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Comparison of the effects of tylosin and tilmicosin as a systemic treatment of dry Holstein cows

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Abstract

The aim of the present study was to evaluate the efficacy of macrolides to eliminate intramammary infection (IMI) caused by *Staphylococcus aureus* (*S. aureus*) and *Streptoccus spp*. 3 weeks before calving time. Eighty Holstein dairy cows with subclinical mastitis pathogens were divided into three groups. Three weeks before expected parturition time, cows in group 1 received tilmicosin (n = 29), cows in group 2 received tylosin (n = 30) and cows in group 3 were left as negative control (n = 21). Milk samples were obtained on 3 and 7 days after calving. Randomly amplified polymorphic DNA (RAPD) method was determined for all of the *S. aureus* isolates that had the same isolates before and after parturition. The total cure rate was 63.33, 75.86 and 66.66% for tylosin, tilmicosin and control groups, respectively. Furthermore, cure rates were not significant, when each type of mastitis causing pathogens were considered separately. The incidence of clinical mastitis during 60 days after calving for tylosin, tilmicosin and Control groups was 23.33, 27.58 and 38.09%, respectively. Only four *S. aureus* isolated before drying-off were similar to post-calving isolate, according to RAPD-PCR method. In conclusion, antibiotic therapy before calving improved the cure rate numerically, however, it was not significant.

Introduction

The goal of dry cow therapy is to have as few infected quarters as possible at the next calving which is achieved by elimination of existing infections and preventing the establishment of new infections during the dry period.[1,2] The new infection rate was higher over the entire dry period than during lactation. First 2 week of the dry period and just prior to parturition heightened susceptibility occurs.[2] At present, the most effective means of achieving these objectives is to administer long-acting dry cow antibiotic therapy (DCT) immediately after the last milking.[2] There are a lot of advantages to treat intramammary infections during the dry period including longer retention time of the drug in the udder, higher cure rate and reduced risk of contamination of milk with antibiotic residues.

Most products are designed to eliminate existing intramammary infection (IMI) caused by *Staphylococcus aureus* (*S. aureus*) and *Streptoccus agalactiae* (*Strep. Agalactiae*) and to prevent the establishment of new IMI caused by these organisms during the non-lactating period. It seems that this treatment could cover the first susceptibility stage of the dry period, however, was not effective for the second susceptibility stage. Although dry cow therapy is > 90.00% effective against *Strep. agalactiae*, efficacy against *S. aureus* ranges from 20.00 to 70.00%.[3]

The fact that macrolides have relatively long half-lives and good tissue penetration in the udder, suggests that they may be advantageous in treatment of mastitis in dairy cows.[4] Tilmicosin is a semisynthetic macrolide antibiotic. Its antibacterial spectrum covers mainly *Pasteurella* spp., *Mycoplasma* spp. and various gram-positive bacteria. This drug possesses properties that might prove favorable in combatting dry period IMI. These properties include rapidly and extensively penetrating the blood stream into milk and slowly eliminating it from mammary tissues. Tilmicosin rapidly accumulates in bovine macrophages and mammary epithelial cells and it is not influenced by either metabolic inhibitors or an anaerobic environment.[5]

Nickerson *et al*. compared tilmicosin and intra-mammary cephapirin benzathine as a treatment of *S. aureus*

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infections at dry-off.[3] The tilmicosin was either administered intramammary (1,500 mg) or via a subcutaneous injection at 5.00 mg kg^{-1} on the day of drying-off, and again 4 days later. The reported quarter cure rates for IMI caused by *S. aureus* at 28 days post calving were 78.10% for intramammary cephapirin, 74.20% for intramammary tilmicosin and 9.10% for subcutaneous tilmicosin. Intramammary tilmicosin was concluded to be as effective as cephapirin benzathine.[3] Zecconi *et al*. showed that systemic administration of tylosin 2 weeks before calving was an effective treatment against *S. aureus* during the dry period.[6]

Genotyping methods are valuable epidemiological tools for determining the differences between isolates. Characteristics of different strains of *S. aureus* were significantly associated with the likelihood of IMI cure rate in the dry period.[7] Sommerhäuser *et al*. who used typing methods on *S. aureus* isolates before and after dry cow therapy showed that actual persistent and new infected animals were able to be realized.[8] Myllys *et al*. using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method have compared the *S. aureus* isolates taken from the same quarter before and after mastitis treatment to verify the persistence of virulent strains in infected quarters.[9] Randomly amplified polymorphic DNA- Polymerase chain reaction (RAPD-PCR) which was used in this study was a simple, fast and cost effective genotyping method for differentiation of microorganisms. Since there is no antibiotic coverage at the end of the dry period, which is one of the most critical times for new infections, systemic injection of antibiotics can be beneficial. The present study was undertaken in order to compare the efficacy of tilmicosin and tylosin for eliminating IMI caused by *S. aureus* and Streptococcus spp. when administered at the end of the dry period.

Materials and Methods

During 13 months in a large Holstein dairy herd, all cows with more than 250,000 somatic cells mL^{-1} at drying off were tested by the California mastitis test (CMT). Milk samples were collected aseptically from all infected quarters. Teats were aseptically prepared prior to collection of all samples according to National Mastitis Council (NMC).[10] Samples were shipped to the Mastitis Research Laboratory at the Ferdowsi University of Mashhad. All laboratory procedures were performed in accordance with NMC recommendations.[10] A sample was considered contaminated if three or more colony types were present on a plate. The present study was confirmed by animal welfare committee of Ferdowsi University of Mashhad (39421) in accordance with institutional and national and/or international guidelines.

Cows were housed in the free-stalls and were milked three times a day. The length of dry period was 60 days. Chlorine tablets were used as pre-milking disinfectant and 1.00% iodine was used for post-milking disinfection.

Eighty Holstein cows with major subclinical mastitis pathogens such as *S. aureus*, *Strep. agalactiae* and environmental streptococcus were selected and divided into three groups according to isolated pathogens. All cows in three groups received an intramammary infusion of an ointment containing 100 mg nafcillin, 300,000 IU penicillin G procaine, and 100 mg dihydrostreptomycin (Nafpencin® DC; Kimia Biotechnology, Arak, Iran) in each quarter at the time of drying off.

Three weeks before expected parturition time, cows in group 1 received 10.00 mg kg^{-1} tilmicosin (30.00%, Razak, Tehran, Iran) subcutaneously (n = 29), cows in group 2 were injected by 10.00 mg kg^{-1} tylosin (20.00%, Razak) once per day for 3 days intramuscularly (n = 30) and cows in group 3 were left intact as negative controls (n = 21).

Milk samples were obtained on 3 and 7 days after calving, if a sample was negative in microbiological culture or had showed a different isolate or genotype than those isolates before the dry period, was considered to be cured.

Other data including dry period length, somatic cell count (SCC), CMT score, estimation of 305-days milk production, parity, history of clinical mastitis in previous lactation period and clinical mastitis in current lactation, until days in milk (DIM) = 60.00, were recorded.

In the present study, matching of fingerprints by RAPD-PCR method was determined for all *S. aureus* isolates in culture before drying-off and after parturition. The objective of this genetic characterization was to find out if a new infection was occurred.

The RAPD analysis was performed as described by Williams *et al*.[11] with some modifications. Amplification was performed in a final volume of 25.00 mL containing 10.00 µL of master mix red (Ampliqon, Odense, Denmark), 0.50 mg mL^{-1} of each primer (DenaZist Asia, Mashhad, Iran) and 2.00 µL template DNA. The PCR conditions consisted of a pre-denaturation step at 94.00 ˚C for 5 min, followed by 45 cycles of 60 sec at 94.00 ˚C, 60 sec at 36.00 ˚C and 120 sec at 72.00 ˚C. A final extension step was performed at 72.00 ˚C for 10 min. Amplified products were analyzed by electrophoresis on 1.50% agarose gel. DNA bands were visualized by staining with ethidium bromide and photographed under UV illumination. A 1.00-kbp DNA ladder and a 100-bp ladder (DenaZist Asia) were used in each gel as molecular size standards. PCR images were analyzed using Gel compare 2 software (Informer technologies inc., Lansdale, USA). Isolates were clustered and displayed in dendrogram and a genetic similarity of more than 80.00% was considered as the same genotype.

Statistical analyses. The rates of intra-mammary infections and new infection rates were compared by Chi square. Data was analyzed using SPSS software (version 21.0; IBM Corp., Armonk, USA).

Results

The total number of cows dried off defined as infected based on culture results was 95. Fifteen cows were excluded from the study because of some reasons such as receiving treatment, culling or contaminated samples. At the beginning of the study, cows that met inclusion criteria were enrolled into the study and divided into three groups based on their bacterial species. Lactation number, dry period length (days), CMT score, DIM milk yield (kg), and number of clinical mastitis in the previous lactation were all recorded for these cows and there was no significant difference between the three groups ($p > 0.05$). Table 1 summarizes the descriptive data related to the infected quarters.

The total prevalence of IMI after parturition in three groups was 48.70%. Post-partum intramammary infections with *S. agalactiae*, *S. aureus*, environmental *Streptococcus* and other microorganisms were 9/39(23.07%), 13/39 (33.33%), 9/39(23.07%) and 8/39(20.51%), respectively. The most common pathogen isolated 3 to 7 days after parturition was *S. aureus* (33.30 %).

The total cure rate was 63.33, 75.86 and 66.66% for tylosin, tilmicosin and control groups, respectively. No significant difference was observed between cure rate of three groups, although that of tilmicosin was higher numerically ($p > 0.05$), (Table 2).

The incidence of clinical mastitis between the day of drying-off and DIM 60.00 for tylosin, tilmicosin and control groups were 23.33%, 27.58% and 38.09%, respectively ($p > 0.05$, Table 2).

Staphylococcus aureus cure rates were 50.00, 62.50, and 40.00% in the tylosin, tilmicosin, and control groups, respectively. *Streptococcus agalactiae* cure rates were 73.33, 88.23, and 76.92% the tylosin, tilmicosin and control groups, respectively ($p > 0.05$). Data of cure rate has been summarized in Table 3. The result of the dendrogram showed that 4 isolates from 11 paired *S. aureus* (before drying-off and after parturition) had more than 80.00% dissimilarity to each other. The RAPD patterns for isolates in this study are shown as a dendrogram in Figure 1.

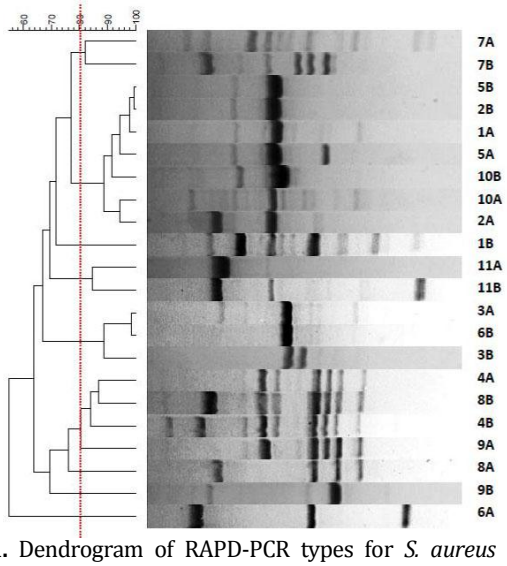

Fig. 1. Dendrogram of RAPD-PCR types for *S. aureus* isolates before drying-off (B) and after calving (A). The genetic similarity is expressed in a dendrogram. Bacteria are considered genetically similar when their band patterns are 80.00% similar.

Table 1. Descriptive and management data of cows included in this study. Data are presented as mean ± SD.

Parameters	Control	Tylosin	Tilmicosin	Total
Lactation number	1.80 ± 1.70	1.80 ± 1.80	1.80 ± 1.30	1.80 ± 1.60
Dry period length (days)	71.10 ± 25.60	69.70 ± 1.70	67.90 ± 17.70	70.50 ± 17.30
CMT score	2.60 ± 0.50	2.60 ± 0.60	2.60 ± 0.50	2.60 ± 0.50
Days in milk	385.50 ± 70.1	308.90 ± 64.70	306.8 ± 182.4	355.70 ± 127.90
Milk yield (kg)	10,547.70 ± 70.10	10,025.60 ± 64.70	10,097.10 ± 182.40	10,371.10 ± 127.90
Clinical mastitis in previous lactation	1.90 ± 1.30	1.50 ± 1.30	1.40 ± 1.90	1.80 ± 1.60

Table 2. Cure rates and incidence of mastitis among tylosin, tilmicosin and control groups.

Parameters	Control (n = 21)	Tylosin (n = 30)	Tilmicosin (n = 29)
Cure rate	14/21 (66.66%) [a]	19/30 (63.33%) [a]	22/29 (75.86%) [a]
No growth	10/14 (71.42%)	14/19 (73.68%)	17/22 (77.27%)
New infection*	4/14 (28.57%)	5/19 (26.31%)	10/14 (71.42%)
Mastitis case **	8/21 (38.09%) [a]	7/30 (23.33%) [a]	8/29 (27.58%) [a]

* Different isolates after parturition; ** Clinical mastitis during 60 days after parturition.
[a] The same letters are not significantly different at the 0.05 level.

Table 3. Cure rates for each bacterial species separately.

Parameters	Control (n = 21)	Tylosin (n = 30)	Tilmicosin (n = 29)
Cure rate	14/21 (66.66%)	19/30 (63.33%)	22/29 (75.86%)
Streptococcus agalactiae	10/13 (76.92%) [a]	11/15 (73.33%) [a]	15/17 (88.23%) [a]
Staphylococcus aureus	2/5 (40.00%) [a]	5/10 (50.00%) [a]	5/8 (62.50%) [a]
Environmental *streptococcus*	2/3 (66.66%) [a]	3/5 (60.00%) [a]	2/4 (50.00%) [a]

[a] The same letters are not significantly different at the 0.05 level.

Discussion

There are two phases of susceptibility within the dry period: The first and the last 2 weeks of dry period and just prior to parturition. Intramammary administration of a long-acting antibiotic ointment called dry cow therapy can cure existing infections and also prevent new infections. Dry cow therapy is beneficial during the first phase of dry-period, however, the second phase of susceptibility when antibiotics are no longer effective is especially important and should be considered. The aim of the present study was to evaluate the pre-calving antibiotic therapy for improvement of the cure rate of dry cows.

In this study, the cure rate after precalving injections of tylosin or tilmicosin was not significantly different compared to that of the control group. Although some authors believed that prophylactic precalving therapy was an accepted recommendation for reducing mastitis in multiparous cows,[12] however, a few studies evaluated pre-calving systemic antimicrobial agents in the cure rate of intramammary infections in dry cows. Zecconi *et al*. reported that administration of systemic tylosin 2 weeks before calving, alongside with traditional dry-cow therapy was an effective supplementary treatment for intra-mammary therapy of *S. aureus*.[6]

In heifers, a systematic review showed that lactating cow antibiotic therapy infused 1 - 2 weeks prepartum improved the cure rate from 26.00 - 31.70% to 59.00 - 76.00%.[13] Naqvi *et al.* showed that occurrence of mastitis was reduced in treated heifers compared to untreated controls with a pooled risk ratio of treated to untreated heifers of 0.56.[12]

Hovareshti *et al.* compared the effects of systemic and local therapy to control staphylococcal IMI in Holstein heifers. Heifers with *S. aureus* intramammary infection 4 to 7 days prepartum were assigned into 4 groups. Group 1 received an intramammary infusion containing sodium nafcillin, procaine benzylpenicillin and dihydro-sptreptomycin, Group 2 and 3 received an intramuscular injection of tylosin or enrofloxacin, and group 4 received no treatment (control). Postpartum cure rates were 93.00, 83.00, 70.00 and 47.00% in heifers of group 1 to 4, respectively ($p < 0.05$).[14]

Cure rate of each quarter was depended on its causative agents and in the present study *S. aureus* cure rate with tilmicosin was higher numerically (50.00%, 62.50% and 40.00% for tylosin, tilmicosin and control, respectively; $p > 0.05$). Erskine *et al.* showed that administration of intramuscular oxytetracycline in combination with intramammary dry cow treatment did not improve the rate of cure for *S. aureus* mastitis.[15]

In this study pre-calving injection of tylosin and tilmicosin only numerically reduced the occurrence of clinical mastitis within 60 days of calving ($p > 0.05$). There is some evidence from literature studies that the intramammary infections during the dry period increased the chances of clinical mastitis in the early subsequent lactation,[16] especially in the first 30 or 60 days of lactation.[17] Ismail *et al*. showed that dry cow therapy of CMT-positive cows using a combination of tylosin and intramammary infusion might result in a significant reduction of clinical mastitis within the first 100 DIM.[18] In contrast, Browning *et al*. have shown that incidence of clinical mastitis in early lactation was almost 50.00% higher for the treated group of uninfected cows compared to the untreated group.[19] The two most common cause of clinical mastitis are *Escherichia coli* and environmental streptococcus, however, dry cow ointments typically contain antibiotics with a predominantly gram-positive spectrum. Therefore, injection of gram-negative spectrum antimicrobial agents in the close-up period may be more beneficial to control clinical mastitis in the early subsequent lactation.

In the present study, based on results of RAPD-PCR, four of 11 pair *S. aureus* strains which were isolated from infected quarters both in the dry off and post-calving periods were similar. On the other hand, 63.60% (7/11) of *S. aureus* isolates have not been persistent in the udder and probably a new infection has been occurred by different genotype of *S. aureus*. Myllys *et al*. compared the *S. aureus* isolated from the same quarter at different times to verify persistence of virulent strains in infected quarters and showed that *S. aureus* strains isolated from the acute phase of the infection persisted in the udder and reinfection by other *S. aureus* strains was not common.[9] Overall, considering the results of RAPD-PCR that showed new infection in 7 of 11 *S. aureus* isolates, the new cure rate of *S. aureus* were 50.00% (8/10), 87.50% (7/8) and 80.00% (4/5) for tylosin, tilmicosin and control groups, respectively. However, if isolates with more than 60.00 percent similarities were taken as different types, only one *S. aureus* isolate was grouped as a new genotype (new infection).

In conclusion, using antibiotic therapy before calving, although the cure rate in the dry period and mastitis rate was improved numerically, the mentioned parameters were not significant. Also, the results of RAPD-PCR showed a considerable change in genotype in *S. aureus* isolated after calving in comparison with that of drying-off period.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Dingwell RT, Leslie KE, Duffield TF, et al. Efficacy of intramammary tilmicosin and risk factors for cure of *Staphylococcus aureus* infection in the dry period. J Dairy Sci 2003; 86(1): 159-168.
2. Eberhart RJ. Management of dry cows to reduce mastitis. J Dairy Sci 1986; 69(6): 1721-1732.
3. Nickerson SC, Owens WE, Fox LK, et al. Comparison of tilmicosin and cephapirin as therapeutics for *Staphylococcus aureus* mastitis at dry-off. J Dairy Sci 1999; 82(4): 696-703.
4. Ziv G, Shem-Tov M, Glickman A, et al. Tilmicosin antibacterial activity and pharmacokinetics in cows. J Vet Pharmacol Ther 1995;18(5): 340-345.
5. Scorneaux B, Shryock TR. Intracellular accumulation, subcellular distribution, and efflux of tilmicosin in bovine mammary, blood, and lung cells. J Dairy Sci 1999; 82(6): 1202-1212.
6. Zecconi A, Piccinini R, Guarini CPB. Tylosin in cows in the dry period. In Proceedings: National Mastitis Council. Arlington, USA 1999; 237-238.
7. Dingwell RT, Leslie KE, Sabour P, et al. Influence of the genotype of *Staphylococcus aureus*, determined by pulsed-field gel electrophoresis, on dry-period elimination of subclinical mastitis in Canadian dairy herds. Can J Vet Res 2006; 70(2): 115-120.
8. Sommerhäuser J, Kloppert B, Wolter W, et al. The epidemiology of *Staphylococcus aureus* infections from subclinical mastitis in dairy cows during a control program. Vet Microbiol 2003; 96(1): 91-102.
9. Myllys V, Ridell J, Björkroth J, et al. Persistence in bovine mastitis of *Staphylococcus aureus* clones as assessed by random amplified polymorphic DNA analysis, ribotyping and biotyping. Vet Microbiol 1997; 57(2-3): 245-251.
10. Hogan JS, Gonzalez RN, Harmon RJ, et al. Laboratory handbook on bovine mastitis. 1st ed. Madison, USA: National Mastitis Council 1999; 1-30.
11. Williams JG, Kubelik AR, Livak KJ, et al. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 1990; 18(22): 6531-6535.
12. Naqvi SA, Nobrega DB, Ronksley PE, et al. Invited review: Effectiveness of precalving treatment on postcalving udder health in nulliparous dairy heifers: A systematic review and meta-analysis. J Dairy Sci 2018; 101(6): 4707-4728.
13. Nickerson SC. Control of heifer mastitis: antimicrobial treatment-an overview. Vet Microbiol 2009; 134(1-2): 128-135.
14. Hovareshti P, Bolourchi M, Tabatabayi AH. Comparison of the effect of systemic and local antibacterial therapy to control staphylococcal intramammary infection in prepartum heifers. J Vet Res 2007; 62(2): 7-9.
15. Erskine RJ, Bartlett PC, Crawshaw PC, et al. Efficacy of intramuscular oxytetracycline as a dry cow treatment for *Staphylococcus aureus* mastitis. J Dairy Sci 1994; 77(11): 3347-3353.
16. Pantoja JC, Hulland C, Ruegg PL. Somatic cell count status across the dry period as a risk factor for the development of clinical mastitis in the subsequent lactation. J Dairy Sci 2009; 92(1): 139-148.
17. Barker AR, Schrick FN, Lewis MJ, et al. Influence of clinical mastitis during early lactation on reproductive performance of Jersey cows. J Dairy Sci 1998; 81(5): 1285-1290.
18. Ismail ZB, Muhaffel MM, Abu-Basha E. The effect of dry cow therapy using systemic tylosin in combination with common intramammary medications on mastitis rate, cull rate, somatic cell count, and milk production in dairy cows affected with subclinical mastitis. Vet world 2018; 11(9): 1266-1271.
19. Browning JW, Mein GA, Barton M, et al. Effects of antibiotic therapy at drying off on mastitis in the dry period and early lactation. Aust Vet J 1990; 67(12): 440-442.