Acta Scientific Veterinary Sciences (ISSN: 2582-3183)

Volume 4 Issue 7 July 2022

Research Article

Diagnosis of Sub Clinical Mastitis in Buffaloes

Keshamoni Ramesh[1], Lakshmi Korsapati[2]*, Ambica Gadige[3] and Swathi Bommu[4]

[1]Ph. D Scholar, College of Veterinary Science, Rajendranagar, Hyderabad, India

[2]Associate Professor, Department of Veterinary Medicine, College of Veterinary Science, Korutla, India

[3]Associate Professor, Department of Veterinary Medicine, College of Veterinary Science, Rajendranagar, Hyderabad, India

[4]Professor, Department of Veterinary Physiology, College of Veterinary Science, Maamnoor, India

***Corresponding Author:** Lakshmi Korsapati, Associate Professor, Department of Veterinary Medicine, College of Veterinary Science, Korutla, India.

DOI: 10.31080/ASVS.2022.04.0425

Received: May 24, 2022

Published: June 15, 2022

Abstract

In present investigation, a total 440 quarter milk samples of 110 Bubalis bubalis were screened for subclinical mastitis (SCM) to find out the occurrence and the causative agents of SCM in and around Hyderabad. Out of these, 76 quarters from 40 buffaloes were found positive based on bacterial culture. Here cultural examination was taken as standard test. Also, milk samples were subjected to California mastitis test, Somatic Cell Count, milk pH and milk fat to determine qualitative changes in milk and cultural examination were undertaken in the laboratory. Results of California mastitis test reveated trace, weak positive and distinct California mastitis test reactions. The mean values of Somatic Cell Count, milk pH was elevated significant ($P < 0.01$) as compared to healthy control buffaloes, While, mean values of milk fat decreased non-significantly as compared with healthy control buffaloes.

Key words: Buffaloes; Sub-Clinical Mastitis; California Mastitis Test; Somatic Cell Count; Milk pH and Milk Fat and Cultural Examination

Introduction

Bubalis bubalis viz., Buffaloes are the large ruminants which are more susceptible to mastitis which causes decreased milk production and thus lead to great loss to the farmers. Mastitis is an important disease of dairy animals particularly Buffaloes caused by several infectious and non-infectious agents and is characterized by inflammation of parenchyma of the mammary gland with physical, chemical and bacteriological changes in the milk and pathological changes in the glandular tissues [4]. Mastitis affects not only the individual animal but, the whole herd or at least several animals within the herd are affected. If left untreated, the condition can lead to deterioration of animal welfare resulting in culling of affected animals, or even death. According to the severity, duration, nature of the exudates and primary cause, mastitis can occur in clinical and subclinical form in buffaloes [7]. Diagnosis of mastitis by various test like California mastitis test, Somatic Cell Count, milk pH and milk fat and cultural examination.

Materials and Methods

The buffaloes with subclinical mastitis were screened by various tests California mastitis test, Somatic Cell Count, milk pH and milk fat and cultural examination.The milk samples were subjected to CMT as per the procedure given by Schalm and Noorlander [8]. The principle of Somatic Cell Count is that the detergent causes rupture of somatic cells when added to a milk sample due to which DNA and other cell contents are released. Released DNA from ruptured cells unites to form a gel, the consistency of which depends upon the number of somatic cells.

The basic principle of the pH meteris to measure the concentration of hydrogen ions. Acids dissolve in water forming positively charged hydrogen ions (H+). The greater this concentration of hydrogen ions, the stronger the acid is. Similarly, alkali or bases dissolve in water forming negatively charged hydrogen ions (OH-). The stronger a base is the higher the concentration of negatively charged hydrogen ions there are. The amount of these hydrogen ions present solution is dissolved in some amount of water determines the pH. A pH value of 7 indicates a neutral solution. Pure water should have a pH value of 7. Now pH values less than 7 indicate an acidic solution while a pH value greater than 7 will indicate an alkaline solution. A solution with pH value of 1 is highly acidic and a solution of pH value of 14 is highly alkaline.

The fat of milk samples was estimated by lacto milk scan the milk samples from the affected quarters were collected by following aseptic precautions into sterile vials and then subjected to bacteriological examination for isolation of etiological agents.

Results and Discussion

In present investigation, occurrence of SCM based on CMT, SCC and bacterial cultural examination were 31.82, 34.55 and 36.36 percent on animal basis and 15.45, 15.90 and 17.27 percent on quarter basis (Table 1) respectively. Bacterial culture was considered as a gold standard to confirm buffaloes with SCM.

California mastitis test (CMT)

The CMT point score in milk samples collected from control and SCM infected animals were presented in (Figure 1). A total of 440 quarter milk samples of 110 lactating buffaloes were screened by CMT. Out of which, 362 (82.73%) quarters have negative (0) CMT score, 11(02.50%) quarters have trace (1), 30 (6.82%) quarters have weak positive (2) and 35 (07.95%) have distinct positive (3) CMT scores.

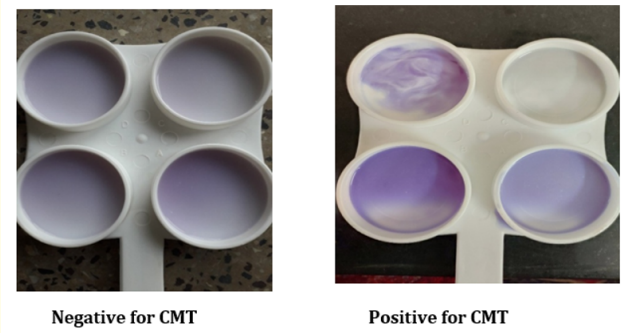

Figure 1: Milk sample with California Mastitis Test negative and various grades of CMT.

Somatic cell count (SCC)

In the present investigation, the mean somatic cell count values of healthy control and subclinical mastitis affected buffaloes were $0.92 \pm 0.01 \times 10^5$cells/ml and $3.61 \pm 0.37 \times 10^5$ cells/ml respectively. There was a significant ($P < 0.01$) increase of somatic cell count in SCM affected buffaloes as compared with healthy control (Table 2).

Milk pH

The mean values of milk pH in healthy control quarters were 6.73 ± 0.02. While the mean milk pH in subclinical mastitis affected quarter milk samples was 7.00 ± 0.05. There was a significant ($P < 0.01$) increase in the milk pH values in SCM affected quarter milk samples as compared to healthy control.

Milk Fat

During the present study, the mean values of milk fat percentage of healthy control and subclinical mastitis affected quarter milk samples were 7.61 ± 0.22 and 6.64 ± 0.19 respectively. There was a significant ($P < 0.01$) decrease in the milk fat in SCM affected animal milk samples as compared to healthy control.

Cultural Examination for bacteria

Out of 440 quarter milk samples, 76 quarter milk samples were found positive for pathogenic bacteria based on cultural examina-

tion. Among these, 76 culture positive milk samples 11.84 (9/76) and 88.16 (67/76) per cent had mixed and mono microbial infection respectively. The bacteria identified as single infection from SCM affected quarter milk samples include *Staphylococcus spp., Streptococcus spp., Escherichia coli, Klebsiella spp., Micrococcus spp.* and *Bacillus spp.* which were isolated in 36.84% (28/76), 18.42% (14/76) ,14.47% (11/76), 9.21% (7/76), 6.58% (5/76), and 2.63% (2/76), respectively, While, the bacteria isolated in mixed infection include *Staphylococcus spp.* in combination with *Streptococcus spp.* (3.95percent), *Escherichia coli* in combination *Streptococcus spp.* (2.63%), *Escherichia coli* in combination with *Staphylococcus spp.* (2.63%), *Bacillus spp.* in combination with *Staphylococcus spp.* (1.32%), and *Klebsiella spp*in combination with *Staphylococcus spp.* (1.32%)respectively (Table 3).

S No	Name of the diagnostic test	No. of animals tested	No. of animals positive	Percentage
1.	California Mastitis Test	110	35	31.82
2.	Somatic Cell Count	110	38	34.55
3.	Bacterial Culture	110	40	36.36

Table 1: Occurrence of SCM in buffaloes using different diagnostic tests.

S. No	Parameter	Apparently healthy animals	Subclinical mastitis
1.	Somatic Cell Count ($\times 10^5$ cells/ml)	0.92 ± 0.01	3.61 ± 0.37**
2.	Milk pH	6.73 ± 0.02	7.00 ± 0.05**
3.	Milk fat percentage	7.61 ± 0.22	6.64 ± 0.19

Table 2: Mean ± SE values of SCC, milk pH and milk fat percent in healthy and SCM affected quarter milk samples.

** Significant at p < 0.01 T- test was performed.

S. No.	Bacteria isolates	Total no. of samples (n= 76)	Percentage (%)
1.	*Staphylococcus spp.*	28	36.84
2.	*Streptococcus spp.*	14	18.42
3.	*Escherichia coli*	11	14.47
4.	*Klebsiella spp.*	7	9.21
5.	*Micrococcus spp.*	5	6.58
6.	*Bacillus spp.*	2	2.63
7.	*Staphylococcus spp. + Streptococcusspp.*	3	3.95
8.	*Escherichia coli + Streptococcus spp.*	2	2.63
9.	*Escherichia coli + Staphylococcus spp.*	2	2.63
10.	*Bacillus spp. + Staphylococcus spp.*	1	1.32
11.	*Klebsiella spp.+ Staphylococcus spp.*	1	1.32

Table 3: Sample wise occurrence of bacterial isolates in SCM affected quarter milk samples.

S No	Name of the test	Percentage accuracy	Sensitivity	Specificity
1.	California Mastitis Test (CMT)	93.18	73.17	97.76
2.	Somatic Cell Count (SCC)	97.27	89.15	99.43
3.	FAT	79.32	24.80	95.23
4.	pH	75.23	25.23	96.69
5.	Bacterial culture	100.00	100.00	100.00

Table 4: Sensitivity, Specificity and Predictive value of different diagnostic tests on the basis of cultural examination as standard.

In present investigation, the percent accuracy of CMT, SCC, milk fat and milk pH for the detection of SCM, with cultural examination as standard was 93.18, 97.27, 75.23 and 79.32 percent respectively. High accuracy of CMT and SCC than milk fat and milk pH might

be due to quick immune response to foreign agents by the immune cells than alteration in the ionic concentration in the milk [4].

Sensitivity of CMT, SCC, milk fat and milk pH for the detection of SCM, with cultural examination as standard was 73.17,89.15, 24.80 and 25.23percent, while specificity of97.76, 99.43, 95.23 and 96.69 respectively. These findings are in agreement with [3] who compared the efficacies of different diagnostic tests for detection of subclinical mastitis in bovines and reported sensitivities of 81.55, 87.38 and 75.73 percent for SCC, bacterial culture and CMT respectively. Sensitivity of CMT and SCC as 86.07 and 88.60 percent with specificity of 59.70 and 97.76% and percentage accuracy of 75.52 and 91.94 percent respectively, taking cultural examination as standard [6]. Comparative efficacy of SCC and CMT with specificities of 84.8 and 62.7 percent respectively, and sensitivities of39.8 and 60.10 percent respectively [1]. In contrary, [5] reported that percentage of accuracy of california mastitis test (CMT) and somatic cell count (SCC) were found to be 73.33and 71.00 percent respectively and also reported that, the false positive reactions were higher with CMT (24.60%) followed by SCC (23.70%) with cultural examination was taken as standard. Reasons for false positive reaction in sub clinical mastitis by CMT and SCC was due to early lactation and may be animals could be in estrus cycle [2].

Out of 440 quarter milk samples, 76 were found culturally positive for pathogenic bacteria. Among these, 36.84% samples were positive for *Staphylococcus* spp. followed by *Streptococcus spp.* (18.42%), *E. coli,* (14.47%), *Klebsiella spp.* (9.26%), *Micrococcus spp.* (6.58%) and *bacillus spp.* (2.63%). Mixed microbial infections of *Staphylococcus* spp.+ *Streptococcus spp*., *Escherichia coli* + *Streptococcus* spp.*, Escherichia coli* + *Staphylococcus spp., Bacillus spp.+ Staphylococcus spp. and Klebsiellaspp*. *+ Staphylococcus spp.* was found accounting to 3.95, 2.63, 2.63, 1.32 and 1.32 percent respectively. These results were in accordance with [6] and [1] who found higher occurrence of *Staphylococcus spp.* and observed to be the most prevalent organism in the quarter milk samples affected with SCM. Whereas [5] observed that95% of mastitis in bovines were caused by *Streptococcus agalactiae, followed by Staphylococcus aureus, Streptococcus dysgalactiae, Streptococcus uberis,* and *Escherichia coli*. While the remaining 5% were caused by other organisms. In contrary, Muhamed., *et al*. 20010 documented that, *Streptococcus spp*. was the most prevalent bacteria along with *Staphylococcus spp*. which could be due to poor managemental dairy practice methods and infected other healthy animals of the herd due to its contagious nature. The higher prevalence of *Staphylococcus spp*. in the present study may be due to their ubiquitous nature and its well adaption to survive in the udder and establish a mild SCM of longer duration [1]. However, Streptococcus spp. had a lower prevalence as compared with Staphylococcus spp. due to poor survival rates of organism outside the environment. Isolation of E. coli in cultural examination in present study could be due to bad managemental practices in the farms, contaminated feed, water and milking machines. During the study, occurrence of mixed bacterial infections was greater than mono-microbial infections [5].

Conclusions

Subclinical mastitis was a hidden problem in buffaloes causing considerable loss to the dairy farmers. Among different diagnostic tests, Cultural examination was taken as standard test followed by Somatic Cell Count, California mastitis test, milk pH and milk fat to determine qualitative changes in milk. *Staphylococcus spp.* was the predominant bacteria isolated from Sub clinical mastitic milk samples.

Acknowledgements

The technical help rendered by the advisory committee, financial help from P.V. Narsimha Rao Telangana Veterinary University and field help from farming community in and around area of Hyderabad are duly acknowledged.

Conflicts of Interest

No.

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