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Prevalence, Bacterial Association and *In Vitro* Antimicrobials Susceptibility of Subclinical Mastitis in Crossbred Cows

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A B S T R A C T

The subclinical mastitis is a more serious concern and is responsible for much greater losses to the dairy industry in India. The prevalence of subclinical mastitis (SCM) was recorded (56.25 %) in cross breed cow, with higher involvement (33.17 %) of right hindquarters. The prevalence was also found highest (19.04 %) in third parity and early stage (41.26%) of lactation. Microbiological examination of milk sample revealed highest involvement of *Staphylococcus* spp. (66.90%), followed by *Streptococcus* spp. (23.23%), *E. Coli* (02.81%) and mixed infection (07.04%). Enrofloxacin was found to be the most effective antibiotic against SCM. For early detection of subclinical mastitis, CMT can be performed regularly as a control measure and emphasis should be given on farm management practices, particularly on milking procedure and udder sanitation.

Introduction

Bovine mastitis is the one of the most prevalent disease of the dairy industry throughout the world. The sub clinical form of mastitis is characterized by having no visible sign either in the udder or in the milk but a negative relationship exists between somatic cell counts (SCC) and the milk yield has been reported (Khan and Khan, 2006). Subclinical Mastitis (SCM) is more important due to the fact that it is 15-40 times more prevalent as compared to the clinical mastitis, it usually precedes the clinical form, is of longer duration, difficult to detect, adversely affects milk quality and production and constitutes a reservoir of microorganisms that lead to infection of other animals within the herd (Shearer and Harris, 2003). The pattern of causative agents and disease prevalence markedly differ from place to place, herd to herd and time to time. It is recognized as one of the most costly diseases with annual losses in the dairy industry touching approximately 2 billion dollars in USA.

In India the losses are estimated to be about Rs. 7,165 crores that include Rs. 4151.16 crores and Rs. 3014.35 crores due to subclinical mastitis and clinical mastitis, respectively (Bansal and Gupta, 2009) and in a crossbred cow Rs. 1, 314.10 (Singh *et al.*, 2014). The present investigation was therefore envisaged with the objective of

screening of the crossbred cows of subclinical mastitis at I.D.F. Pantnagar by using field diagnostic test i.e. California mastitis test.

Materials and Methods

112 lactating crossbred cows at Instructional Dairy farm (IDF), G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand were screened for SCM using California Mastitis Test (CMT).

The milk samples (about 10 to 15 ml) from individual quarter were collected in sterilized test tubes after cleaning the teat orifice with 70% ethyl alcohol and after discarding the first few streams of milk.

California mastitis test was conducted and interpreted as per standard method described by Pandit and Mehta (1969).

The results were read as negative (-), trace, one plus (+), two plus (++) and three plus (+++) depending upon the degree of gel formation and graded as 0, 1, 2, 3 and 4, respectively.

The prevalence of subclinical mastitis were estimated as overall, quarter wise, age wise (less than 5 years, 5 to 8 years and more than 8 years of age), parity wise, lactation stage wise i.e. early (<60 days post parturition), mid (60-90 days post parturition) or late (>90days post parturition) and Lactation yield wise viz. high (>12L), average (5-8L) and low (< 5L) milk yielders.

The different milk samples were analyzed for bacterial investigation viz. *Staphylococcus aureus, Sreptococcus agalactiae, E. coli* and mixed infection in positive cases of subclinical mastitis. The sensitivity and resistance patterns of different bacteria's against the different antibiotics were performed using Kirby -Bauer discs.

Results and Discussion

Out of 112 crossbred cows studied, 63 were positive for subclinical mastitis. Among a total of 428 quarters, 142 quarters were found positive for subclinical mastitis. The overall and quarter wise prevalence of subclinical mastitis was computed to be 56.25% and 33.17% respectively (Table 1) which was in agreement to the findings of Gupta (2010) who found an overall prevalence of subclinical mastitis as 57.72% and quarter wise prevalence as 32.98%. In the individual quarter wise prevalence study (Table 1), the highest prevalence was in right hind quarters (43.80%) followed by left hind quarters (43.68%), left fore quarters (23.85%) and right fore quarters (22.52%) which is in very close agreement to Kumar (2010). The probable reason for more involvement of hind quarters might be the frequent exposure of rear teat tips to urine and dung during their excretion. Study also revealed that out of 20 (4.67%) blind quarters, 4 (20.00%) were left fore, 5 (25.00%) were right fore, 6 (30.00%) were left hind and 8 (40.00%) were right hind quarters. It might be due to their more susceptibility for mastitis. The findings of the study are in agreement with the findings of Khan and Muhammad (2005) who also reported that the prevalence of blind quarters in cows was higher in hindquarters as compared to the forequarters and slightly higher in right quarters than left ones. Prevalence of subclinical mastitis was 36.50%, 47.61% and 15.87% in the age groups of <5 years, 5 - 8 years and >8 years respectively, which was very much in accordance to the findings of Islam *et al.*, (2011) who found the prevalence of subclinical mastitis as 33.33%, 40.90%, 28.57% and 0.00% respectively in the age group of 3 years to 5 years, 5 years to 8 years, 8 years to 12 years and >12 years in cross breed cows. The weak immune system with age could probably be considered as the

reason behind this relationship of age with the increasing incidence of subclinical mastitis in crossbred cows. The highest number of subclinical mastitis cases were detected in cows between third to fifth parity (52.38%) followed by cows of first and second parity (38.09%) and cows in sixth parity or above (15.87%), which is in close agreement to Gupta (2010). The possible reason for the highest incidence during third, fourth or fifth lactation may be the decreased resistance of animals and reduced effectiveness of streak canal as a barrier of infection with increasing lactation age (Nauriyal, 1996). Highest prevalence of subclinical mastitis was seen in the animals in early period of lactation (41.26%) followed by mid (33.33%) and late (25.39%) lactation periods. The present findings were in corroboration with the findings of Kumar (2010) who recorded maximum subclinical mastitis cases in cows between 31-60 days after parturition followed by 60-90 days of lactation period. This could be due to the fact that in early stage of lactation cows are under stress due to parturition causing decreased resistance (Houben *et al.,* 1993).

Erskine *et al.,* (1988) opined that most new infections occur in the first 2 months of lactation, especially the environmental infections. The prevalence of subclinical mastitis recorded was 20.63%, 30.15%, and 49.20% in lactating cows with daily milk yield of 5-8L, 8-12L and more than 12L, respectively. Kayesh *et al.,* (2014) found that the highest prevalence of subclinical mastitis based on daily milk yield was 71.43% found in the cattle producing milk within a range of 5-7L and cows with a daily milk yield of 3-5L and 5-7L had significantly ($p < 0.05$) higher prevalence of subclinical mastitis. The prevalence of subclinical mastitis in crossbred cows were 28.57%, 35.48%, 40.00% and 42.85%, respectively 1-2L, 2-5L, 5-10L and 10L milk production per day (Islam *et al.,* 2011). The probable reason of increased chances of SCM in high yielders may be their increased susceptibility for the infections. Cattle producing high quantity of milk are susceptible for intramammary infection and there was rising trend of subclinical mastitis as reviewed by Radostits *et al.,* (2005). Out of total 142 positive samples, 95 (66.90%) samples were found positive for *Staphylococcus* spp. followed by *Streptococcus* spp 33 (23.23%).*E. coli* 04 (02.81%) and mixed infection 10 (07.04%). Patel *et al.,* (2012) found that the most prevalent pathogen isolated from 235 milk samples was *Staphylococcus aureus* 72 (30.64%), followed by *Streptococcus agalactiae* 47 (20.00%), *E. coli* 24 (10.21%), *Pseudomonas* 22 (9.36%), *Proteus* 6 (2.55%), *Pasteurella* 6 (2.55%), *Salmonella* 4 (1.70%) and mixed growth 54 (22.98%). Such a high involvement of *Staphylococcus* spp. observed during present investigation would be attributed to the ability of the bacteria to colonize on the teat ends and teat canal weeks before invading the upper parts of mammary gland and to their survival on the skin for longer period causing new intramammary infection of subclinical in nature (Eberhart *et al.,* 1982).

Quarter wise highest (31.57%) infection of *Staphylococcus* spp. was observed in right hind quarter followed by left hind quarters (29.47%), right fore quarters (20.00%) and left fore quarters (18.94%). *Streptococcus* spp. infection was found maximum (36.36%) in left hind quarter followed by right hind quarters (33.33%), and left fore quarters (18.18%) and right fore quarters (12.12%). Two cases (50%) of *E. coli* were seen in right hind quarter while one case each was seen in both left hind and right front quarter. Mixed infection was recorded greatest (40.00%) in left hind quarter (Table 2). All these findings are in close agreement to the findings of Kumar (2010).

Table.1 Overall and quarter wise prevalence of subclinical mastitis affected cows

Observations	No. of animals	Total No. of functional quarters examined	Quarter wise prevalence			
			LF	RF	LH	RH
Cows examined	112	428*	109	111	103	105
SCM positive	63	142	26	25	45	46
Prevalence (%)	56.25	33.17	23.85	22.52	43.68	43.80

Table.2 Quarter wise association of bacteria in subclinical mastitis affected cows

Bacteria	Quarter affected				Total
	RF	LF	RH	LH	
***Staphylococcus* spp.**	19 (20%)	18 (18.94%)	30 (31.57%)	28 (29.47%)	95 (66.90%)
***Streptococcus* spp.**	4 (12.12%)	6 (18.18%)	11 (33.33%)	12 (36.36%)	33 (23.23%)
E. coli	1 (25%)	0 (0%)	2 (50%)	1 (25%)	4 (2.81%)
Mixed infection	1 (10%)	2 (20%)	3 (30%)	4 (40%)	10 (7.04)

During the investigation, *Staphylococcus* spp. exhibited reasonably higher sensitivity to enrofloxacin, cefotaxime, gentamicin, amikacin, and choramphenicol, and moderate to amoxicillin, ampicillin, erythromycin, streptomycin. *Streptococcus spp*. showed higher sensitivity to cefotaxime and enrofloxacin and moderate to amoxicillin, choramphenicol and gentamicin and least to streptomycin. *E. coli* showed higher sensitivity to cefotaxime, enrofloxacin, amikacin and moderate to amoxicillin, gentamicin and low to choramphenicol. Mixed infection had higher sensitivity to enrofloxacin and cefotaxime, and moderate to gentamicin, tetracycline, choramphenicol, and least sensitive with ampicillin and amoxicillin. Enrofloxacin was found to be the most effective antibiotic against SCM in the present study. Sharma (2000) also reported highest *in-vitro* sensitivity of enrofloxacin (93.07%) followed by cloxacillin (64.61%) against microbes associated with mastitis in Himachal Pradesh. The sensitivity pattern would vary depending on the intensive use of antibiotics in particular region for therapeutic uses in livestock. Enrofloxacin which has been developed for exclusive use in veterinary practice, belonged to fluoroquinolones, is a potent synthetic antimicrobial agent with long lasting bactericidal effect with extended spectrum of antibacterial activity (Neer, 1998). Some mastitis pathogens exhibited poor sensitivity to certain antimicrobials and this can be attributed to indiscriminate and prolonged use of antibacterial drugs in the absence of *in vitro* drug susceptibility testing of mastitis pathogens as has earlier been reported by Sobiraj *et al.,* (1999).

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