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Coagulase Negative *Staphylococci* and *Staphylococcus aureus*, the Main Organisms Causing Pre and Post Calving Heifer Mastitis in a Holstein Dairy Farm

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Abstract: The main microbial causes of IMI in heifers in a dairy farm with long history of high incidence of mastitis were studied in 53 pregnant heifers. Mammary secretions (52 samples) and milk (53 samples) were taken on day 5±5 before and day 10±5 after calving, respectively. Each sterile composite sample from 4 quarters was obtained, refrigerated and transported into the lab for culture and SCC. Results showed that in the pre calving samples, the most frequent bacteria found were: *CNS*, *Staph. aureus, E.coli, Strep. dysgalactia* and *Strep. uberis* at the rate of 71, 68, 57, 35 and 32% of the samples, respectively. In post calving samples, prevalence of *CNS, Staph. aureus, E.coli, Strep. dysgalactia, agalactia, bovis* and *uberis* was 69, 47, 18, 37, 24 and 5%, respectively. *Streptococci* were found in all the pre and/or post parturition samples. Yeasts were isolated from 7.7% of the pre and 9.43% of the post calving samples. The most prevalent CNS was *Staph. chromogenes* which was found in 20% of the pre and 28% of the post parturition samples. SCC in all the samples were higher than the local standards (2×10^5), showing high contamination of the mammary glands with environmental and/or contagious microorganisms.

Key words: Heifer mastitis, CNS, *Staph. aureus*, SCC, TBC

INTRODUCTION

Microorganisms responsible for IMI in heifers have been studied in different regions and countries (Aarestrup and Jensen, 1997; Daniel *et al*., 1986; Fox *et al*., 1995; Munch-Petersen, 1970; Myllys, 1995; Myllys and Rautala, 1995; Pankey *et al*., 1991). Coagulase Negative *Staphylococci* (CNS) have been determined to be the most prevalent bacteria. In some studies, *Streptococcus dysgalactia* and *uberis* have been isolated but the rate of incidence was low. Prevalence of *Staphylococcus aureus* IMI in pre calving heifers was variable in different regions and dairy farms so that, in some studies it was found in few cases (Daniel *et al*., 1986; Pankey *et al*., 1991) or not found at all (Aarestrup and Jensen, 1997), while others reported a high prevalence (Nickerson *et al*., 1995; Trinidad *et al*., 1990).

Mammary glands of heifers are generally considered free of infection before or during parturition. However, high incidence of heifer IMI caused by CNS have been reported (Oliver *et al*., 2005; Oliver 1992). These cases are not severe (Trinidad *et al*., 1990) and when clinical signs are present, it is not as severe as the mastitis produced by the major bacteria like *Staph. aureus* (Birgersson *et al*., 1992; Myllys *et al*., 1994). However, IMI and mastitis in the first parity heifers is an important devastating disease. Health of the udders of the newly entering heifers into the herd affects the quantity and quality of the milk produced in the future (Trinidad *et al*., 1990) and intact quarters can not compensate loss of milk production by unhealthy quarters (Woolford, 1985). IMI increase SCC (Trinidad *et al*., 1990) and infected heifers may act as a bacterial reservoir including *Staph. aureus* in dairy herds (Matthews *et al*., 1992; Roberson *et al*., 1994). Studies on the mammary secretions of the heifers before calving showed that mammary glands of many of them are potential source of mastitis bearing bacteria. Although, the results are contradictory but rate of IMIs between 30-50% in the quarters before and/or during parturition is not unexpected (Aarestrup and Jensen, 1997; Fox *et al*., 1995). It has been reported that *Staph. aureus, Strep. dysgalactia, agalactia, Arcanobacter pyogenes, E.coli* and CNS are the most prevalent bacteria pertaining to clinical and sub-clinical IMIs in heifers (Jonsson *et al*., 1991; Myllys and Rautala, 1995; Waage *et al*., 1990).

The aim of the present study was to determine rate of heifer IMI before and after parturition and to identify the main causative microorganisms in a Holstein dairy farm with long history of high incidence of mastitis.

MATERIALS AND METHODS

Fifty three pregnant healthy heifers with no pre-parturient problems were chosen. Composite mammary

gland secretions (52 samples) and milk (53 samples) were taken on day 5±5 before and 10±5 days after calving, respectively. Each sterile sample was obtained from 4 quarters according to the procedure explained by Fox *et al.* (1995) for bacterial culture and SCC. Refrigerated samples were transported into the lab for culture and SCC. At the same time CMT was performed for all the quarters and grade 1 to 3 positive samples were sent to the lab for SCC (Fossomatic™ FC counter; Foss Electric, Hillerød, Denmark). In the dairy farm, all the sanitary precautions including washing, drying, strip cup test and teat dipping were practiced routinely. In order to separate the major mastitis bearing microorganisms from secondary contaminants, milk samples were cultured in the primary and specific media, successively. Major microorganisms were identified by use of biochemical tests.

SPSS package was used for statistical analysis. Comparison of TBC and SCC of the pre and post calving milk samples was made by paired Student's t-test and mean TBC was compared by ANOVA. Chi square was used for the comparison of number of contaminated samples.

RESULTS AND DISCUSSION

Feeding milk contaminated with *Strep. agalactia* (Schalm, 1942) and *Staph. aureus* (Roberson *et al*., 1994) to calves and suckling other calves teats in highly contaminated herds causes contamination of the mammary glands which remains dormant until parturition time or after that. Importance of IMI control in young heifers arose from the fact that mammary gland development and future milk production are affected seriously (Nickerson *et al*., 1995; Oliver and Sordillo, 1988; Pankey *et al*., 1991). Table 1 shows the rate of IMI before and after parturition in the present study. Almost, all of the heifers were contaminated both before and after parturition. *Staph. aureus* was found in lower rates after calving due to evacuation of milk and reduction in bacterial population of the mammary glands. High incidence of IMI in non inseminated (86.7%) and/or pregnant heifers (70%) had been reported (Trinidad *et al*., 1990). Rate of incidence is mainly dependent on the prevalence of contagious bacteria especially *Staph. aureus* in the herd (Roberson *et al*., 1994). In the present study almost 100% of the samples were contaminated. Trinidad *et al*. (1990) found 8 different species of *Staphylococci* and highest rates belonged to *Staph. aureus*, *chromogens* and *hyicus* with CNS being prevailed in 67% of the samples. In the present study 6 species of *Staphylococci* was found while *Staph. aureus, chromogens* and *epidermidis* having the highest rates. Prevalence of CNS in the pre and post calving samples were 71.15 and 69.81%, respectively. Mixed contamination was found in many of the samples.

Table 1: Microorganisms isolated from pre and post calving samples. Significant differences are show by different letters ($p<0.05$)

	Pre calving samples		Post calving samples	
Microorganism	Sample size	(%)	Sample size	(%)
Staph. aureus	35	67.30	25	47.17
Coagulase -ve Staphylococci	37	71.15	37	69.81
Staph. Epidermidis	9	17.30	10	18.87
Staph. Chromogens	20	38.46	15	28.30
Staph. Hæmoliticus	4	7.70	5	9.43
Staph. Saprophyticus	4[b]	7.70	7[a]	13.21
Staph. Hyicus	6	11.54	7	13.21
Corynebacteriom sp.	7	13.46	2	3.77
Enterococcus sp.	19	36.54	22	41.51
E. coli	30[b]	57.70	10[a]	18.87
Klebsiella sp.	13[b]	25.00	3[a]	5.66
Pseudomonas sp.	13	25.00	14	26.42
Strep. agalactia	-	-	13	24.53
Strep. dysgalactia	19	36.54	20	37.74
Strep. uberis	17[b]	32.70	3[a]	5.66
Strep. bovis	11	21.15	13	24.53
Other strep.	52	100.00	53	100.00
Yeasts	4	7.70	5	9.43

Strep. agalactia was not found in pre calving samples but, was isolated in 24.53% of post calving samples showing contamination after machine milking was started. *Staph. aureus* and *Strep. agalactia* were previously isolated from infected milk samples from the herd. Rate of contamination with environmental *Streptococcal* and *non-streptococcal* bacteria was 100% indicating severe contamination of the dairy environment. Treatment of infected heifers after proper diagnosis is a very helpful control measure for the whole herd. If mastitis is constantly present in the herd, dry cows and heifers might be routinely treated with dry and milking cow preparations on day 45 and 14 pre calving, respectively. Sensitivity of *Staph. aureus* to this treatment regime has been proven (Jaenicke *et al*., 1999).

Candida was also isolated from the samples, verifying previous sporadic isolations from milking cows. Presence of mycotic IMI in the farm might be due to the extensive and continuous use of antibiotics for the treatment of mastitis in cows. Fungi and yeasts frequently may cause IMI (Gancedo *et al*., 2000) and may be responsible for wide morbidity and even mortality. Most of the fungi and yeasts invade mammary glands of chronically infected cows as an opportunist and grow *in-situ* but, infection is normally mild (Gancedo *et al*., 2000). Entrance into the teats usually happens during non-aseptic intra-mammary injections (Malinowski *et al*., 2002). When mycotic IMI has been prevailed in the herd, milking machine may be the other rout of infection. Moreover, fungi and yeasts can easily grow in the suitable environment provided by bedding (Malinowski *et al*., 2002).

Table 2: SCC of the pre and post calving samples. No significant differences was present

Most prevalent microorganisms	Mean SCC before calving	Mean SCC after calving
Mixed infection with ≥ 2 microorganisms	2.3×10^6	2.2×10^5
Staph. aureus	7.6×10^6	6.9×10^6
Strep. agalactia	9.2×10^6	10.6×10^5
E. coli	2.5×10^6	3.2×10^5
Yeasts (Candida)	8.7×10^4	4.8×10^4

SCC: SCC was determined in the pre calving samples, either from mastitis or non mastitis cases. SCC of the pre and post calving samples containing most prevalent bacterial contamination are shown in Table 2.

From the Table 2 it might be inferred that SCC in the pre calving samples were generally higher than post calving samples. Considering the fact that pre calving samples are more concentrated (Hallberg, 1997), milking of heifers reduce somatic cell population and bacteria as well (Owens and Oliver, 1998). Milking heifers 2-3 weeks before calving is one way to reduce rate of mastitis and SCC (Daniels *et al*., 2007). SCC was high in the samples having a mixed contamination, indicating the importance of these bacteria in heifer mastitis. Nickerson *et al.* (1995) reported that SCC in the mammary glands contaminated with *Staph. aureus* was 9.2×10^6 mL while in the present study, there were 7.6×10^6 and 6.9×10^6 somatic cells per ml in the pre and post calving samples, respectively. Results show that *Staph. aureus* has increased SCC tremendously as compared to other bacteria.

CONCLUSION

Dairy farmers should be aware of the heifer mastitis in their farm by monitoring situation constantly. Regulations should be set when new heifers are supposed to enter into the herd (Waage *et al*., 1998). If there are no conditions set, these heifers may transfer harmful microorganisms into the environment and the milking machine, therefore, healthy heifers are at risk and mammary gland growth and development might be retarded (Woolford, 1985).

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