SUBCLINICAL MASTITIS – DIAGNOSIS AND ISOLATION OF ORGANISMS*

P. C. Siji[1] and K. Vijayakumar[2]

Department of Veterinary Epidemiology and Preventive Medicine
College of Veterinary and Animal Sciences
Mannuthy - 680 651, Thrissur, Kerala

Abstract

Bovine subclinical mastitis in an organized dairy farm was diagnosed using different tests like California Mastitis Test (CMT), Modified White Side Test (MWST) and Modified Aluendorfer Mastitis Probe Test (MAMP). Somatic cell counts for different grades of CMT and types of bacterial organisms causing subclinical infection and their antibiogram were found. Bacterial isolates obtained were Staphylococcus aureus, Coagulase negative staphylococci, Streptococcus agalactiae and Escherichia coli. Sensitivity for these bacteria was maximum for ciprofloxacin, enrofloxacin and trimethoprim.

Key words : *Bovine subclinical mastitis, mastitis diagnostic tests, somatic cell count and antibiogram*

Bovine subclinical mastitis is one of the most common diseases causing huge economic loss to dairy farmer as it invariably goes undetected and may cause up to 80% milk loss in a herd (Singh *et al.*, 1994). Predisposing factors like poor management and hygiene, teat injuries and faulty milking machines hasten the entry of infectious agents into the udder. Indiscriminate use of antimicrobials in the treatment of mastitis is common and this might generate an increase in the resistance level of many micro-organisms to different antibiotics. So a thorough knowledge about the causal organisms and their profile of susceptibility to drug is essential to initiate suitable therapy and reduce the losses in a farm.

With this objective a study was undertaken to assess the occurrence of subclinical mastitis using different tests like California Mastitis Test (CMT), Modified White Side Test (MWST) and Modified Aluendorfer Mastitis Probe Test (MAMP), in an organized dairy farm. Somatic Cell Count (SCC) for different grades of CMT and types of bacterial organisms causing subclinical infections and their antibiogram were also determined.

Materials and Methods

A total of 200 quarter milk samples collected from 50 dairy crossbred cows maintained at an organized dairy farm were screened for subclinical mastitis in February 2002, using CMT (Schalm *et al.*, 1971).The midstream milk samples from the positive quarters were collected in sterile glass vials, maintaining all aseptic precautions. The samples were transported in ice packed container to laboratory. Isolation and identification of bacteria was done using standard procedures (Barrow and Feltham, 1993). *In vitro* antibiotic sensitivity of the organisms was studied using disc diffusion technique (Baur *et al.*, 1966). Smears for SCC were prepared within an hour of collection to count the somatic cells. The number of somatic cells in milk was estimated as per the method described by Prescott and Breed (1910). Staining of smears was done by Broadhurst-Paley triple step procedure described by Schalm *et al.* (1971). MWST was also performed on the same milk samples as per Murphy and Hanson (1941). MAMP test was conducted

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1. Lecturer(LSM),VHSS, Ayyanthole, Thrissur

2. Associate Professor & Head

as per the method cited by Buragohain and Dutta (1991). Results were interpreted after 24 h at room temperature.

Results and Discussion

Out of the 200 quarter milk samples, CMT detected 68 (34%) quarters positive and 132 (66%) quarters negative for the test. Depending upon the intensity of reaction, 18 (9 %), 28 (14 %), 21 (10.05 %) and 1 (0.5 %) quarter milk samples were graded as 0, 1, 2 and 3. SCC determined for these different grades of reactions were having a mean (± SE) value of 4.35 ± 2.87 L/ml, 10.72 ± 3.16 L/ml, 61.17 ± 10.69 L/ml for 0, 1+, 2+ grades. There was only one quarter sample for the grade 3+ which recorded a SCC of 67.5 L/ml of milk. Corresponding SCC values indicated by Schalm *et al.* (1971) were 1.5 L to 5 L, 4 L to 15 L, 8 L to 50 L and >50 L cells/ml. Basan *et al.* (1997) scored CMT reaction into +, ++, +++ with SCC 2 to 3 L, 3 to 10 L and >10 L cells/ml respectively. MWST performed on 68 CMT positive samples yielded 10 negative (14.71%), 36 (52.94%) 1+, 19 (27.94%) 2+ and 4 (5.88%) 3+ reactions with mean (± SE) SCC values as 7.06 ± 3.05, 12.45 ± 1.61, 18.57 ± 2.22 and 49.27 ± 5.58 L cells/ml. These values were in agreement with the values suggested by Sebastian (2001). It is to be noted that MWST detected the infection only at a higher range of SCC values. CMT was reported to be more sensitive and specific than MWST (Santhos *et al.*, 1995), both being specific for the leukocyte counts. MAMP detected 42 out of 68 CMT positive samples.

Only 39 (57.35%) of the milk samples were positive in cultures. A total of 17 *Staphylococcus aureus* (43.59%), 13 coagulase negative Staphylococci (33.33%), 5 *Streptococcus agalactiae* (12.82%) and 4 *Escherichia coli* (10.26%) were the bacterial isolates determined. These milk samples had a mean (± SE) SCC of 18.60 ± 3.64, 19.29 ± 5.94, 26.3 ± 8.01 and 11.08 ± 9.63 L cells/ml. High SCC values were found in Gram positive infections than Gram negative infections. This was in agreement with the findings of Vijayalakshmi *et al.* (2001). Statistically significant difference in SCC was observed between Gram positive and Gram negative bacterial organisms of mastitis milk. Thus SCC can be used as a predictive indicator for the determination of mastitis of Gram positive or Gram negative bacterial etiology. SCC is actually an indication of the body's defence mechanism. Barkema *et al.* (1998) noted that low bulk milk SCC occurred in Gram negative pathogens causing clinical mastitis with more severe systemic signs of illness and vice versa. In cows, infection with major mastitis pathogens is reported to have higher average SCC (Eberhart *et al.*, 1979). There was a dominance of Gram positive organisms among the bacteria identified.

Staphylococcus aureus is a contagious pathogen and its major reservoir is the infected udder. Usually the infections are spread among cows during the milking process (Harmon, 1993). Hence hygiene at milking is of paramount importance in the control of this infection. Control of this type of mastitis is often difficult in a herd because it is contagious and infection is usually subclinical and often resistant to antibiotic treatment, which results in chronic carrier animals (Enevoldsen *et al.*, 1995). Though coagulase negative Staphylococci are regarded as minor pathogens, 33.33 per cent of bacteria isolated belonged to this category. Humans are the natural hosts of certain species of coagulase negative Staphylococci. Aarestrup and Jensen (1997) opined that the most likely source of infection is the human reservoir. *Streptococcus agalactiae* was isolated from five samples. Streptococci are mainly supposed to cause mild forms of mastitis, mainly chronic in nature (Saikir *et al.*, 1989).

In vitro antibiotic sensitivity studies of different bacterial isolates are shown in Table.

All the Staphylococcal organisms isolated were sensitive to ciprofloxacin, enrofloxacin and trimethoprim whereas all the Streptococcal organisms were sensitive to gentamicin also, in addition to the above. Cent percent *Escherichia coli* isolated were sensitive to ciprofloxacin, enrofloxacin, chloramphenicol, gentamicin and trime thoprim.

Overall sensitivity pattern of bacterial isolates showed higher sensitivity to ciprofloxacin, enrofloxacin and trimethoprim, followed by gentamicin, oxytetracycline, chloramphenicol and amoxicillin. More than half of the isolates were resistant to streptomycin.

Table. Bacterial isolates and their antibiogram (Percent in parenthesis)

Sl. No.	Bacterial isolates	No. of isolates	Am	C	RC	Ex	Gm	Ox	St	Tr
1	*Staphylococcus aureus*	17	12 (70.58)	13 (76.47)	17 (100)	17 (100)	17 (100)	13 (76.4)	7 (41.17)	17 (100)
2	Coagulase negative *Staphylococci*	13	12 (93.3)	12 (93.3)	13 (100)	13 (100)	10 (76.92)	13 (100)	12 (93.3)	13 (100)
3	*Streptococcus agalactiae*	5	4 (80)	4 (80)	5 (100)	5 (100)	5 (100)	4 (80)	2 (40)	5 (100)
4	*Escherichia coli*	4	2 (50)	4 (100)	4 (100)	4 (100)	4 (100)	2 (50)	2 (50)	4 (100)

Am – Amoxycillin RC – Ciprofloxacin Ex – Enrofloxacin C – Chloramphenicol
Gm – Gentamicin Ox – Oxytetracycline St – Streptomycin Tr – Trimethoprim

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