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Effect of systemic antibacterial administration during prepartum period on coagulase negative *Staphylococcal intramammary* infection in Holstein Heifers

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Summary

A total of 183 coagulase negative Staphylococcal infected quarters and 64 uninfected quarters were randomly allotted to 4 experimental groups: infected quarters that did not receive any treatment (positive control group; n = 60); infected quarters that received Tylosin (n = 61) or Cefquinome (n = 62) and also uninfected quarters that did not receive any treatment were considered as negative control group (n = 64). Treated heifers received Tylosin or Cefquinome 10 to 14 days before the expected calving date. The bacteriological cure rate based on sampling obtained 3 days after calving was significantly higher (P<0.01) in mammary glands treated with systemic Tylosin (85.3%) than in the positive control group and mammary glands treated with systemic Cefquinome (69.4%). At the same time Tylosin treated heifers had significantly lower (P<0.01) somatic cell count scores compared to the positive control and Cefquinome groups. With the exception of one heifer that calved 8 days earlier than the expected calving date, there was no detectable antibiotic residues in the milk of the treated heifers on the 3rd day of lactation.

Key words: Heifer mastitis, Coagulase negative Staphylococcal, Systemic antibacterial administration

Introduction

Heifers represent the future of dairy herds for the producion of high quality milk. Current practices for the control of intramammary infections (IMI) were developed for mature cows (Aaerstrup and Jensen, 1997). In replacement heifers, emphasis was placed on genetic improvement, reproduction, nutrition and immunization against diseases without particular attention to mammary glands (Trinidad *et al*., 1990b; Pankey *et al*., 1991). Oliver and Mitchell (1983) showed that a high percentage of pregnant heifer mammary glands were infected during late gestation, at calving and during early lactation. Studies during the recent two decades have revealed that intramammary infection in nulliparous heifers is not uncommon (Oliver *et al*., 2003). Minor pathogens, especially coagulase negative staphylococci, are the most common cause of IMI in heifers (Trinidad *et al*., 1990a; Fox *et al*., 1995).

Mammary glands of heifers infected with coagulase negative *staphylococus* species exhibited greater leukocyte infiltration and increased connective tissue (Trinidad *et al*., 1990a) which in turn may impair the growth and development of mammary glands, thereby influencing future milk production (Oliver *et al*., 2003). The most common and effective practice in controlling prepartum intramammary Staphylococcal infection of pregnant heifers is to use intramammary antibiotic infusions (Nickerson *et al*., 1995; Oliver *et al*., 2003, 2005).

Intramammary infusions may introduce bacteria resulting in secondary infection due to stretching the teat canal and the sphincter

muscle (Radostits *et al*., 2006). Therefore, the administration of antibiotics by a parenteral route would be preferred. The objective of the present study was to evaluate the effectiveness of parentral administration of Tylosin and Cefquinome in controlling IMI due to CNS in dairy heifers during early lactation period.

Materials and Methods

This study was conducted at a commercial dairy farm in Tehran province. In our earlier study mammary secretions of 913 quarters from 229 pregnant Holstein heifers were obtained aseptically to determine the prevalence of coagulase-negative Staphylococci isolated during the periparturient period. Quarter samples were collected 10 to 14 days before expected parturition. Results showed that 187 (20.5%) quarters from 105 (45.9%) heifers contained coagulase-negative staphylococci from which *S. chromogenes* (16.3%) was commonly isolated (Ataee *et al*., 2007).

In the present study, coagulase negative staphylococcal infected (n = 101) and un-infected (n = 32) heifers from our previous study were randomly allotted to 4 experimental groups, 2 × 2 factorial design, including: 1) negative control group (64 uninfected quarters from 32 heifers), 2) positive control group (60 CNS infected quarters from 34 heifers), 3) Tylosin group (61 CNS infected quarters from 38 heifers) which received a daily intramuscular injection of 10 mg/kg of 20% Tylosin for 3 days (Tyloject; Razak Co, Tehran, Iran), starting 10 to 14 days before expected day of calving, 4) Cefquinome group (62 CNS infected quarters from 29 heifers) received a daily intramuscular injection of 1 mg/kg of 2.5% Cefquinome (Cobactan; Intervet, Boxmeer, Netherland) for 2 days, starting 10 to 14 days before expected day of calving. On Day 3 after parturition, mammary secretion samples from experimental quarters were aseptically collected and examined for bacterial infection, as recommended by the National Mastitis Council (NMC, 1999), SCC and antibiotic residues. Somatic cell count was determind significant difference in the frequency of by direct microscopic method (Marshal, 1992) and samples were analyzed qualitatively for antibiotic residues using Copan test kits (Hansen, 2004). Mean 305 day milk production was calculated based on 180 days actual milk production data.

The presence of quarter infection after parturition with binomial distribution was analyzed using the Genmod procedure of SAS (2001). The season of the year and the age of the heifers at the time of calving were included as the main effect and covariate in the statistical model, respectively. Homogeneity of variances and deviances from normality for parametric data were tested and subjected to proper transformation when required. For statistical analysis purposes, SCC was transformed to somatic cell score (SCS) and analyzed using the GLM procedure in SAS. The differences between the groups were examined using least square means. A p-value less than 0.05 was considered as statisically significant.

Results

On Day 3 after parturition, the cure rates of the quarters treated with Tylosin and Cefquinome were 85.3% (52 of 61) and 69.4% (43 of 62), respectively, and the self cure rate in the positive control and the new infection rate in the negative control were 53.3% (33 of 60) and 6.2% (4 of 64), respectively (Table 1).

There was a 12.9 fold greater odds of new intramammary infections after parturition for the positive control than the negative control (Table 1; $P<0.0001$). The odds of new intramammary infections after parturition were not significantly different between Tylosin and negative control ($P = 0.19$), whereas it tended to be significant between Cefquinome and negative control ($P = 0.049$).

The bacteriological cure rate was higher in mammary glands treated with systemic Tylosin during the prepartum period than in the positive control group and the mammary glands treated with systemic cefquinome ($P<0.0001$; Table 1). Four negative control mammary quarters (6.3%) showed new IMI due to CNS after parturition. There was no postpartum infected quarters between the

Tylosin treated and negative control groups (P>0.05).

On Day 3 after parturition, the average SCS in the milk of heifers treated with Tylosin (4.2) was lower than the positive control group (6; P<0.0001) and those heifers treated with cefquinome (5.1; P<0.001; Table 2). Mean 305 days milk production was 8658.9, 8323.9, 8251.7 and 8583.6 kg for Tylosin, Cefquinome, control positive and control negative groups, respectively (P>0.05; Table 2).

On Day 3 after parturition, only one sample from the heifers treated with Tylosin was positive for antibiotic residue.

Discussion

The present study was conducted to evaluate the effectiveness of systemic antibiotic administration to control IMI due to CNS in dairy heifers.

An alternative to prepartum intramammary antibiotic infusion, which is a common approach in reducing the postpartum prevalence of IMI in heifers (Oliver *et al*., 2003, 2005) is systemic antibiotic therapy. This method might be advised due to the better distribution of drug in the udder tissue, which may lead to a higher cure rate (Ziv, 1980a) and prevention of a new infection which is a possible risk following intramammary infusion (Boddie and Nickerson, 1986).

Tylosin is a weak basic compound with the structure of a macrolide antibiotic. Macrolides easily penetrate from blood to milk, reaching concentrations several times higher than that in serum, due to ion trapping (Ziv, 1980b). Cefquinome, a 4th generation cephalosporin, with a broad spectrum activity, is distributed very quickly in the treated animals, resulting in a rapid anti-bactericidal effect following injection (Limbert *et al*., 1991). Since a heifer's mammary glands development continues up to first and even second lactation, this could possibly be related to the fact that the milk blood barrier is under development, in the present study cefquinum was adminestrated by the parenteral route to control IMI due to CNS. Regardless of the route of administration, the result of our experiment is in agreement with many researchers who demonstrated that prepartum antibiotic therapy is an effective procedure for eliminating many infections in heifers during early lactation (Oliver *et al*., 2003, 2005).

In the last 10 years some reports have been published dealing with systemic dry cow therapy. Bolourchi *et al*. (1995) found that systemic enrofloxacin or tylosin at drying off approached, but did not exceed, the efficacy of the local treatment with a combination of nafcillin, penicillin and

Table 1: Adjusted odds ratio, 95% confidence limits and cure rate (n, %) of infected quarters treated with Tylosin and Cefquinome compared to positive and negative controls in dairy heifers

Groups	Infected quarters		Cure rate	Risk of IMI 3 days after parturition		
	Day 10-14 Prepartum	Day 3 Postpartum		AOR	95% CL	P-Value
Tylosin	61	9 (14.7)	52 (85.3)[a]	2.5	0.7-9.8	0.19
Cefquinome	62	19 (30.6)	43 (69.4)[b]	6.4	2.2-23.5	0.049
Positive control	60	28 (46.7)	32 (53.3)[b]	12.9	4.5-46.8	<0.0001
Negative control	0	4 (6.3)	0[a]	Referent	Referent	NA

[a, b] Values with different superscripts within the column differ (P<0.01)

Table 2: Somatic cell count score (SCS) on Day 3 after parturition and 305-day milk production in controls (positive and negative) and prepartum antibiotic-treated (Tylosin and Cefquinome) heifers. Mean ± SEM (Range)

	Tylosin	Cefquinome	Positive control	Negative control
SCS	4.2 ± 0.2[a] (1.06-8.64)	5.1 ± 0.23[b] (2.38-10.17)	6.0 ± 0.21[b] (3.0-8.64)	4.4 ± 0.24[a] (0.8-11.54)
Milk production (Kg)	8658.9 ± 198.53 (5820-10591)	8323.9 ± 202.01 (6182-10279)	8251.7 ± 205.24 (5633-9938)	8583.6 ± 184.48 (5708-10549)

[a, b] Values with different superscripts within raw differ (P<0.01)

dihydrostreptomycin. O'Boyle *et al*. (2006) have also shown that tylosin given systemically during the dry period significantly reduced the number of Gram positive IMI at the begining of the next lactation. The *in vitro* trial of our earlier study revealed that all isolates of CNSs were extremely suceptible (100%) to cefquinome; whereas, the succeptibility to Tylosin was relatively low (60-92.6%) depending on the isolate (Ataee *et al*., 2007).

However, in the present study the cure rate of IMI on the third day of lactation in the Cefquinome treated group was less when compared with the control and Tylosin treated groups. This suggested that prepartum systemic therapy by Cefquinome, as done in the present study, had no significant effect in eliminating IMI due to CNS or reducing SCS. This is in agreement with Ziv *et al*. (1980b) who showed that some of the most active drugs *in vitro*, are poorly and unevenly distributed in the udder and are absorbed only to a limited extent.

One disadvantage of prepartum anibiotic administration for controlling mastitis in heifers is the potential for antibiotic residues in milk. Only one of 61 milk samples obtained 3 days after parturition (the time when milk would likely be marketed for human consumption) was positive for Tylosin residue. This sample was from a heifer that calved 8 days earlier than the expected calving date.

Data presented herein indicated that prepartum treatment of heifers with systemic Tylosin significantly reduced the prevalence of early lactation IMI caused by CNS. There was no significant increase in the 305 days milk production period with the Tylosin treatment, but significant reduction in early lactation SCC was observed. Lack of Tylosin residues in milk meant consumer safety issues were ensured.

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