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Prevalence and Etiology of Subclinical Mastitis among Buffaloes (*Bubalus bubalus*) in Namakkal, India

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Abstract: Milk samples from 206 apparently healthy buffaloes of marginal farmers maintained under the prevailing field conditions were screened for Subclinical Mastitis (SCM) to determine its prevalence and etiology by White Side Test (WST), California Mastitis Test (CMT), Somatic Cell Count (SCC) and Bacteriological examination. 26.20% of animals were positive for SCM in SCC and bacteriological examination. Prevalence in graded Murrah buffaloes were found to be higher compared to graded Surti and Non-descript breeds. The prevalence of SCM was highest in above 6th stage of lactation. Single quarter infection (51.85%) was more common compared to multiple quarter infection. Hind (83.34%) and left side (57.41%) quarters were more prone to SCM. The indirect tests such as WST and CMT were found to be closely agreement with SCC and bacteriological examination. Due to their efficacious, easy to perform and interpret, these tests can effectively be employed to detect SCM under field conditions. Somatic cell count of = 3,00,000/ mL of milk was regarded as the upper limit for normal buffaloes. The SCC of = 3,00,000/mL of milk with positive bacterial growth were used to diagnose SCM in the present study. *Staphylococcus* sp. 25(46.30%) was the most common pathogens isolated from SCM followed by *Streptococcus* sp. 11(20.37%) and *E. coli* 06 (11.11%) of the 54 bacterial isolates. Monobacterial and mixed bacterial infections were observed in 47 (87.04%) and 7 (12.96%) cases respectively.

Key words: Buffalo, subclinical mastitis, california mastitis test, white side test, somatic cell count, bacteriological examination

INTRODUCTION

Mastitis is recognised worldwide as one of the most costly diseases of dairy animals because it incurs great financial loss due to reduced milk yield, increased culling rates and treatment costs as well as lowering nutritive value of milk (Radostits *et al*., 2007). In contrast to visible changes in the acute form of mastitis there is absence of gross abnormalities in the milk or udder in case of subclinical mastitis which leads to a greater loss to the dairy industry. In India, Singh and Singh (1994) and Dua (2001) reported the economic loss due to SCM was higher (483.10 crores and 1723.32 crores) than the acute mastitis (234.59 and 696.29 crores) in buffaloes. Apart from causing huge economic losses, this disease also possesses the risk for the transmission of zoonotic diseases to human beings (Radostits *et al*., 2007).

Namakkal, a district of Tamil Nadu having buffalo population of 1,18,202 with milk production of 118.675 tons and contributing 57.76% of total milk production i.e., 205.448 tones (Census, 2004). However, limited information regarding the prevalence and etiology of SCM in clinically normal buffaloes in this region was available. Keeping the above facts in view, the present investigation was undertaken to find out the prevalence and etiology of SCM in buffaloes of marginal farmers maintained under prevailing field conditions by indirect chemical tests, somatic cell count and bacteriological examination.

MATERIALS AND METHODS

In the present study, a total number of 206 (824 teats) apparently healthy buffaloes without any clinical signs of mastitis were screened for SCM in and around Namakkal district during a period of one year from March 2007 to February 2008. All the screened buffaloes were housed in stables during rainy days and allowed to move freely in spring and summer. All the tested animals were apparently healthy during the preceding lactations. Full hand method of milking was performed twice a day (6 and 18 h). Of the 206 animals 68, 50 and 88 were graded Murrah, graded Surti and Non descript breeds, respectively. Information

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regarding, stage of lactation, parity, milking methods and previous incidence of mastitis were collected. The animals were divided into three groups on the basis of stage of lactation viz., early (up to 120 days), mid (121- 240 days) and late (above 240 days) stage. The parity of the buffaloes were grouped into 1, 2, 3, 4, 5, 6 and above 6.

Collection of milk samples: A total of 824 quarter milk samples were collected from 206 apparently healthy lactating buffaloes. Teat end were cleaned with chlorhexidine solution. After discarding first few streams of fore milk, 5 mL of milk from each quarter was collected into sterile screw caped vials for laboratory examination. Each vial was labeled as Left Front (LF), Left Hind (LH), Right Front (RF) and Right Hind (RH). Individual quarter milk samples was subjected to California Mastitis Test (CMT), White Side Test (WST) and SCC, where as the composite or pooled milk from each buffaloes were utilized for bacteriological examination. The WST and CMT were done in the spot i.e., milking byre itself and for confirmatory tests, milk samples were taken to laboratory in a thermal flask containing crushed ice.

White side test: The WST was done according to Doxey (1985). One drop of 4 percent sodium hydroxide and five drops of milk were placed on the glass and mixed with a glass rod. The results were read after 20 sec, according to the change in viscosity of milk as negative, 1+, 2+ and 3+.

California mastitis test: The test was carried out according to the method of Schalm and Noorlander (1957). Equal volume of milk and CMT reagent (obtained from IVPM, Ranipet, Tamil Nadu) were mixed on a test plate. The formation of viscous gel was evaluated by rotating the plate gently. According to the changes of colour and formation of gel, the result was interpreted as negative, 1+, 2+ and 3+ as described by Schalm *et al.* (1971). Quarters with CMT score of 1+ and above were judged as positive. Buffaloes were considered positive for SCM, when at least one quarter turned out to be positive for CMT.

Somatic cell count: It was done as described by Schalm *et al.* (1971). Milk was mixed thoroughly before testing. Ten microliter (One loopful) of milk from each quarter was spread over 1 cm^2 marked square area on a glass slide. The milk film was left undisturbed at room temperature until it dried, then the smear was fixed in Xylol for 5 min and stained with Loffer's methylene blue reagent. Cell counting was made under oil immersion as per the procedure described by Dhakal (2006).

Microbiological examination: The CMT and WST positive samples were held in ice box until transported to the laboratory, where the samples were kept at room temperature for 30 min before streaking on the culture plates. One hundred microliter of each buffalo pooled milk from indirect test positive sample were streaked on the Mac Conkey's and blood agar plates for bacterial culture and isolation. Pure colonies were identified on the basis of colony characters, Gram's reaction, morphological findings and biochemical tests as described by Barrow and Feltham (1993).

The bacteriological examination was reported to be most reliable method for diagnosis of subclinical mastitis. Hence an animal was considered positive for SCM irrespective of the number of quarters affected if the milk samples showed increased SCC along with bacteriological growth.

Statistical analysis: The data were subjected to Chi-square test of analysis and completely randomized block design as per Snedecor and Cochran (1994).

RESULTS

In the present study, total number of animals affected with SCM were 54 (26.21 percent) out of 206 buffaloes. Among the different breeds Graded Murrah (GM), graded Suruti (GS) and Non-descript (ND) showed 15.33, 5.83 and 4.85% positivity to SCM, respectively on SCC and bacteriological examination (Table 1). The breed wise analysis showed no significant difference in subclinical mastitis among different breeds.

The prevalence of SCM in different stages of lactation has been shown in Table 2. The prevalence of SCM was 17.44, 19.29 and 44.44% in early, mid and late stages of lactation, respectively. Among the different breeds also the prevalence was high during late stage of

Table 1: Breed wise prevalence of subclinical mastitis in buffaloes

		WST		CMT		SCC		Bacteriology	
Breed	No. of animals examined	1	2	1	2	1	2	1	2
GM	68	33	16.02	32	15.53	32	15.53	32	15.53
GS	50	15	7.28	14	6.80	12	5.83	12	5.83
ND	88	13	6.31	12	5.82	10	4.85	10	4.85
Total	206	61	29.61	58	28.15	54	26.21	54	26.21

1: Indicate number of animals positive for SCM, 2: Indicate percent of positivity to total number of animals screened (i.e., 206)

Table 2: Prevalence SCM in different stages of lactation

Stage of lactation	No. of animals tested	No. of positivity	Percent of positivity	Breed		
				GM	GS	ND
Early	86	15	17.44	8 (9.30)*	4 (4.63)	3 (3.49)
Mid	57	11	19.29	6 (10.53)	3 (5.26)	2 (3.50)
Late	63	28	44.44	18 (28.56)	5 (7.94)	5 (7.94)

*Number of animals positive and figures in parenthesis indicate percent of positivity in different breeds

Table 3: Prevalence of SCM in different parities of buffaloes

Parity	No. of animals tested	No. of positivity	Percent of positivity
1	39	07	17.94
2.	34	07	20.58
3.	39	09	23.07
4.	40	13	32.50
5.	36	11	30.55
>6.	18	07	38.89

Table 4: CMT and Mean SCC×10^5/mL of milk in SCC affected buffaloes

CMT reaction	No. of observation	SCC×10^5/mL of milk
Negative	20	5.25±0.002[a]
+	46	5.93±0.005[b]
++	9	6.56±0.002[c]
+++	3	7.02±0.000[d]

Means bearing different superscript differ significantly ($p<0.01$)

Table 5: Microorganisms isolated from cases of SCM in buffaloes

Organism	Number isolated	Percent
Staphylococcus sp.	25	46.30
Streptococcus sp.	11	20.37
Escherichia coli	06	11.11
Bacillus sp.	03	05.56
Klebsiella sp.	02	3.70
Staphylococcus sp.+*E.coli*	04	7.41
Staphylococcus sp.+*Klebsiella* sp.	01	1.85
Streptococcus sp.+*E.coli*	02	3.70

lactation. The Chi-square analysis of SCM at different stages of lactation revealed a significant difference ($p>0.05$) among different stages of lactation with regard to percentage of occurrence of mastitis. However, there is no significant difference between different breeds studied.

The percentage of SCM prevalence in different parities has been shown in Table 3. The prevalence of SCM was observed to increase along with parity. The percent of prevalence being 17.94, 20.58, 23.07, 32.50, 30.55 and 38.89 in first, second, third, fourth, fifth and above sixth parity, respectively. The overall incidence was highest during above 6th lactation and it was found to increasing after 4th lactation. However, there is no significant difference among different parities.

Quarter (s) involvement in buffaloes showed that maximum number of positive animals (51.85%) was having infection in single quarter. The percentage involvement of two, three and four quarter (s) was 33.33, 9.26 and 5.56, respectively. The prevalence of SCM in LF, LH, RF and RH quarters were 12 (11.11%), 50 (46.30%), 06 (5.5%) and 40 (37.04%), respectively. There was higher prevalence in hind quarters and among which left one (46.30%) was found to be more susceptible. In case of fore quarters, left quarter (11.11%) was more susceptible.

The indirect tests were compared with SCC and bacteriological examination in order to know their efficacy. The percentage of agreement of indirect tests i.e., WST and CMT with SCC and bacteriological examination were 88.52 and 93.10 percent, respectively. Based on the above observation, SCC and bacteriologically positive samples were considered as SCM positive animals. Hence, in the present study, total number of animals affected with SCM were 54 (26.21 percent) out of 206 animals.

The mean values of milk SCC in different scores of CMT are given in Table 4. Out of 206 animals tested, 58 showed CMT positive reaction and the score varied from Negative to 3+. The mean SCC in Negative, +, ++ and +++ were 5.25±0.002, 5.93±0.005, 6.56±0.002 and 7.02±0.000, respectively and they differed highly significantly ($p<0.01$). In the negative samples, the SCC ranged from 0.72 to 2.80×10^5. Two samples which showed positive reaction (+) in CMT revealed SCC with in the negative range values (2.08 and 2.64×10^5).

The pooled milk samples of 58 and 61 buffaloes which showed positive reaction for SCM by CMT and WST respectively were tested bacteriologically, of which 54 revealed different bacterial infections (Table 5). Among them 47 (87.04%) showed monobacterial infection and 7 (12.96%) revealed mixed bacterial infection. The major pathogen isolated from milk samples were *Staphylococcus* sp. 25 (46.30 %) followed by *Streptococcus* sp. 11 (20.37%), *E.coli* 6 (11.11%), *Bacillus* sp. 3 (5.56%) and *Klebsiella* sp. 2 (3.70%) in pure culture. *Staphylococcus* sp. and *Streptococcus* sp. were also the chief etiological agents associated with mixed infections. In this study 73.79% of the samples did not yield any bacteria.

DISCUSSION

Infections of mammary gland by pathogenic bacteria result in decreased milk production and compositional changes that vary with the intensity and duration of the infection. Subclinical mastitis are those for which no visible changes occur in the appearance of milk or the udder but milk production decreases, bacteria are present in the secretion and composition is altered (Harmon, 1993). It is very difficult to detect SCM in the udder

without the help of laboratory diagnostics. Among the various tests, isolation and identification of pathogenic organisms from milk is most reliable and direct indicator for diagnosis of SCM, but it is cumbersome and expensive for regular testing. Hence indirect tests such as WST and CMT were employed to screen this form of mastitis and the results were compared with bacteriological examination.

In the present study, total number animals affected with SCM were 54 (26.21%) out of 206 buffaloes. Sigh and Baxi (1980) and Dhakal (2006) were reported 23.86 and 21.7% of SCM respectively which might be comparable with the present findings.

Among the different breeds, graded Murrah (15.53%) showed higher incidence. The significant difference between the different breeds may be due to their milk production potentials. Radostits *et al.* (2007) stated that high yielding animals are more susceptible to mastitis than low yielding ones.

The prevalence of SCM was higher in later stage of lactation (44.44%). The finding of the present study was contrary to earlier observation (Palanivel *et al*., 2005), in which higher incidence during early (70.80%) and low in late (36.8%) stage of lactation were reported. Comparison of the available information on the incidence of clinical and subclinical infections of udder indicates that incidence of clinical mastitis may be more during early and mid lactation (Bendixen *et al*., 1988). The incidence of SCM may show the opposite trend (Trisanram *et al*., 1994).

Comparison of the incidence of SCM in different parity groups showed in general the incidence of infection increased as the lactation number increased. The incidence was minimum during first (17.94%) and maximum during above 6th (38.89%) lactation which was in agreement with Palanivel *et al.* (2005), who found that the risk of SCM increases significantly with advancing the age of animals which approximate to the parity number. The most likely cause of the higher prevalence of the SCM in multiparous animals might be due to prolonged period of risk.

In most of the animals it was observed that only one quarter (51.85%) was affected which was in agreement with Saini *et al.* (1994). The possible reason could be that the pathogenic organisms might not have entered all the quarters at the same time. The predisposing factors like injury, defective sphincter and prevalence of pathogens and immune status of different quarters also play a role in the development of the disease (Saini *et al*., 1994).

Quarter wise presence of SCM was found to be higher in hind quarters (83.34%) than fore quarters (16.36%). Naiknaware *et al.* (1998) and Chisty *et al.* (2007) also observed higher rate of hind quarter affections. The higher prevalence in hind quarters may be due to their frequent exposure to dung and urine, while milking they are pulled forward and sideways which may lead to undue stress on them, the left side quarter get first chance of contamination by milkers, being milking approach side in buffaloes and comparatively more milk yield from hind quarters increases their susceptibility to mastitis.

The bacteriological test was reported to be most reliable method for diagnosis of mastitis. Keeping this in view the indirect tests were compared with them in order to know their efficacy. The percent agreements of WST and CMT with SCC and bacteriological examination were 88.52 and 93.10%, respectively. The present finding was close agreement with Saravanan *et al.* (2008). This indicates that any one of the test can be safely resorted to detect the incidence of SCM in buffaloes under field condition.

In the present study, SCC increased with the increasing severity of SCM as determined by CMT. The SCC in SCM cases were above 3,28,000 with an average of 10.04×10^5/mL of milk. Our results are in conformity with Gosh *et al.* (2004), who also recorded higher SCC in SCM buffaloes compared to normal non infected buffaloes. Increase in SCC in SCM might be due to increased number of leukocytes and epithelial cells in milk. The great variation in SCC in SCM (3.28 to 22.80×10^5/mL) might be due to management, breed, age, stage and parity of lactation and infectious status (Harmon, 1993).

Staphylococcal (46.30%) and *Streptococcal* sp. (20.37%) were predominant subclinical mastitis pathogens in buffaloes in the present study. These findings were in close agreement with earlier workers (Palanivel *et al*., 2005; Piepers *et al*., 2007). The higher incidence of Staphylococcal infection might be due to their ubiquitous nature and its well adaptation to survive in the udder and establish a mild subclinical infection of long duration from which it is shed in milk, facilitate transmission to healthy animals during milking procedure and the emergence of drug resistant strains which destroy penicillin through the production of enzyme pencillinase. Moreover, these organisms survive better in the environment and are widely distributed at different body sites of lactating animal leads to easy infection. Of the 54 buffaloes infected with SCM, 47 (87.03) infected with monobacterial and 7 (12.97%) with concurrent bacterial infection which confirming the earlier results of Naiknaware *et al.* (1998).

CONCLUSION

In conclusion, SCM was observed in a considerable percentage of buffaloes in Namakkal area of Tamil Nadu.

Hence, a study on the risk factors associated with this problem will help in control and reduce the possible economic losses.

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