

Prevalence, Diagnosis and Therapeutic Management of Subclinical Mastitis in Deoni Cows

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ABSTRACT

The study was carried out to know prevalence, diagnosis and therapeutic efficacy of Marbofloxacin and Vitamin E and Selenium against the subclinical mastitis from lactating Deoni Cows at Organized and Unorganized dairy farms in and around Bidar. Screening of subclinical mastitis was done by California Mastitis Test (CMT), Electrical Conductivity (EC) and Somatic Cell Count (SCC) in milk. Positive samples for subclinical mastitis were considered for isolation and identification of bacterial pathogen by cultural examination. Ninety (56.25%) out of one hundred sixty lactating cows were found positive for subclinical mastitis. Affected quarter-wise single, two, three and four prevalence was given a prevalence of 28.13, 25, 1.88 per cent. Out of 21 positive milk sample 16 and 9 samples were found positive for *Staphylococcus spp.*, *Streptococcus spp.* (76.19%) and *E.coli* (42.86%) organism. The antibiogram has a of the bacterial isolates to standard antibiotic discs determined by disc diffusion method revealed the highest sensitivity to Gentamicin and Enrofloxacin followed by Tetracycline, Moxifloxacin, Cefoperazone, Ceftriaxone, in decreasing order and least sensitive to amoxicillin and clavulanic acid. Positive animal were treated with single dose of Marbofloxacin (8 mg/kg.BW) alone, Marbofloxacin with Vitamin E and Selenium and Vitamin E and Selenium alone on '0'day and 3rd day with (1 ml/25 kg.BW) given intramuscularly. Therapeutic efficacy of Marbofloxacin along with Vitamin E and Selenium showed (86.67%), Marbofloxacin (84.62%) and Vitamin E and Selenium (50%).

Keywords: Subclinical mastitis; Deoni cows; Prevalence; Diagnosis; Treatment

INTRODUCTION

Mastitis, inflammation of the mammary gland, is a major disease affecting dairy animals worldwide. Based up on severity of inflammation, it can be classified into subclinical, clinical and chronic forms. Out of which subclinical form is difficult to detect due to the absence of any visible indications and has major cost implications associated with decreased milk production. In India, annual economic loss to dairy industry due to SCM is estimated to be Rs 43,653 million. Incidence of subclinical form of mastitis was found to be more common in India, when compared to clinical mastitis (1%-10%). The disease not only decreases the quantity, but also decreases the quality of the milk, thus resulting in significant losses to the dairy farmer. The loss is further aggravated by the cost of treatment and culling of the animals due to the permanent damage of quarters

in certain cases [1]. Apart from being concern to the dairy industry, the disease is also of public health significance.

The prevalence of subclinical mastitis in dairy herds is often surprising to producers, moreover, subclinically infected udder quarters can develop clinical mastitis and the rate of new infection can be high. Cows with subclinical mastitis are those with no visible changes in the appearance of the milk and/or udder, but milk production decreases by 10%-20% with there in undesirable effect on its constituents and nutritional value rendering it of low quality and unfit for processing [2]. Although there are no visible or palpable external changes, the infection is present and damage to udder occurs due to inflammation.

Subclinical mastitis being the most common form of mastitis is 15 to 40 times more prevalent than clinical mastitis and its prevalence varies from herd to herd and place to place. The

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incidence of subclinical mastitis was higher in cows than in buffaloes.

Identification of intramammary pathogens found in milk is the gold standard for the diagnosis of mastitis. However, this is time-consuming, costly and of limited applicability under field conditions. According to the International Dairy Federation (IDF) recommendations, microbiological status of the quarter and the Somatic Cell Count (SCC) are the most common tests to detect changes in the milk because of an inflammatory process.

The unnoticeable changes in subclinical mastitis can be recognized indirectly by several diagnostic methods including the California Mastitis Test (CMT), Electrical Conductivity (EC), White Side Test (WST), and Somatic Cell Count (SCC), pH, chloride and catalase tests [3]. These tests are preferred to be screening tests for subclinical mastitis as they can be used easily, yielding rapid as well as satisfactory results. The CMT gives an indirect estimate of SCC in which gelling reaction between nucleic acid of the cells and detergent reagent is the basic principle in several investigations because it is more reliable than other chemical tests for diagnosis of SCM. Milk Somatic Cell Count (SCC) is a diagnostic parameter for subclinical mastitis. A SCC level below 1,00,000 cells/mL is accepted to represent a healthy quarter, SCC levels \geq 2,00,000 somatic cells per mL of milk is considered to indicate subclinical mastitis in animals. In India, the most common causative agents of clinical mastitis in cattle are *Staphylococcus* and *E.coli* while *Streptococcus aureus* and *Staphylococcus dysgalactia* cause most of those in the various subclinical cases. Mastitis is often caused by bacteria; insufficient contact between antibiotic and causative bacteria at the site of infection is important cause for failure of mastitis treatment. Single Injection Short Acting Antibiotic (SISAAB). protocols have been developed for the treatment of mastitis with marbofloxacin in cattle. Subclinical mastitis is associated with release of free radicals and decreased total antioxidant capacity in milk. Beneficial effect of antioxidant Vitamin E along with antibiotics was proved in mastitis. Hence, the present study was undertaken to evaluate the prevalence, diagnosis and therapeutic efficacy of subclinical mastitis in Deoni cows.

MATERIAL AND METHODS

Animals

Livestock Research Instructional Complex, (Deoni) Hallikhed B of Bidar district during the period from 2018 to 2019. Total of 160 Deoni cows of different age groups, parities and stage of lactation (early, mid and late) were included in the study group [4]. All cows were housed in tie stall barns and milked twice daily by hand.

Sample collection

The proper disinfection of teat surface with 70% ethanol. After letting out few strips, the milk samples about 30 ml were collected separately from each quarter in sterile bottles which were labelled as Left Front (LF), Left Hind (LH), Right Front (RF) and Right Hind (RH). The milk samples were transported to the laboratory by using readymade nutrient broth manufactured by Himedia Laboratories and milk samples which

are positive for California Mastitis Test and Electrical conductivity, further subjected to somatic cell count and culture of milk samples for isolation of bacteria and antibiogram pattern of pre and post treatment subclinical mastitic cow's milk [5].

Diagnostic test

The subclinical mastitis by California Mastitis Test (CMT) as per. Milk Somatic Cell Count was calculated as per the standard method by and also by Electrical conductivity of milk sample by using hand held Ecotester. EC value more than 5 ms/cm is there considered as positive for subclinical mastitis.

Microscopic examination

In CMT, few samples positive for subclinical mastitis were picked randomly and were further examined by the Newman Lampert Staining. The methodology described by Coles for the purpose of Newman Lampert Staining and by studying the morphological features the bacteria were identified as *Staphylococcus*, were in the *Streptococcus Bacilli* and *Diplococci* in this study.

Culture examination

These nutrient broth aseptically and kept in a incubator at an a temperature of 37° for 24 hours. After incubation, a smear was prepared from the inoculums of nutrient broth on a clean glass slide and staining was done by Grams staining method. Organisms like *Staphylococci spp*, *Streptococci spp*, and *E.coli spp*.in were identified in the stained smear.

Study of antibiotic sensitivity

The collected were inoculated into nutrient broth in fully aseptic condition and kept in the incubator at a temperature of 37°C for 24 hours. After incubation, a smear was prepared on a clean glass slide from the inoculum of nutrient broth. The smear was stained by Gram staining method to identify Gram positive or Gram negative bacteria. After staining and confirming bacterial growth, antibiotic sensitivity test was done using Kirby-Bauer disk diffusion method.

Antibiotic discs

These Antibiotics in field condition were selected for this study. Following antimicrobial discs procured from Hi-Media were stored at 2-8 degree Celsius in the refrigerator and were kept outside 1-2 hours before use to equilibrate to the room temperature. The antimicrobial discs with known concentration as noted in microgram were used for this study.

Treatment

In EC tests were divided into two groups of 10 each. Cows of the group-I were treated with Marbofloxacin through intramuscular route as single dose at the rate of 8 mg/kg body weight. Cows of group-II were treated with Marbofloxacin through intramuscular rate as single dose at the rate of 8 mg/kg body weight along with vitamin E and selenium intramuscularly as single dose at the rate of 1 ml/50 kg body weight. The response to treatment was evaluated on the basis of examination of milk samples by CMT and EC tests before and after 5th day of treatment.

RESULT AND DISCUSSION

Prevalence

Deoni cows in the present research study was 56.25, 25 and 20 per cent on California Mastitis Test, Electrical Conductivity and

Somatic Cell Count, respectively. Similar finding were reported. However, several workers have reported comparatively low prevalence rates i.e, 21.96 to 41 percent. Contrary to the mainly present study high prevalence rates of subclinical mastitis were reported, i.e, 51 to 73.3 percent [6]. Such variation in there rates prevalence may be ascribed to the varied hygienic practice in vogue in different farms or village conditions of different regions.

Parity-wise prevalence

This highest 4th parity (69.44%) followed by an 3rd (62.07%), 1st (53.33%), 5th and above (50.80%) and least in 2nd parity in there (41.18%) on cow basis (Table 1). The increase in prevalence with lactation number in the present study collabates with the findings of earlier workers. The increase in the prevalence of SCM could be attributed to increase in milk yield up to 6th lactation [7]. In addition, it could be due to the animals might have been exposed to infection for longer periods, as the period of lactation advanced there was simultaneous decline in the defence mechanism of the animal during the advanced age.

Parity	Total animals	Positive animals	%	Total Quarters	Positive quarters	%
First	15	8	53.33	60	13	21.67
Second	17	7	41.18	68	10	14.71
Third	29	18	62.07	116	25	21.55
Fourth	36	25	69.44	144	43	29.86
Five and above	63	32	50.8	252	54	21.43
Total	160	90	56.25	640	145	22.66

Table 1: Parity-wise prevalence of subclinical mastitis in Deoni cows.

Age-wise prevalence

Deoni cows was 6-8 years (24.43%), 9-11 years (21.88%), 3-5 years (19.05%) and least in above 11 years of age group on quarter basis. (Table 2). These finding were similar to the ones reported. The highest prevalence in the older animals of the present study could be due to increased exposure to the infection or due to the increased susceptibility or combination of these two factors [8].

Age (years)	Total animals	Positive animals	%	Total Quarters	Positive quarters	%
44319	21	10	47.62	84	16	19.05
44414	88	54	61.36	352	86	24.43
44509	40	20	50	160	35	21.88

> 11	11	6	54.55	44	8	18.18
Total	160	90	56.25	640	145	22.66

Table 2: Age-wise prevalence of subclinical mastitis in deoni cows.

Quarter-wise prevalence

This were found positive for subclinical mastitis recorded. Overall quarter-wise prevalence was 22.66 per cent, followed by single quarter (27.50%), two quarter (25%), three and four quarter (1.88%) (Table 3). These prevalence rates are in accordance with earlier report. In SCM affected Deoni cows, involvement of single quarter was significantly higher than the multiple quarter involvement followed by two quarter, four and three quarter.

Quarters affected	No. of animals screened	No. of positive animals	Percentage
Single quarter	160	44	27.5
Two quarter	160	40	25
Three quarter	160	3	1.88
Four quarter	160	3	1.88
Total		90	56.25

Table 3: Prevalence of subclinical mastitis in deoni cows according to number of Quarters affected.

The prevalence rate of SCM in cows was significantly higher in left half (53.79%) of the udder than right half (46.21%) of the udder. Significantly higher prevalence of SCM in cows was observed in hind quarters (67.59%) when compared to the fore quarters (32.41%). This finding is similar to the findings. The higher involvement of hind quarter may be due to greater chance of contamination by dung, urine and higher number of bacteria in them. While milking, rear quarters are pulled forward and sideways which may lead to undue stress on them.

Culture isolation

Deoni cows was collected and subjected into culture and isolation, 80 percent of samples revealed *Staphylococcus spp.* 80 percent of samples revealed *Streptococcus spp.* and 40 percent was samples revealed *E.coli* organism. Further subjected into culture and isolation was revealed that, an *Staphylococcus spp.* was there a major pathogen accounting for 76.19 percent followed by subject *Streptococcus spp.* was second largest pathogens accounting for the 76.19 percent and lastly *E.coli* accounting for 42.86 percent use. These finding are in agreement with earlier reports.

The antibiogram of bacterial isolates revealed highest sensitivity to Gentamicin (80%) followed by Enrofloxacin (60%), tetracycline (45%), Moxifloxacin (40%), Cefoperazone (30%), Ceftriaxone (30%), in decreasing order and least sensitive to amoxicillin and clavulanic acid, Similarly, the higher sensitivity with Gentamicin was recorded and Enrofloxacin [9]. Poor sensitivity to amoxicillin and clavulanic acid may be attributed

to production of beta lactamase enzyme by resistant strains of isolates due to their frequent use.

Treatment

The cows treated of single dose marbofloxacin, 11 quarters were cured giving an efficacy of 84.62%. This is in agreement with earlier reports of in clinical mastitis in cows [10]. Out of 15 quarters of seven cows treated with single dose of marbofloxacin along with Vitamin-E and Selenium 13 quarters were cured giving an efficacy of 86.67%. Similarly higher efficacy rates were observed with single dose of marbofloxacin along with Vitamin-E and Selenium in mastitis in cows. This could be attributed to increased phagocytic activity of vitamin-E when provided with antibiotic [11]. Out of 10 quarters of seven cows treated with single dose Vitamin-E and Selenium 5 quarters were cured giving an efficacy of 50.00%. This is an agreement with earlier reports. Beneficial effect of vitamin-E and Se could be attributed to decreased somatic cell count and electrical conductivity.

CONCLUSION

From the present study found that prevalence was more in 6-8 years of the age group (61.36%), 4th parity (69.44%), hind-quarters (67.59%), left half of the udder (53.79%) and single quarter (27.50%), respectively. Highest prevalence of SCM in Deoni cows was due to open space rearing, rough floor, improper drainage in the village, milking without hand washing are the basic cause for the disease. Among the chemical tests California Mastitis test was better diagnostic test compared to Electrical conductivity and Somatic cell count. Combination of Marbofloxacin along with vitamin-E and Selenium gave higher cure rate than the drugs used alone in sub-clinical mastitis.

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