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Prevalence of Dairy Heifer IMI and the Associated Bacteria Before and after Parturition as Determined by Use of Bacterial Culture, SCC, CMT and TBC

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Abstract: To determine pre and post calving major infectious microorganisms in dairy heifer mammary glands, a study was conducted for a period of 18 months in 6 large industrial dairy farms in Mashhad, north east of Iran. Composite sterile milk samples were taken on day 5±5 before and 10±5 after calving (168 and 157 samples, respectively) from 168 Holstein dairy heifers. Heifers had no apparent pre-parturient problems. Milk samples were refrigerated and sent to the food microbiology lab of the faculty of veterinary medicine for culture and antibiotic sensitivity test. At the time of sampling, CMT was done for all the quarters and positive samples were sent to the lab for SCC. If a heifer was showing signs of clinical mastitis before or during parturition, milk sample was taken and sent to the microbiology lab. Washing, drying, milking and teat deeping was practiced regularly in all the farms engaged in the research. Pre-calving milk samples had either thick, thin, or normal consistency. Normal samples had mostly milky-creamy appearance. Average milk pH of the 3 groups had no significant difference ($p>0.05$) but, pH variations in the samples from normal group were much less than the other 2 groups. Thick and thin samples had a pH of 6.74 and 7.31, respectively. Mean TBC of the thick and thin samples were 3.1×10^5 and 4.9×10^5, respectively. Mean TBC of the thin samples was different from the other two groups ($p<0.05$) but it was not different between normal and thick samples ($p>0.05$). Thirty five percent of the pre-calving and 47.11% of the post-calving milk samples contained mastitis bearing bacteria and the difference was significant ($p<0.05$). Post calving samples had lower but not significant SCC than the pre calving samples ($p>0.05$). Mean SCC of the samples infected with coagulase negative *Staph. chromogens*, *Hyicus*, *Haemolyticus*, *Saprophyticus* and *Epidermidis*, were lower than those infected with *Staph. aureus*. Mean SCC in *E. coli* containing samples collected after calving was lower than those samples containing *Staph. aureus*. Mean SCC in post calving samples from quarters infected with *Staph. chromogens*, *hyicus* and *aureus* were, 7.8×10^5, 8.5×10^4 and 9.2×10^6, respectively. In conclusion, a high proportion of the peri-parturient heifers had infected quarters. Coagulase negative *Staph.*, *Staph. aureus*, *Enterobacter* sp. and *E.coli* were important microorganisms causing IMI in heifers.

Key words: *Staph*, strep, environmental, mastitis, heifers, SCC, TBC

INTRODUCTION

Heifer mastitis has been noticed from many years ago (Palmer *et al*., 1941; Schlam, 1942). Dairy heifers are exposed to mastitis from early stages of their life (Trinidad *et al*., 1990b). This may happen during 4 stages: while keeping calves in individual or group pens, after weaning, around insemination and before parturition (Boddie *et al*., 1987). Most of the IMIs occur in 8-9 months of age at the time the mammary glands start to produce secretions (Crist, 1997). Invasion of microorganisms may occur in 5 different routs: from the environment, suckling of the mammary glands by infected calves, consumption of contaminated milk, trauma to the mammary glands and biting of the insects (Nickerson, 1998; Owens *et al*., 1998). Results of some of the researches in commercial and experimental herds show IMI rates as high as 97% (Nickerson, 1990) but, mean rate has been determined to be around 8.1%. Pankey *et al*. (1991) claimed rate of contamination to be 46% for the heifers and 19% for the quarters. In some other experiments, infected heifers were showing clinical signs of mastitis immediately or within a few days of parturition (Jaenicke *et al*., 1999). Presence of *Staph*ylococci, *Streptococci*, *Coliforms*, *Corynebacteria*, *Pseudomonas*, *Nocardia*, my*coplasma* and fugi were demonstrated in cases of heifer mastitis (Hogan, 1999; Oliver *et al*., 2000). Coagulase negative *Staph. chromogens* and *epidermidis* are determined to be the most prevalent causes of heifer mastitis but *Staph. aureus*

is the highest cost bearing while environmental microorganisms have their own merit (Nickerson, 1998; Roberson, 1998).

Species of CNS that are routinely cultured from milk samples are normal habitat of skin and include *Staph. chromogens*, *hyicus*, simulance and *epidermidis*. Most of the IMIs due to the *Streptococci*, Mycoplasma and *Coliforms* usually disappear within first two weeks of life (Owens *et al*., 1998). Rate of infections are quite different in various herds reasonably, due to diversity of management standards, degree of environmental contamination, bedding type and material, udder sanitation, degree of contamination of milk fed to calves, fly control and so on (Bowen and Lawrence, 2005).

Highly concentrated pre calving secretions of the udder is high in SCC (Nickerson *et al*., 1995). In quarters infected with *Staph. aureus*, SCC is higher than those infected with CNS and environmental *Streptococci* (Hallberg, 1997). Greater numbers of SCC due to chronic IMI, affects growth and development of the udders and reduces milk production potential significantly (Owens *et al*., 1993).

MATERIALS AND METHODS

To determine pre and post calving major infectious microorganisms in dairy heifer mammary glands and rate of heifer IMI, a study was designed for a period of 18 months in 6 large industrial dairy farms in Mashhad, north east of Iran. Composite sterile milk samples were taken on day 5±5 before and 10±5 after calving (168 and 157 samples, respectively) from 168 Holstein dairy heifers according to the procedure explained by Fox (1995). Heifers had no apparent peri-parturient problems. The teat ends were cleaned with alcohol swabs and allowed to dry. The first few streams were discarded and then 2-4 mL of secretion was collected in sterile tubes. Samples were cooled and immediately transported to the food microbiology lab of the faculty of veterinary medicine for culture and antibiotic sensitivity tests. At the time of sampling, CMT was done for all the quarters and positive samples were sent to the lab for SCC (Fossomatic™ FC counter; Foss Electric, Hillerød, Denmark). If a heifer was showing signs of clinical mastitis before parturition, milk sample was taken and sent to the microbiology lab. In all the farms engaged in the research, washing, drying, milking and teat deeping were a routine practice. In order to separate the major mastitis microorganisms from secondary contaminants, milk samples were cultured in the primary and specific media, successively. Genus and species of the microorganisms were determined by use of biochemical tests.

Statistical analysis was done by SPSS package. For the comparison of TBC and SCC of the pre and post calving milk samples, paired Student's t-test was used and for comparison of mean TBC and pH, ANOVA was performed. Chi square was used for the comparison of number of samples contaminated with microorganisms.

RESULTS AND DISCUSSION

Pre-calving samples

Physical properties: Pre calving samples were different in color, consistency and volume. Samples with thicker and creamy-yellowish appearance were less contaminated (colostrum consistency) while more contaminated ones had thinner consistency (Table 1). Mean TBC was different in thin samples as compared with other 2 types of samples ($p<0.05$). Normal samples had a milky-creamy color without any clot. The difference between TBC of normal and thick samples were not significant ($p>0.05$). Mean pH of the three groups were not different ($p>0.05$) but, variations of the pH was lower in the normal samples. Mastitis bearing microorganisms were separated from 35% of the pre and 47.11% of the post calving samples and the difference was significant ($p<0.05$).

Nickerson *et al*. (1995) demonstrated that 10% of the samples with honey consistency and yellowish color were contaminated while 78% of the samples that had clots with thin consistency were infected. In the present study mean TBC of the thick and thin samples were 3.094×10^5 and 4.885×10^5 mL^{-1}, respectively. In the same samples, pH was 6.74 and 7.31 in the thick and thin samples, respectively. Normal pH of the cow milk is 6.6-6.9 (Bowen and Lawrence, 2005) therefore, thick samples mixed with colostrums, had a normal pH but, the thin samples had alkaline pH that was probably due to growth of microorganisms (Table 1).

Microorganisms found in the samples: Table 2 shows microorganisms separated from pre calving samples. With regard to the diversity of the microorganisms found, the major contagious, environmental microorganisms and the rate of infection are shown.

Staph. chromogens, *epidermidis*, *saprophyticus* and *haemolyticus* were among the most prevalent CNS in the pre calving samples (>84%), environmental *Streptococci* have also been separated from the same samples. Three of the samples contained *Pediococcus*. This is a Gram positive, selective anaerobic, non-motile and non spore forming bacteria (Garvie, 1986). Likewise, in 26 of the pre and post calving samples a bacterium from genus Leuconostac have been cultured. This cocci is anaerobic and grows well in temperatures around 1-5 Celsius,

Table 1: Physical properties and rate of contamination of the pre calving samples

Physical appearance	Mean pH of the samples	Mean TBC ($10^5\times$)	Number of samples containing germs	% of the samples	Sample consistency
Dark/light yellow or brown	6.74	3.094	96	13.09	Thick
Light yellow, thin milky, colorless with/without clot	7.313	4.885	150	18.45	Thin
White, milky-cream	6.72	3.6	120	68.45	Normal

Table 2: Microorganisms separated from pre calving samples and their prevalence (%)

Most prevalent microorganisms and rate of contamination of the samples (%)		Less prevalent and non significant microorganisms found in the samples
Name	%	Name
yeasts (Candida)	7.65	*Bacillus cereus*
Strep. dysgalactia	1.89	*Bacillus* sp.
Strep. bovis	1.89	*Clostridium* sp.
Staph. epidermidis	32.96	*Lactobacillus* sp.
Staph. chromogens	31.08	*Leuconstoc* sp.
Staph. haemoliticus	13.2	*Micrococcus* sp.
Staph. saprophyticus	7.65	*Other Strep.*
Corynebacteriom sp.	1.89	*Paracolobacterium* sp.
E. coli	1.89	*Pediococcus* sp..
		Strep. lactice
		Klebsiella sp.
		Pseudomonas sp.
		Enterococcus sp.

Table 3: Microorganisms separated from post calving samples

Most prevalent microorganisms and rate of contamination (%)		Less prevalent and non significant microorganisms found in the samples
Name	%	*Bacillus cereus*
yeasts (Candida)	1.92	*Bacillus* sp.
Strep. dysgalactia	7.7	*Clostridium* sp.
Strep. agalactia	5.77	*Lactobacillus* sp.
Staph. epidermidis	29.73	*Leuconostoc* sp.
Staph. hyicus	1.92	*Micrococcus* sp.
Staph. aureus	6.73	*Other Strep.*
Staph. chromogens	24	*Paracolobacterium* sp.
Staph. haemoliticus	17.27	*Pediococcus* sp.
Staph. saprophyticus	3.85	*Strep. lactice*
E. coli	0.96	*Klebsiella* sp.
		Pseudomonas sp.
		Enterococcus sp.

produces lactic acid but is not mastitis bearing and probably is a secondary contaminants after sampling. *Candida* sp., have been separated from 7.65% of the pre and 1.92% of the post calving samples (Table 2 and 3). *Candida* had also been cultured from the samples taken from one of the herds before the present experiment. In order to confirm the results, new samples were taken and presence of *Candida* was verified in the same animals.

More than 90% of the microorganisms separated from pre calving samples were environmental and non contagious microorganisms. *Staph. aureus* and Stre. agalactia have not been found in those samples. Our results are similar to the results of others (Oliver *et al*., 2000) that could separate *Staph*. sp., *Strep*. sp., *Coliforms*, *Corynebacteria*, *Pseudomonas*, *Nocardia*, *Mycoplasma* and *yeasts*.

Post calving samples: *Staph. aureus* (6.73%) and *Strep. agalactia* (5.77%) were separated from post calving samples, indicating that infection have been introduced into the udders after parturition (Table 3). These two bacteria were not present in the pre calving sam;es. Moreover, infection with *yeasts* and environmental bacteria were decreased from 7.65-1.92% and 90.56-84.46%, respectively, showing diminishing population of environmental microorganisms in IMIs.

Researchers in the U.S. demonstrated that rate of infection before parturition was around 7.5% but increased to 24% after calving (Trinidad *et al*., 1999a). In the present study pre and post calving rate of IMIs were 35 and 47.11, respectively. In another experiment conducted in 2000-2003 in the University of Minnesota, on 104812 dairy cows, 80% of the causative microorganisms were environmental, 14% contagious and 6% miscellaneous (Oliver *et al*., 2005). In the present study, 4 cases (2.38%) of pre calving clinical mastitis were observed and one case (0.595%) died immediately after parturition. Seven (4.46%) cases of post calving clinical mastitis were treated and recovered completely. Increase in the number of post calving clinical mastitis is an indication of physiological changes that happen during the transition period in the mammary glands. This, along with the calving and milking stress, diminishes mammary glands resistance against microorganisms, therefore, mastitis reveals during or after calving. Reasonably, it is preferable to treat pre calving IMIs before parturition rather than after (Oliver *et al*., 1992; Trinidad *et al*., 1990c).

It is not possible to control skin bacterial flora and these opportunist bacteria may penetrate teat canal at proper time. On the other hand, feeding calves with milk from cows suffering from IMI and suckling of the teats of the pen-mate calves by contaminated mouth, increases risk of IMI in non infected calves. Keeping calves in individual pens is a good control measure for this problem (Nickerson *et al*., 1995, 1992; Nickerson, 1998). Due to a major role played by insects in spreading IMIs into healthy animals, use of insecticides especially at larval stage is crucial in the dairy barns (Owens *et al*., 2001). In all of the herds under this research, lack of regular insect control measures were noticed.

Waage *et al*. (1999) demonstrated that *Staph. aureus*, along with some environmental bacteria are the most prevalent causes of pre-calving mastitis in heifers. They did not find *Strep. agalactia* but, in our experiment we found this microorganism in pos-calving samples only. SCC When possible, pre and post calving sample SCC were determined (Table 4).

Table 4: Pre and post calving SCC

Most prevalent microorganisms	Mean SCC after parturition	Mean SCC beforeparturition
yeasts (Candida)	8.7×10^4	$10^4 \times 4.8$
Strep. dysgalactia	8.1×10^3	$10^5 \times 4.5$
Strep. agalactia	-----	$10^5 \times 10.6$
Staph. epidermidis	3.8×10^4	9.8×10^4
Staph. hyicus	-----	7.3×10^4
Staph. aureus	-----	8.9×10^6
Corynebacterium sp.	7.3×10^3	-----
E. coli	2.5×10^6	3.2×10^6
Staph. chromogens	7.8×10^4	7.1×10^5
Staph. haemoliticus	2.8×10^4	3.3×10^4
Staph. saprophyticus	6.6×10^3	7.7×10^4
Strep. bovis	9.4×10^3	7.9×10^4
Mixed infection with = 2 microorganisms	2.3×10^6	2.2×10^5

Trinidad *et al*. (1990b) found higher rates of IMIs in non-pregnant heifers (86.7% vs 70% in the quarters of non-pregnant and pregnant heifers, respectively). They found 8 species of *Staph*. sp. and *Staph. aureus*, *chromogens* and *hyicus* were the most prevalent ones. In our post calving samples we found 6 species of *Staph*. sp. with 32.65% of them due to the above named 3 species. In the Trinidad experiment, CNS comprised 67% of the separated bacteria while in our experiment this rate was 76.77%.

Mean SCC of pre calving samples were higher than post calving samples. In general, with the start of milking, population of somatic cells and bacteria diminishes (Owens *et al*., 1998) and it should be considered that pre calving samples are more concentrated in somatic cells (Hallberg, 1997). One of the ways to reduce rate of IMI and SCC in peri-calving heifers is to start milking 2-3 weeks before calving (Daniels *et al*., 2007).

In the samples containing CNS, mean SCC was lower than those containing *Staph. aureus* and *E.coli*. This indicates the importance of the latter two bacteria in heifer IMIs. Nickerson *et al*. (1995) reported that mean SCC in the quarters infected with *Staph. chromogens*, *hyicus* and *aureus* were, 7.8×10^6, 8.5×10^6 and 9.2×10^6 mL^{-1}, respectively. Our results for the same bacteria are 7.1×10^5, 7.3×10^4 and 8.9×10^6 mL^{-1}, respectively. From the above results it is obvious that *Staph. aureus* and *E.coli* are responsible for the elevation of SCC as compared with other bacteria.

A major reason for unfavorable mastitis control and eradication is that, control measures for dairy cows are not practiced at the time heifers enter the herd. Therefore, infected heifers act as a large reservoir of mastitis bearing microorganisms and contaminate the milking equipment (Roberson *et al*., 1994). Schlam (1942) reported that feeding contaminated milk to dairy calves and suckling of mammary glands of pen mates may spread infection that remain dormant until parturition time. There are frequent reports from other researchers showing the same sequence in herds with high incidence of *Staphylococcus* mastitis so that pre and post calving heifer mastitis is higher in those herds (Roberson *et al*., 1994). Early age infection, reduces growth and development of the mammary glands and consequently diminishes future production, therefore, heifer mastitis control is crucial for dairy farmers (Oliver, 1992; Oliver *et al*., 2005, 1997a).

Results of the present study and other researches indicate the importance of monitoring heifer IMIs by applying routine pre-calving sampling from udders (Fox and Chester, 1994; Nickerson and Boddie, 1992). Some people may be anxious about breaking the teat canal keratin seal while sampling from heifers before calving but, there are reports indicating that if, proper sanitation measures are considered before, during and after sampling by pre and post deeping of the teats, sampling would create no problem (Fox, 1995). Regular and routine udder inspection in heifers and dry cows would help detecting unusual situations in the mammary glands. Early diagnosis and monitoring the rate of IMIs in heifers and dry cows is extremely critical in dairy herds (Jaenicke *et al*., 1999) and makes it possible to conduct proper preventing and cure disciplines in affected animals (Oliver, 1992; Oliver *et al*., 1992, 1997, 2000). There are effective antibiotic preparations present in the market for the treatment the of IMIs in heifers and dry cows (Oliver *et al*., 2000; Owens *et al*., 1991, 1993).

There are vaccines against *E. coli* (Hogan, 1999) and *Staph. aureus* (Nickerson, 1998) that may help control heifer mastitis. It has been demonstrated that supplementation of vitamin E and selenium to the animals in deficient areas may decrease IMI incidence (Smith *et al*., 1985). It has been concluded that in the deficient areas, providing vitamin E and Selenium should be a routine practice either in the feed or as an injection. This procedure is practiced in khorasan province.

CONCLUSION

From the above results it can be concluded that heifer mastitis is a serious problem in dairy herds and it is necessary to consider preventive and treatment measures. Care should be taken when purchasing heifers from outside. One should also be cautious when mixing first parity heifers into the herd from the same herd, reasonably, because they may be a big reservoir of contamination. *Staph. aureus* and enterobacteriacea have their own merit in heifer mastitis.

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