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Effects of Precalving Antibiotic Treatment on Mastitis and Individual Somatic Cell Count in Heifers

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Abstract: In this experiment, effectiveness of antibiotic treatment was evaluated by bacteriology and Somatic Cell Count (SCC). Ninety-six heifers were divided randomly into two groups: either to be treated with antibiotics (200 mg cephalexin monohydrate and 250 mg neomycin) 45 days prior to expected calving date (Group 1, n = 41) or not treated (Group 2, n = 55). Bacteriologic tests were used for detection of infected quarters. Of the quarters, 58.3% had infection at least in one quarter before calving. Mastitis pathogens were isolated from 31% of the quarters. In the treatment group, 43 of 52 infected quarters (82.6%) were cured but new infections (n = 43) were detected in the beginning of the lactation. Spontaneous cure rate was 69.3% in the control group and new infection rate (24.2%) was lower than the treated group. The treatment was effective for reducing prepartum Intramammary Infections (IMI) and persistent IMI; it was however inadequate to protect the quarters from new IMI in the beginning of lactation ($p>0.05$). Precalving antibiotic treatment was however quite effective for reducing individual SCC. There were less quarters (n = 5) with high SCC (>400.000 cells mL^{-1}) in the treated group than the control group. In conclusion, antibiotic treatment prepartum may be necessary to reduce incidence of mastitis and improve quality of milk.

Key words: Mastitis, precalving intramammary antibiotic treatment, somatic cell count, lactation, heifer

INTRODUCTION

Prepartum heifer mastitis is a well-known herd problem in dairy operations (Oliver and Mitchell, 1983; Oliver *et al*., 1992; Fox *et al*., 1995; Aarestrup and Jensen, 1997; Sampimon *et al*., 2009a). Although, previous reports mentioned about primigravid heifer mastitis, control programs did not widely involve heifers (Kreiger *et al*., 2007). Recently, some prevention strategies have been recommended, which covers animal and environmental hygiene, administration of external and internal teat sealants, precalving milking, control of insects, application of teat antiseptics, segregation of pregnant heifers from older cows, separation of preweaned calves to prevent suckling and avoidance of feeding mastitic milk to calves.

The aims of these regulations are to minimize the bacterial invasion. (Shearer and Harmon, 1993; Heinrichs *et al*., 2009; McDougall *et al*., 2009).

Bacterial invasion occurs during late gestation and microorganisms cause glandular damage in parechymatous tissue. The tissue damage leads to increase Somatic Cell Counts (SCC) and reduce milk production in the first lactation. Additionally, these bacteria can be persistent and they can increase the risk for clinical mastitis during early postpartum (Trinidad *et al*., 1990a; Nickerson *et al*., 1995; Zhao and Lacasse, 2007; Sampimon *et al*., 2009b). The Coagulase Negative Staphylococci (CNS) and *Staphylococcus aureus* have been reported to be the most isolated bacteria in heifer mastitis (Oliver and Mitchell, 1983; Fox *et al*., 1995; Malinowski *et al*., 2003; Piepers *et al*., 2009). Coagulase negative staphylococci and *S. aureus* can penetrate into the epithelial cells and provide a microenvironment free from immunologic response. Although, CNS infections can recover spontaneously, *S. aureus* become usually persistent (Owens *et al*., 2001; Deluyker *et al*., 2005; Fox, 2009).

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The previous studies report that the cure rates of these infections for non-lactating heifers are higher than lactating cows and pre-calving antibiotic treatment is suggested for reduction of mastitis incidence during early postpartum. Additionally, economic losses due to discarded milk can be prevented by precalving treatment (Nickerson *et al*., 1995; Watts *et al*., 1995; Owens *et al*., 2001; Andrew *et al*., 2009).

The aim of this study was to evaluate effectiveness of precalving antibiotic treatment on mastitis before and after calving in heifers. The efficacy of the treatment was attained by bacteriologic results and test day Individual Somatic Cell Counts (ISCC).

MATERIALS AND METHODS

Heifers and experimental groups: In the presented study, 369 mammary quarters from 96 Holstein-Friesian pregnant (6-7 months) heifers belonging to 6 commercial dairy farms were used in Turkey. The heifers were chosen if they had no signs of clinical mastitis, had four quarters free of teat abnormalities and had not received antibiotic or anti-inflammatory treatment during the previous 30 days. The pregnant heifers were detected by using rectal palpation and insemination data.

The heifers in each herd were divided randomly into two groups: dry period (last 45 days prepartum) antibiotic treatment (200 mg cephalexin monohydrate and 250 mg neomycin; Rilexine 500, Virbac, France (Group 1, n = 41) or no dry period antibiotics administration (Group 2, n = 55).

Sample collection and laboratory analysis: Glandular secretion samples were collected from pregnant heifers according to recommended procedures for milk samples (Harmon *et al*., 1990). Teat ends were cleaned by 70% ethyl alcohol-soaked gauze and nearly 3 mL udder secretions were collected using a gentle milking into 15 mL sterile plastic tubes. After sampling, 1% iodine solution was applied to teat-ends. Samples were transported to the laboratory at +4°C.

Milk samples were collected aseptically for bacteriology and SCC day 10 postpartum. The samples were collected from fresh cows according to National Mastitis Council (NMC) protocols (Harmon *et al*., 1990). Dirty teats were washed and dried with a towel. Teat ends were sanitized with 70% ethyl alcohol-soaked gauze. A few streams of milk were discarded to reduce the number of contaminating bacteria in the teat canal. Plastic tubes were held as near the horizontal as possible and milk samples were collected into the 15 mL plastic tubes. After sampling, the teats were dipped in 1% iodine solutions. The samples were carried to the laboratory at +4°C.

All samples (milk and glandular secretion) were homogenized at room temperature and bacteriologic tests were performed according to NMC procedures (Harmon *et al*., 1990). The samples (0.01 mL) were spread on 6% sheep blood and MacConkey agar by using disposable plastic loops. Plates were incubated at constant temperature (37°C) for 24 and 48 h. Gram staining was performed and gram positive colonies examined by catalase tests. Catalase positive and negative colonies accepted to be staphylococci and streptococci, respectively. Coagulase tests were used for differentiation of *S. aureus* and CNS colonies. *Staphylococcus aureus* colonies had coagulase positive reactions. Streptococci were classified according to colony morphology, hemolytic properties, CAMP (Christie, Atkins, Munch-Petersen) test, Lancefield group and hydrolysis of esculin and hippurate. *Streptococcus dysgalactiae* had CAMP negative reaction and it was positive for lancefield group C. *Streptococcus agalactiae* was positive for group B and hippurate test. *Streptococcus uberis* hydrolyzed the esculin. *Escherichia coli* had positive reactions for catalase, indole, methyl red and lactose tests. *Bacillus* sp. and *Corynebacterium bovis* were identified by comparing of colony morphologies on blood agar. At the same time with culturing procedure, test day ISCC were detected by using flow cytometry method (Bactocount IBCm, Bentley Instrument, USA).

Prepartum infections were accepted cured if the same quarters had negative bacteriologic results postpartum. If infected and uninfected quarters were infected by different microorganism postpartum, these were considered a new infection. If the same bacteria were isolated in the same quarters before and after calving, this infection was considered persistent.

Statistical analysis: The percentages of infected quarters were evaluated by using descriptive statistics. Differences in prevalence of infected and uninfected quarters within the treatment and control groups after calving were evaluated using Pearson Chi-square test and $p<0.05$ was considered significant (PASW statistics version 18.0, SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

In the presented study, 56 (58.3%) of 96 heifers had infection at least in one quarter before calving. Mastitis pathogens were isolated from 114 (31%) of 369 quarters before treatment (Table 1). These findings were similar to those previous reports. Borm *et al*. (2006) found that 34.1% of mammary quarters and 63.4% of heifers were infected before calving. Malinowski *et al*. (2003) reported

Table 1: Prevalence of intramammary infection in heifers before the treatment

	Treatment group		Control group	
Heifers	n	%	n	%
Infected quarters	52	32.1	62	30.0
Healthy quarters	110	67.9	145	70.0
Total	162	100.0	207	100.0
Infected heifers[1]	27	65.9	29	52.7
Healthy heifers	14	34.1	26	47.3
Total	41	100.0	55	100.0

[1]The heifers had at least one mammary quarter infected

Table 2: Intramammary infections in all quarters before the treatment

	Treatment group		Control group	
Bacteria	n	%	n	%
CNS	28	53.8	45	72.5
S. aureus	17	32.7	15	24.2
Other pathogens[1]	7	13.5	2	3.3
Total	52	100.0	62	100.0

[1]The mammary quarters infected with *E. coli* (n = 1), *C. bovis* (n =1) and *Bacillus* sp. (n = 5) in the treatment group and with *E. coli* (n = 1) and *C. bovis* (n = 1) in the control group

34.8% of the quarters with infection in pregnant heifers. In some studies, the prevalence of infections was recorded higher than presented study (Trinidad *et al*., 1990b; Oliver *et al*., 1992, 1997, 2004; Owens *et al*., 2001; Sampimon *et al*., 2009b).

According to bacteriologic results, (CNS) (64%) and *S. aureus* (28.1%) were the most isolated bacteria prepartum. *E. coli*, *C. bovis* and *Bacillus* sp. were the other pathogens isolated from infected quarters (Table 2). Previous reports also confirmed these findings. Coagulase negative staphylococci were reported to be the most detected bacteria in heifer mastitis and CNS infections were observed in nearly half of the quarters (Trinidad *et al*. 1990b; Oliver *et al*., 1992). In the current study, prevalence of *S. aureus* was detected higher than other reports (Trinidad *et al*. 1990b; Fox *et al*., 1995; Aarestrup and Jensen, 1997; Owens *et al*., 2001; Malinowski *et al*., 2003).

Studies dealing with the effects of prepartum intramammary antibiotic treatment showed that 90% of staphylococci were found susceptible to antibiotics. Especially, the treatment was reported to be very effective on CNS and *S. aureus* infections (Oliver *et al*., 1992, 2004; Owens and Ray, 1996; Owens *et al*., 2001; Borm *et al*., 2006; Nickerson, 2009). Several products were used and different cure rates were reported ranging from 60-100% according to bacterial species and duration of pregnancy (Owens *et al*., 1994, 2001; Owens and Ray, 1996; Oliver *et al*., 2004; Borm *et al*., 2006). To the knowledge, the effects of antibiotic treatment on heifer mastitis were not reported in Turkey. In the presented study, cure rates were 82.1, 76.5 and 100% for CNS, *S. aureus* and other pathogens in group 1, respectively. The average cure rate was 82.6% in this group (Table 3).

Table 3: Intramammary infections in the treatment group after calving (n)

Bacteria	Quarters before calving	[2]Quarters after calving	Cured	Quarters with persisted IMI	Quarters with new IMI
CNS	28	26	23	5	21
S. aureus	17	7	13	4	3
Others[1]	7	13	7	-	13
Streptococcus sp.[3]	-	6	-	-	6

[1]The mammary quarters infected with *C. bovis* (n = 2) and *Bacillus* sp. (n = 11). [2]One-hundred sixty two mammary quarters were evaluated after calving in group 1. [3]*Streptococcus uberis* (n = 4), *Streptococcus dysgalactiae* (n = 1) and *Streptococcus agalactiae* (n = 1)

Although, antibiotic treatment reduced the Intra Mammary Infection (IMI) rates before calving, it was not sufficient for reduction of new infections (Oliver *et al*., 1992, 2004; Middleton *et al*., 2005; Roy *et al*., 2007). Oliver *et al*. (2004) stated 51% new infection rates in early lactation for antibiotic treated group. Oliver *et al*. (1992) found 13.6% persistent IMI after antibiotic treatment 7 days before expected calving. Additionally, chronic infection rates were detected 67 and 27% for CNS and *S. aureus*, respectively (Oliver *et al*., 1992). In the presented study, CNS were the most detected bacteria postpartum in group 1. Persisted IMI caused by CNS and *S. aureus* were detected in 9 (17, 3%) of 52 infected quarters. Of the 52 infected quarters postpartum, 43 quarters had new IMI. New infection rates (82.6%) were obviously high in the treatment group, suggesting that precalving antibiotic treatment was not effective on reducing the new IMI in the beginning of the first lactation (Table 4). In both groups, *Streptococcus* sp. was isolated after calving, which is in agreement with the results reported by Aarestrup and Jensen (1997). It seemed that increased environmental infections was related with milking hygiene and decreased immunologic response (Table 3).

Oliver *et al*. (1997) reported 55.6% infection rates for untreated quarters after calving. Roy *et al*. (2007) stated 37 and 75% spontaneous cure rates for *S. aureus* and CNS infections in control group. Oliver *et al*. (2004) detected 56 and 63.3% spontaneous cure and persisted IMI rates for untreated control group. In the present study, spontaneous cure rates were 62.2 and 86.6% for CNS and *S. aureus* in group 2. Average spontaneous cure rate was 69.3% in this group. Of the quarters untreated in the control group, 32% were infected with mastitis pathogens and CNS and *S. aureus* were the most isolated group of bacteria after calving. The infection rates were 8.2 and 19.1% for *S. aureus* and CNS after calving. New IMI and persisted IMI rates were found 24.2 and 26.3% for group 2 (Table 5).

Previous studies indicated that precalving antibiotic treatment reduced the quarter milk SCC in early lactation (Hallberg *et al*., 1995; Nickerson *et al*., 1995; Oliver *et al*.,

Table 4: Effectiveness of the precalving antibiotic treatment on new IMI after calving (n)

	Bacteriologic result		
Groups	Uninfected quarters	Infected quarters	p<
1	110	52	0.87
2	147	72	
[1]Total	257	124	

[1]Two quarters in group 1 and 1 quarter in group 2 were nonfunctional

Table 5: Intramammary infections in the control group after calving (n)

Bacteria	Quarters before calving	[2]Quarters after calving	Cured spontaneously	Quarters with persisted IMI	Quarters with new IMI
CNS	45	42	28	17	25
S. aureus	15	18	13	2	16
Other pathogens[1]	2	10	2	-	10
[3]*Streptococcus agalactiae*	-	2	-	-	2

[1]The mammary quarters infected with *Bacillus spp.* (n = 10). [2]Two-hundred nineteen mammary quarters were evaluated after calving in group 2. [3]Streptococcus agalactiae was not isolated in quarters before calving

Table 6: Individual Somatic Cell Counts (ISCC) in quarters for group 1 and 2*

ISCC×1,000 cells mL^{-1}	Treatment n (%)	Control n (%)
<200	187 (91.2)	150 (68.4)
200-400	13 (6.3)	13 (5.9)
>400	5 (2.4)	56 (25.5)

*162 and 219 mammary quarters were evaluated in the treatment and control groups, respectively

2003; Sampimon *et al*., 2009b). Contrary to these reports, Borm *et al*. (2006) did not report significant effect on SCC. In this study, the test day ISCC were classified as <200,000, 200,000 to 400,000 and >400,000 cells mL^{-1} and compared between two groups. According to results, group 1 had more quarters with <200,000 cells mL^{-1} than group 2. On the other hand, there were more quarters with high SCC (>400,000 cells mL^{-1}) in group 2. The intramammary antibiotic treatment before calving was effective to reducing the ISCC (Table 6).

CONCLUSION

Intramammary antibiotic treatment (200 mg cephalexin monohydrate and 250 mg neomycin) 45 days prior to parturition was effective for heifer mastitis. The treatment was also preventive against high SCC. However, it was insufficient to prevent new IMI at the beginning of the first lactation.

Perhaps, new infections were related with milking and environmental hygiene in this study. Heifers are the genetic future and future productivity of dairy herds. Antibiotic treatment before calving may be the key point in heifer mastitis prevention program due to minimum residues and maximum effectiveness on bacteria.

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