ORB; n = 65), internal teat sealant (Orbeseal®); and Group 3 (ANTB-ORB; n = 66) intramammary antibiotic and teat sealant combination were performed. All of the quarters were checked for subclinical *mastitis* on the 20th, 40th and 60th days of lactation. The percentage of animals found to be CMT negative on the 20th day of lactation was 80.65% in G1 (ANTB), 83.60% in Group 2 (ORB), and 87.50% in Group 3 (ANTB-ORB), respectively. There was no quarter with CMT +3 score in G1 and G3, but it was determined in one quarter in G2 (ORB). On the 60th day there were no quarters with CMT +2 and +3 scores in both G1 and G2. No statistically significant difference was found between the CMT results of treatment groups on the 20th, 40th and 60th days of lactation (p > 0.05). In conclusion, it was determined that bismuth subnitrate-based teat canal sealer provided good protection against subclinical *mastitis* rate in drying healthy udder quarters. However, especially in high risk groups it is important to closely monitor udder health with SCC and/or CMT applications before the dry period and to choose the dry period treatment.

Keywords: cow, dry period, subclinical *mastitis*, antibiotic, teat sealant

Mastitis is one of the most common and complex diseases that adversely affects dairy farming. It can cause poor quality of milk and dairy products, decrease milk yield, sales value of milk, discarding of milk, and removal of cows from the herd. In addition, cow's milk with *mastitis* is a risk factor for public health due to its high number of microorganisms and antibiotic residues (30).

An important factor in the formation of *mastitis* in the dry period is the teat canal that remains open. Keratin plug, which mostly acts as a natural teat barrier during the dry period, plays a role in preventing the formation of new infections. However, the formation of the keratin plug can sometimes take weeks and may be expelled from the teat in response to increased intramammary pressure in the late dry period. Thus, the natural defense mechanism of the teat canal weakens, and the risk of infection increases (13). Preparations covering the outside of the teat with a film barrier paraffin-based and bismuth subnitrate-based teat plugs have been found to be protective against environmental *mastitis* (18, 21, 26).

In dairy farms, antibiotic therapy has been used successfully to treat subclinical *mastitis* in dry cows and to protect the udder against new infections in the early dry period (10, 20, 33). However, in addition to its negative economic effects such as treatment costs and loss of milk, alternative approaches are needed in *mastitis* control due to antibiotic residue and increased risk of bacterial resistance (4, 25, 28).

Teat sealants are materials of very great density. Following the last milking in lactation, Teat sealants are infused into the teat canal and prevent the infectious agents from reaching the teat canal to the mammary gland until the beginning of lactation. The purpose of the use of these plugs is to protect the quarters from

infectious agents in the dry period without depending on antibiotics (31).

Researchers have found that the combined use of coating and antibiotic applications is more successful than the use of antibiotics alone in reducing new infections in the dry period. However, it has been reported that the combined use of teat sealer and antibiotic applications are costly (2, 8, 27).

Within the framework of the literature information compiled above, dry period antibiotic therapy, which is among the routine applications in *mastitis* control programs in dairy farms, is an application that carries the risk of antibiotic resistance and may have weak effectiveness depending on the antibiotic used. At the same time, internal teat sealers developed in recent years have been found to be effective as an alternative to dry-term antibiotic therapy due to the serious economic burden to the enterprise. Furthermore, studies show that the number of somatic cells is reduced in animals treated with teat sealers when compared to untreated groups (22, 27).

Our main hypothesis in the planning of this study is that the need for alternative prophylactic methods will increase due to the increasing public concern about the use of high-level antimicrobials in the livestock industry and antibiotics may not be used to prevent disease in healthy animals according to new European Union (EU) regulations (24). However, the efficiency of the usage of internal teat sealers as an alternative to antibiotic therapy needs to be proved.

Thus, an effective dry period application that is inexpensive and minimizes the use of antibiotics can be included in *mastitis* control programs. Therefore, the objectives of this study were to assess the efficacy of an internal teat sealant applied at the dry-off in preventing the formation of subclinical *mastitis* during the first 60 days of lactation and to investigate whether this product could be a non-antibiotic alternative to total dry cow treatment in small dairy farms.

Material and methods

Animals and housing conditions. This study was carried out in a semi-open dairy farm with free circulation and a feeding system (TMR). A total of 195 quarters from 52 Holstein and Holstein Cross-breed dairy cows were used in the study. The mean body condition scores of the cows at the beginning of the dry period were 3.28 ± 0.03 (2.75--3.75) and the average annual milk yield was 24.2 ± 0.23 kg. During the study, the same researcher visited the herd every two weeks to check up and sample the cows on the day of dry-off. The selected cows were late gestation (expected to calve within 60 days) did not have a clinical mammary infection, with good clinical condition (i.e., no symptoms of disease or visible abnormality in the milk), and did not receive systemic or intramammary antibiotic treatment or anti-inflammatory drugs 30 days before drying off.

Treatments. The animals in the study groups were examined for subclinical *mastitis* with the California Mastitis Test

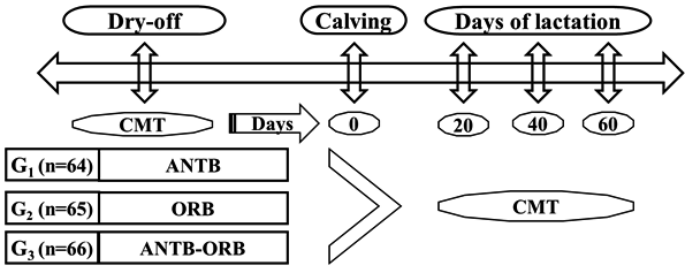

Fig. 1. Study design and treatment protocols

(CMT) before the dry period treatment and all teat sealings were carry out in healthy quarters. CMT was performed on each udder quarter of all cows and results were classified as negative (–) or positive (1+) (traces), 2+ (gel), and 3+ (clumps). Animals with varying degrees of subclinical *mastitis* in more than one quarter were not included in the study. Udder quarters were randomly divided into 3 groups. In Group 1 (ANTB; n = 64), all quarters were treated with an intramammary antibiotic combination (Ubrostar®, Boehringer-Ingelheim Animal Health, UK) containing penetamate hydriodide 100 mg + Benetamine penicillin 280 mg + Framycetin sulfate 100 mg. In Group 2 (ORB; n = 65), an internal teat sealant (Orbeseal®, Zoetis Animal Health, USA) consists of a 4 g intramammary syringe containing 2.6 g (65%) of bismuth subnitrate in an oily base was used. In Group 3 (ANTB-ORB; n = 66) intramammary antibiotic and teat sealant combination were performed at the time of dry-off (Fig. 1). All of the quarters were checked for subclinical *mastitis* (CMT, Kerba Test Liquid®, Kerbl East sp, Wola Rasztowska, Poland) at 20, 40 and 60 days of lactation.

The study was approved by Kafkas University Animal Experiments Local Ethics Committee (KAÜ-HADYEK) with the number 2020-161 and all clinical applications were carried out in accordance with the principles of the ethics committee.

Statistical analysis. Scoring of subclinical mastitis examination was evaluated as –, +1, +2 and +3 by CMT and categorical results were obtained. Due to the insufficient number of cases (n = 1), a separate statistical evaluation was not performed for quarters with +3 CMT scores. For this reason, the Chi-Square Test, in which the frequencies are compared in a 3 × 3 table, was used. Pearson Chi-Square result was evaluated because there was no frequency below 5 in the expected values. The data were analyzed using IBM SPSS Statistics 26.0 (SPSS®, IL, USA) statistical software. $p < 0.05$ was considered statistically significant.

Results and discussion

Family-type dairy farms are milk production centers that are common in Turkey and where udder health control programs can be applied less frequently compared to large dairy farms. The present study examines the protective effect of the use of internal teat sealer on the development of subclinical *mastitis* during the first 60 days of lactation while entering the dry period in a medium-sized dairy farm.

Long-acting intramammary antibiotic preparations applied at the dry-off provided a strategic advantage over other methods in the treatment of *mastitis* originat-

ing from the lactation period (especially *Staph. aureus*). Intramammary infusion of antibiotics provides some protection during the dry periods, but this protection does not last until calving, resulting in a "prophylaxis gap". Compared to the cows that were not treated during drying, in the cows that received the dry period treatment it has been revealed that the frequency of new infections in the dry period and the incidence of periparturient clinical *mastitis* are decreased (5). Although "collective dry period treatment" based on the application of all cows is preferred as the dry period treatment method in most of the farms in our country, "selective treatment" has been recommended as a more advantageous method in recent years (19). In addition, according to the new EU regulations from 28 January 2022 (24), the use of antibiotics in healthy animals was limited. Thus, alternative methods and management strategies have to be determined in future dry-period treatments in milk production. Thus, important advantages such as reducing the cost of antibiotics, the development of resistance to antibiotics, and the rate of contamination from possible pathogens are provided.

The risk of new dry period intramammary infection in cows is highest immediately after dry-off and just before calving. More than 50% of clinical *mastitis* cases originate in the dry period (9, 32). According to Dingwell et al. (14) the ductus papillaris is open in more than 50% of the cows at the end of the first week of the dry period and in 23% after 6 weeks and reports suggest that 50% and 5% of teats had incomplete keratin plugs present 7 and 50 days after dry-off, respectively (33). The early absence of the keratin plug in the dry period and just before calving creates an opportunity for *mastitis* pathogens. This should be considered as an important factor in increasing the incidence of *mastitis* in herds of cows with high milk yield and a weak teat canal sphincter. The development of teat sealants is considered as an option to reduce the mentioned risk factors and the protective effect against intramammary infections has also been reported by different researchers (1, 3, 6, 10, 15). Teat sealant is considered to be an effective option among mammary health programs that can be used as a dry period treatment alone in uninfected quarters (15) or as an adjuvant in combination with dry period antibiotic therapy in infected quarters (11).

In the presented study, the clinical mastitis percentage in the ORB group was found to be proportionally higher than the ANTB and ANTB-ORB groups at the 20th day of lactation (6.15% vs 3.12% and 3.03%, respectively). Clinical signs of *mastitis* were detected in a total of 8 quarters (4.10%) from the all groups. Data from these quarters are excluded from the study (Tab. 1). No new clinical *mastitis* cases were found in any study groups at the 40th and 60th day of lactation. However due to the insufficient number of quarters in the groups, it was not possible to interpret the clinical *mastitis* results and compare them with the literature.

Tab. 1. Numbers of quarters detected with clinical *mastitis* on the 20th day of lactation in all groups

Clinical mastitis	Group			Overall
	G 1 (ANTB)	G 2 (ORB)	G 3 (ANTB-ORB)	
	2	4	2	8
%	3.12%	6.15%	3.03%	4.10%
x/n*	2/64	4/65	2/66	

Explanations: *n – number of quarters; x – number of CMT positive quarters

Tab. 2. Total CMT scores during the first 60 days of lactation

CMT	Days of lactation % (x/n)*								
	20			40			60		
CMT Negative	83.96% (157/187)			89.30% (167/187)			92.51% (173/187)		
CMT Positive	16.04% (30/187)			10.70% (20/187)			7.49% (14/187)		
	+	++	+++	+	++	+++	+	++	+++
	21	8	1	15	5	0	13	1	0

Explanations: *n – number of quarters; x – number of CMT positive quarters

The lack of bacterial examinations and SCC in this study is an important factor limiting the comparison of results with other studies. However, controlling the presence of infection in the quarters before dry-off with CMT, which is an easy-to-apply, inexpensive and effective method, provides an advantage for the farm in terms of determining the dry period treatment option and performing selective dry period treatment. Other methods, requiring laboratory conditions and labor, are considered to be economically disadvantageous for the farm. Therefore, in the presented study, mammary quarters that were determined to be healthy according to CMT results were selected.

On the 20th, 40th and 60th days of lactation, a total of 30 (16.04%), 20 (10.70%) and 14 (7.49%) quarters were found to have positive CMT scores in varying degrees, respectively. The distribution of subclinical *mastitis* cases determined within the first 60 days of lactation according to different CMT score levels is given in Table 2.

The present study was designed to investigate the effect of dry period treatment on healthy quarters on the percentage of encountering subclinical *mastitis* cases on different days of the milking period, not the dry period cure rate. Therefore, CMT results of different days obtained from the study were evaluated separately. Findings from the study showed that the percentage of protection from subclinical infection on the 20th day was higher in the group treated with teat sealant and antibiotic combination ($p > 0.05$). This finding is similar to that of other researchers who reported lower rates of new infections in combined treatment compared to antibiotic or teat sealant results alone (3,

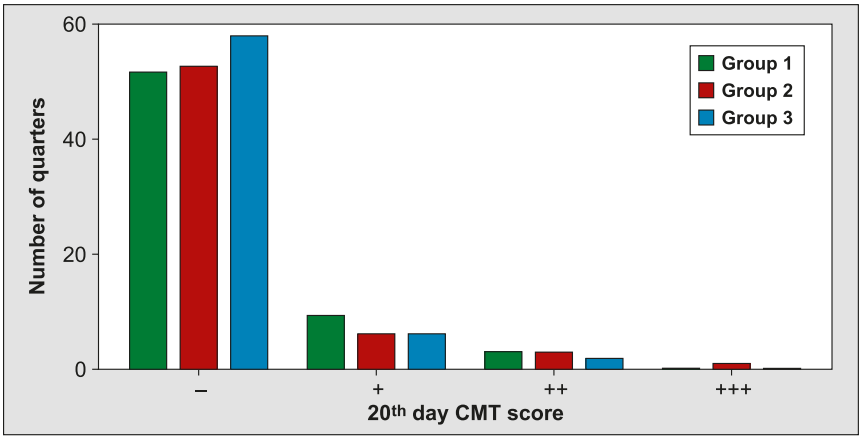

Fig. 2. CMT scores on the 20th day of lactation

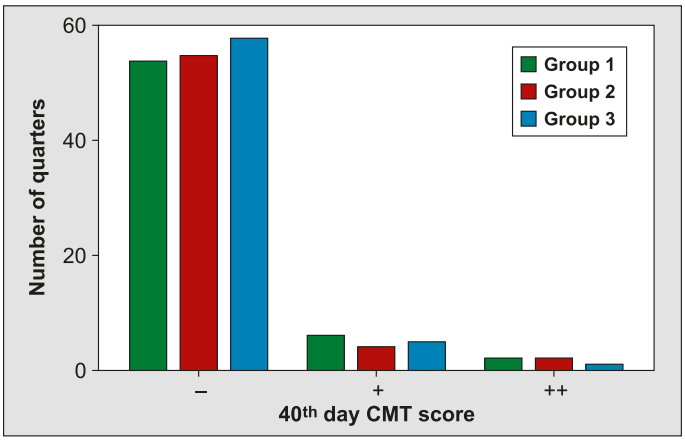

Fig. 3. CMT scores on the 40th day of lactation

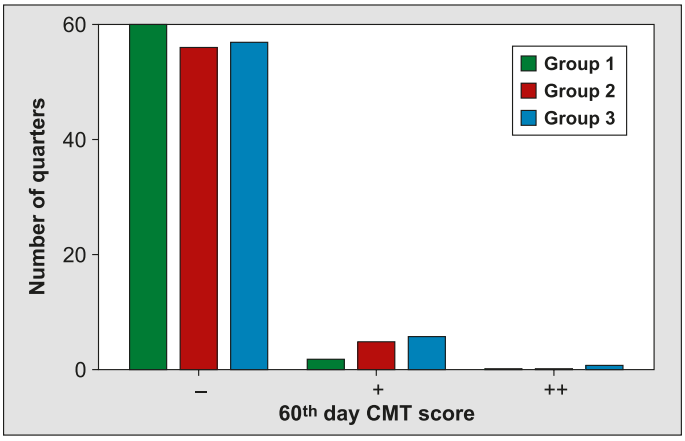

Fig. 4. CMT scores on the 60th day of lactation

11, 17, 29). On the 20th day of lactation, CMT results in the groups were found to be 80.65% negative in Group 1 (ANTB), 83.60% in Group 2 (ORB), and 87.50% in Group 3 (ANTB-ORB) ($p = 0.765$). While no quarter with a CMT+3 score was found on the 20th day of lactation in Group 1 (ANTB) and Group 3 (ANTB-ORB), where intramammary antibiotics were administered, this score was determined in one quarter in Group 2 (ORB) (Fig. 2). On the 40th day of lactation, 87.10% in Group 1 (ANTB), 90.16% in Group 2 (ORB) and 90.62% in Group 3 (ANTB-ORB) showed that the CMT score was negative ($p = 0.923$). No quarter with a CMT+3 score was found in any group on that day (Fig. 3).

On the 60th day of lactation, the quarters were found to have negative CMT scores at a percentage of 96.78% in Group 1 (ANTB), 91.80% in Group 2 (ORB), and 89.06% in Group 3 (ANTB-ORB) ($p = 0.379$). No quarter with a CMT+3 score was found in any group on that day (Fig. 4). The percentage of quarters with CMT +1 score on the 60th day of lactation in Group 1 (ANTB) was 3.22%, the lowest level compared to Group 2 (ORB) (8.20%) and Group 3 (ANTB-ORB) (9.38%) ($p > 0.05$). While no quarters with CMT +2 and +3 scores were found in both Group 1 and Group 2 on the 60th day, it was determined that the percentage of healthy quarters was the highest in all groups. However, this proportional difference between the groups was not found to be statistically significant. Based on these results, it can be said that the subclinical *mastitis* rates formed in the first 60 days were similar between the treatment groups. No statistically significant difference was found between the treatment groups in the proportional comparisons of the CMT results performed on the 20th, 40th and 60th days of lactation in order to compare the protective efficacy of the different treatment groups ($p > 0.05$).

Unlike the studies mentioned above, in the study of Booth et al. (7) carried out in "breeding heifers", the rates of protection from new infection with teat sealant, antibiotic treatment and combined treatment were determined as 90.9%, 100% and 100%, respectively, which were found to be higher compared to our study (CMT negative on day 20; 80.65%, 83.60% and 87.50%, respectively). Compared to similar studies including the present study, the remarkably high success rates in this study are probably due to the animal material (> 4-year-old cows instead of heifers) and the difference in sampling time/method (pp. 20 days instead of pp. 3-10 days). The most interesting result of the mentioned study in question was that the infection resolved in a significant number of quarters (93.8%) after the application of teat sealants, although they do not have any antimicrobial properties. According to the researchers, this improvement is related to the elimination of the existing infection as a result of the detection of teat sealant as a foreign substance in the cow's immune system and the formation of a local immune response. While there are no studies using teat sealants that have reported a significant reduction in infections caused by specific pathogens, most studies agree that teat sealants significantly reduce intramammary infections from environmental streptococcus (12, 16, 18, 23).

It should be noted that non-antibiotic dry-period applications such as teat sealant used in this study were

not designed to treat infections present during the dry period. Dry period treatment using antibiotics in high milk yielding animals is the most effective way to avoid an increase in the incidence of clinical/subclinical *mastitis* in the herd.

We recommend grouping cows with different milk yields and "selective dry period treatment" in farms where an effective *mastitis* control program is implemented. Thus, animals can be dried by using antibiotic or combined treatment for cows with high risk of dry period infection, and internal teat sealant alone for healthy cows in low risk group. Bismuth subnitrate-based teat sealer could be a non-antibiotic alternative to total dry cow treatment in small dairy farms. However, more studies are needed to examine both the efficacy and long-term effects of inorganic compounds used in the udder, including bismuth subnitrate, in this new and developing field of dry period applications.

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