SOMATIC CELL COUNT AND BIOCHEMICAL COMPONENTS OF MILK: RELATION TO UDDER HEALTH AND DIAGNOSIS OF SUBCLINICAL QUARTER INFECTIONS IN BUFFALOES

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ABSTRACT

The current investigation determines the occurrence of subclinical mastitis and its diagnosis in relation to milk SCC and biochemical composition in buffaloes. The results showed that taking quarter foremilk (QFM) somatic cell count (SCC) threshold limit of $\leq 200\times10^3$ cell for defining healthy quarters, 16.48% of buffaloes and 5.79% of quarters were positive for specific subclinical mastitis. In total, 57/363 (15.70%) of quarters were bacteriological positive with 21 (5.79%) representing specific and 36 (9.92%) latent infections. The major pathogen isolated were 28 (49%) coagulase-negative staphylococci followed by 16 (28%) *S. aureus*, 09 (16%) *Streptococcus* and 4 (7%) *Corynebacterium* spp. The average QFM SCC was found as 52.50×10^3 cells /ml for the quarter with no bacteria, and 356.51×10^3 cell /ml for the quarters infected with one or the other bacteria ($P<0.05$). The milk electrical conductivity (EC), Fat and Lactoseshowed significant alterations with the quarter infection; the EC and Fat increased while Lactose decreased in infected quarters ($P<0.05$). The California mastitis test CMT) at a cut off score of >0.5 for diseased quarters showed 68.75% sensitivity with 99.09% specificity. The EC with 87.50% sensitivity and 74.02% specificity and Lactose with 84.37% sensitivity and 66.16% specificity also showed significant ($P<0.01$) discrimination ability to differentiate healthy and mastitis quarters in buffaloes. The milk pH was not found to be a good parameter to identify diseased quarters.

Keywords: *Bubalus bubalis*, buffaloes, subclinical mastitis, milk SCC, biochemical components, diagnosis

INTRODUCTION

Mastitis is major animal welfare and economic concern in the dairy industry (Medrano-Galarza *et al*., 2012). It is characterized by physical, chemical and bacteriological changes in the milk and pathological changes in the glandular tissue (Sharma, 2007). Its occurrence could be clinical and subclinical. The subclinical mastitis due to its hidden form and higher occurrence results in higher economic losses (Bansal and Gupta, 2009) and forms a constant reservoir of intramammary infections in the dairy herd. Mastitis refers to an inflammation within the udder but not always necessarily infection; however, the bacterial infection is the usual cause of mastitis (Pyörälä, 2003; Oliveira *et al*., 2004). In response to infection and injury, there is the shedding of somatic cells in

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milk. Therefore, the guidelines for the diagnosis of mastitis include both the microbial culture and the milk SCC as a measure of udder inflammation. Milk SCC is a key component of international and national regulations for milk quality and is an indicator of udder health and the prevalence of clinical and sub-clinical mastitis in dairy farming (Sharma *et al*., 2013). Normally, in milk from a healthy mammary gland, the SCC is lower than 10^5 cells/ml, while in bacterial infection it increases above 10^6 cells/ml (Bytyqi *et al*., 2010). Buffalo is considered to be less susceptible to mastitis than cattle (Mustafa *et al*., 2013). Some important udder morphological characteristics of the buffalo may influence any difference from the cow in the predisposition for infections and inflammations, e.g. the tighter teat sphincter of buffaloes (Uppal *et al*., 1994). On the other hand, more pendulous udders and longer teats with larger teat diameter in buffaloes may contribute to a greater risk of mastitis (Kaur *et al*., 2018).

In mastitis, the biochemical composition of milk gets altered; an increase in EC and SCC, and a decrease in lactose content in milk from mastitis udders was reported in dairy cows (Bansal *et al*., 2005). Concerning milk fat, no effect of mastitis was observed by Rogers *et al*. (1989). Whereas a reduction in the fat content of milk in subclinical mastitis was noted in cows (Auldist *et al*., 1996). The mastitis-related alterations in the biochemical components of milk have been used in the identification of diseased quarters/ udders (Bansal *et al*., 2005). For example, electrical conductivity is frequently used in the identification of mastitis quarters by cow-side and milk line systems. However, the discrimination ability of EC is considerably affected by the fat level and fraction of milk used (Holdaway *et al*., 1996; Bansal *et al*., 2005). Lactose was found to lead to good differentiation of healthy and mastitis quarters in one study (Bansal *et al*., 2005) but it did not show promising results in other investigations (Holdaway *et al*., 1996). Singh *et al*. (2018) observed the highest level of discrimination for CMT (86.74%) in differentiating healthy and mastitis quarters in Sahiwal cows. The use of EC showed a 36.30% positive prediction with 33% misclassifications.

The purpose of the present study is to evaluate the udder health in buffaloes particularly in relation to milk SCC and biochemical composition and determine the efficacy of various biochemical parameters in the diagnosis of subclinical quarter infections.

MATERIALS AND METHODS

The study involved 91 lactating buffaloes in an organized dairy farm. The animals were milked twice daily at 12 h milking intervals with test day average milk production of 7.9 Kg. The weaning of calves was practiced at birth, and daily morning and evening milking was recorded. The udder preparation included pre-milking washing and drying of the udder and post milking teat dipping. The prompt therapy of clinical mastitis cases and use of dry therapy were followed as a farm management practice.

The milk samples were collected at the regular afternoon milking (3.30 pm). It was ensured that no animals involved in the study suffered from clinical mastitis or were treated for mastitis during the last 21 days. The proper cleanliness and dryness of the udder and teats were ensured. The teat orifice was disinfected with a spirit swab. The two types of milk samples were collected: quarter foremilk (QFM, about 10 to 15 ml) from each functional quarter in sterilized glass vials and the

udder composite milk (about 30 to 40 ml) in clean disposable plastic vials. No samples from freshly calved (before the fifth day after calving) or late lactation buffaloes (after 270 days of lactation) or animals with day milk production less than 3 Kg were used. The milk samples after collection were straightaway transferred and processed for various parameters in the mastitis designated lab.

Microbiological analysis

The standard microbial procedures prescribed by the National Mastitis Council (Hogan *et al*., 1999) were employed for milk culturing and isolation and identification of microbial organisms. After thorough mixing, the milk sample was streaked on the 5 to 7% ovine blood agar plates. Each plate was divided into four equal parts marked for the individual quarters and 0.05 ml of quarter milk was streaked on the respective part with a sterilized platinum loop. The plates were incubated at 37°C and examined after 24 to 48 h for the presence of any bacterial growth. The growth if any was evaluated in terms of colony shape, size, color, texture and hemolytic pattern. Further classification and identification of bacteria followed Gram staining and Catalase tests.

The Catalase test could distinguish the Gram-positive cocci into Catalase-negative *Streptococcus* and catalase-positive *Staphylococcus* or *Corynebacterium* spp. The corynebacteria growth was visible after 36 to 48 h of incubation, mainly in the fatty areas of milk-streaked plates and was non-hemolytic. The Mannitol salt agar and Baird Parker agar were used for the differentiation of *Staphylococcus aureus* from other *Staphylococcus* spp. On Mannitol salt agar, *Staphylococcus aureus* resulted in yellow zones with yellow colonies, whereas other staphylococci showed no change in colour of medium, and produced small pink or red colonies. On Baird Parker agar, *Staphylococcus aureus* caused an opaque zone of precipitation with dark grey to black colonies. The tube coagulase test was also done to identify the coagulase-positive and coagulase-negative staphylococci by using rabbit plasma.

Somatic cell count

Soma Scope Smart (From DELTA Instruments, The Netherlands) measured the somatic cells in milk. Both absolute ($\times 10^3$cells/ml) and somatic cell score (SCS) values were calculated.

California mastitis test

In this test, the sodium lauryl sulfate was used as a test reagent. The test was conducted in a plastic paddle with four cups marked for individual teats. The milk and reagent were added in equal quantities (about 3 ml each). The contents were mixed by giving a circular motion to the paddle along the horizontal plane. The results were recorded within 10 seconds and interpreted as:

No mastitis (-, CMT score 0): Liquid with no precipitate.

Trace (CMT score 0.5): Traces of precipitate which disappeared quickly

Doubtful (+, CMT score 1): Traces of precipitate with little gel formation

Positive (++, CMT score 2): Precipitate thickens and moves towards the center on swirling.

Strong positive (+++, CMT score 3): A well-defined gel which a tendency to stick to the base of the cup and a central peak was seen during swirling. When movements stopped, the mixture leveled again at bottom of the cup.

Electrical conductivity

The milk electrical conductivity was measured using a digital conductivity meter from Mettler-Toledo Five Easy™ Plus. The results were expressed in mS/cm.

pH

A digital pH meter (Mettler-Toledo Five Easy™ Plus) was used for evaluating the pH of the milk.

Biochemical composition of milk

The milk biochemical components viz., fat, protein, solids not fat (SNF) and lactose were tested by using milk analyser lactoscan LA from Milkotronic Ltd., Bulgaria. The basic operation is that of an infrared spectrophotometer in which an infrared single beam is focused to pass through the samples and strike a detector. The energy detected is amplified through a microprocessor and converted to a corresponding readout. The results were expressed in % fat, protein, lactose, and SNF.

Definition of quarter/ udder health

The health status of individual quarters was defined following IDF criteria which considered at a time both the culture result and cell count of quarter foremilk (Table 1A).

The buffaloes with all the four healthy quarters were categorized into Healthy udders. Whereas the buffaloes with one or more specific/ non-specific mastitis quarter (s) were assigned the Mastitis udders group.

Statistical analysis

The statistical evaluation and interpretation of the data were done by applying ANOVA with post HOC (Tuckey's method) using SAS version 9.3 (SAS Institute, Cary, USA). The statistical analyses to determine the diagnostic efficacy of the tests (kappa coefficient, the area under the receiver operating characteristic (ROC) curve, sensitivity, specificity, Youden index, positive and negative likelihood ratios, and positive and negative predictive values were performed. The SCC threshold of 200×10^3 cells/ml was used to determine the sensitivity and specificity of different parameters in discriminating individual mammary quarters into healthy and diseased ones. The level of significance was set at $P<0.05$.

RESULTS AND DISCUSSION

The occurrence of Subclinical mastitis in Buffaloes

At QFM cutoff $\leq200\times10^3$ cells/ml for healthy quarters, 15/91 buffaloes (16.48%) and 21/363 quarters (5.79%) were found positive for specific mastitis. The 11 quarters (3.03%) had nonspecific, and 36 (9.92%) showed latent infections, with the remaining 295 (81.27%) revealing no bacterial infection (Table 1B). The occurrence of disease in our study falls in line with the one reported by Joshi and Gokhale (2006) who observed the disease prevalence in buffaloes varying from 5 to 20% in India. They further observed disease prevalence much higher in Jersey and Holsteins cows as compared to that in buffaloes and local cattle. The incidence of latent infections was found lower than reported previously 26.63% (Bansal *et al*., 1995) and 23.08% (Kumar *et al*., 2012). Dhakal (2006) identified subclinical mastitis quarters in buffaloes based on positive milk cultures and SCC 200×10^3 cells/ml and observed 21.7% of buffaloes and 8% of the quarters positive for mastitis. Using IDF criteria, Supriya *et al*. (2010) estimated that 14.17, 18.33 and 8.35% of

quarters in buffaloes had subclinical, latent and nonspecific mastitis, respectively. Overall, 53.33% of animals were positive for bacteriology and 35% revealed milk SCC above 500×10^3 cells/ml. The comparatively lower disease prevalence in the herd under our study may be probably attributed to the organized management system including hygienic milking procedure, post milking teat dipping, and dry therapy.

The coagulase-negative staphylococci (CNS) causing almost half (49%, 28/57) of the intramammary infections (IMI) were found the major organism in the present study, followed by *S. aureus* (28%, 16), *Streptococci* (16%, 09) and *Corynebacteria* (7%, 04).

The predominance of CNS as pathogens of subclinical mastitis in buffaloes has been reported previously (Chavoshi and Hussain, 2012; Pankaj *et al*., 2013; Bansal *et al*., 2015). In a 10 year evaluation of data, Bhutia *et al*. (2019) observed the staphylococci chief etiological agents in clinical cases of mastitis also. However, the distribution of mastitis isolates seen in the current investigation differed from that documented previously in cows (Abdel-Rady and Sayed, 2009) and buffaloes (Mitra *et al*., 1995) where these workers reported the *Escherichia coli* as one of the major isolates in subclinical mastitis cases. The predominance of staphylococci may be attributed to several factors. First, staphylococcal mastitis being one of the most common contagious nature, these organisms may spread from infected to healthy buffaloes on hands or equipment from one udder to another.

Second, the *S. aureus* persists in the mammary glands and result in chronic lifelong infection (Bannerman *et al*., 2004; Riollet *et al*., 2000; Yang *et al*., 2008). The *S. aureus* is also a common commensal of skin. They inhabit the teat skin, enter through the teat canal into the gland and resist phagocytosis. This mechanism includes a capsule, surface protein, and biofilm-associated protein which usually causes subclinical infection, causing higher cell counts but no detectable changes in milk or the udder (Ruegg *et al*., 2015).

Variation in milk somatic cell count and biochemical composition with quarter infections

The Mean±SESCC and biochemical composition of the QFM in the healthy and infected quarter are presented in (Table 2). A significant difference was found for milk SCC, EC, fat, and lactose between the infected and healthy quarters; the SCC, EC and fat increased while the lactose decreased with intramammary infections. The pH and total protein did not show any change while the SNF content decreased marginally. An increase in SCC of the infected quarter is due to a response towards invasive agents (pathogens). According to Bytyqi *et al*. (2010), in the healthy mammary gland, the SCC is lower than 1×10^5 cells/ml, while bacterial infection can cause it to increase above 1×10^5cells/ml. The difference in milk composition with higher somatic cell count has been reported previously in cows (Hamann, 2002; Bansal *et al*., 2005; Berglund *et al*., 2007) and buffaloes (Bansal *et al*., 2007). Mastitis increased the concentration of ions mainly Na^+ and Cl^- and decreased the concentration of K^+ and lactose in milk and hence increases the EC of the milk. (Fahmid *et al*., 2016).

The decrease in lactose contents with quarter infections is generally accepted. Ingalls (2003) found out lower content of lactose (0.69%) with a rise of per ml milk cell count to 4.21×10^3. Similarly, a high negative association was observed of SCC with the lactose content of milk (Sawa and Piwczynski, 2002). The reduction in lactose content may be attributed to the decreased synthesis caused by damage to mammary glandular tissue

(Schallibaum, 2001). Also, the passing of lactose from the mammary gland into the blood due to the increased permeability of tissues between the milk duct of the udder and the blood plays a part.

A review of the literature shows contradictory reports concerning concentration changes in total protein contents due to mastitis. While we did not find any change, Rawdat and Omaima (2000); Ullah *et al*. (2005); Thakur *et al*. (2018) observed a substantial decrease in total protein contents of milk. On another side, an increase (Auldist *et al*., 1996) or no change (Mitchell *et al*., 1986; Rogers *et al*., 1989) in total protein values of milk due to mastitis was seen. These variable outcomes may be explained on the basis that "total protein" comprises diverse fractions. The fractions originating from blood e.g. bovine serum albumin and immunoglobulins upturn due to increased alveolar permeability, while protein fractions of mammary gland origin such as lactalbumin, casein, and lactoglobulin decline due to impaired glandular synthesis. Thus, the magnitude of alteration in total protein content of milk depends upon the type and amount of pathological response in mastitis.

Our findings on increase in the fat values in mastitis fall in line with earlier recordings (Paura *et al*., 2002; Sawa and Piwczynski, 2002) where a positive correlation between the Log SCC and fat content of milk was observed. However, it is unlike where a decrease (Bansal *et al*., 2007; Thakur *et al*., 2018) or no change (Rogers *et al*., 1989) was observed in the fat content of milk with mastitis in buffaloes/ cows.

While decreases in the fat content of milk may be explained by the decreased synthesis owing to damage to the gland, the increase in fat could be justified by a concurrent large reduction in milk production rather than decreased fat synthesis, telling as a result, a seeming rise in the fat percentage (Schultz, 1977). The decrease in the SNF content of milk with mastitis is agreed generally (Bansal *et al*., 2007).

Ability of biochemical parameters in distinguishing healthy and mastitis quarters

The CMT at the cut off point of >0.5 for diseased quarters showed 68.75% sensitivity with 99.09% specificity (Table 3). The EC with 87.50% sensitivity and 74.02% specificity and Lactose with 84.37% sensitivity and 66.16% specificity were also found good parameters to differentiate healthy and mastitis quarters in buffaloes. Fosgate *et al*. (2013) compare the sensitivity and specificity of milk electrical conductivity (EC) to the California mastitis test (CMT). They observed that the CMT was more accurate than EC for the classification of cows having somatic cell counts >200, 000/ml and for isolation of a bacterial pathogen. Reddy *et al*. (2014) found that EC had the highest specificity (84.84%) and predictive value (79.59%) with the lowest sensitivity (56.62%) compared with the other diagnostic methods of subclinical mastitis in cattle thus it can be used as the criteria to treat and cull the animals in herds with a high prevalence of subclinical mastitis. Pyorala (2003) and Joshi and Gokhale (2006) determined the California mastitis test as the gold standard for screening of mastitis in dairy bovines with higher (>95%) sensitivity and specificity. Whereas, Tiwari *et al*. (2018) determined the milk SCC as a better parameter to distinguish healthy and mastitis udders at a threshold of 245×10^3 cells/ml with 99.97% accuracy. In our study, for the cutoff pH value of 6.9, sensitivity in QFM was 59.38% with a specificity of 79.46 %. The AUC (area under curve) of the ROC graph was 0.703, indicating that the QFM pH could be considered to differentiate

Table 1A. Defining the health status of udder quarters.

Milk SCC (cells/ ml)	Milk culture result	
	Microbial pathogen (Not detected)	Microbial pathogen (Detected)
≤200×10³ cells/ ml	Healthy	Latent infection
≥200×10³ cells/ ml	Nonspecific mastitis	Specific mastitis

Table 1B. The occurrence of subclinical mastitis in buffaloes.

Threshold limit QFM SCC for healthy quarters	Animal basis		Quarter basis				
	Animals examined	Positive for specific mastitis in at least one qut.	Quarters examined	Specific[1] mastitis	Nonspecific[2] mastitis	Latent[3] infection	Healthy[4]
≤200×10³ cells/ml	91	15 (16.48)	363	21 (5.79)	11 (3.03)	36 (9.92)	295 (81.27)

Figures in parenthesis indicate percentages

[1]QFM bacteriological Positive and SCC > defined threshold limit

[2]QFM bacteriological Negative and SCC > defined threshold limit

[3]QFM bacteriological Positive, but SCC not more than the defined threshold limit

[4]QFM bacteriological Negative and SCC not more than the defined threshold limit

Table 2. Milk SCC and biochemical composition in healthy and infected quarters.

Variable	Healthy (n = 306)			Infected quarters (n = 57)		
	Mean±SE	Lower 95% CL for Mean	Upper 95% CL for Mean	Mean±SE	Lower 95% CL for Mean	Upper 95% CL for Mean
SCC ($\times 10^3$ cells/ml)	52.50±3.58	45.46	59.54	356.51±73.42*	209.42	503.60
SCS	1.49±0.07	1.36	1.63	3.47±0.27*	2.93	4.01
EC (mS/cm)	3.88±0.04	3.79	3.97	4.62±0.19*	4.23	5.01
PH	6.78±0.02	6.74	6.83	6.78±0.04	6.69	6.86
Fat (%)	6.37±0.20	5.98	6.75	7.81±0.48*	6.84	8.78
SNF (%)	11.35±0.09	11.17	11.53	11.02±0.12	10.78	11.27
Total Protein (%)	4.19±0.04	4.11	4.27	4.18±0.07	4.05	4.31
Lactose (%)	6.15±0.04	6.07	6.22	5.89±0.07*	5.74	6.03

*P<0.05

Table 3. Ability of biochemical parameters in distinguishing healthy and mastitis* quarters in buffaloes.

Parameter	Criteria of diseased	Sensitivity	Specificity	The area under the ROC curve	95% CI	Z-statistic
CMT score	≥0.5	68.75	99.09	0.840	0.798 to 0.876	8.138 (P<0.0001)
Electrical conductivity	>4.10	87.50	74.02	0.878	0.839 to 0.910	11.859 (P<0.0001)
Lactose	≤5.98	84.37	66.16	0.784	0.738 to 0.825	7.253 (P<0.0001)
Ph	>6.9	59.38	79.46	0.703	0.653 to 0.749	3.623 (P<0.001)

*Includes both specific and nonspecific mastitis quarters with QFM SCC >200×10^3 cells/ml

healthy and diseased quarters in buffaloes. This is in agreement with the similar findings reported for pH as an indicator of subclinical mastitis in cows (Bansal *et al*., 2005). However, Kandeel *et al*. (2019) concluded that at the pH meter cutoff point at dry off ≥6.67 and freshening ≥6.52, the pH had poor sensitivity, specificity, positive likelihood ratio, and kappa coefficient so that it is not used for diagnosing the SCM or IMI in dairy cattle. Patbandha *et al*. (2016) used milk lactose to discriminate infected and healthy udder quarter based on CMT. They estimated an optimum threshold value of 5.31g% (Sensitivity 58.82%; Specificity, 58.28%) in moderate infection and 5.23 g% (Sensitivity 70.97%; Specificity 64.41%) in severe infection by ROC analysis.

CONCLUSIONS

It may be determined that buffaloes harbor fewer quarter infections and possess lower milk SCC levels than that generally reported in cows. As such a physiological threshold of milk SCC in buffaloes may be defined at 100×10^3cells/ml and pathological at $>200\times10^3$cells/ml. The intramammary infections, even at a subclinical level, may result in a considerable rise in SCC and EC, and a decrease in lactose content of milk. The alterations in these parameters could be used in the diagnosis of subclinical quarter infections with significant discrimination ability.

REFERENCES

Abdel-Rady, A. and M. Sayed. 2009. Epidemiological studies on subclinical mastitis in dairy cows in Assiut Governorate, *Vet. World*, **2**(10): 373-380. Available on: http://www.veterinaryworld.org/Vol.2/October/Epidemiological%20Studies%20on%20Subclinical%20Mastitis%20in%20Dairy%20cow.pdf

Auldist, M.J., S. Coats, B.J. Sutherland, J.J. Mayes, G.H. McDowell and G.L. Rogers. 1996. Effects of somatic cell count and stage of lactation on raw milk composition and the yield and quality of Cheddar cheese. *J. Dairy. Res*., **63**(2): 269-280. DOI: 10.1017/s0022029900031769.

Bannerman, D.D., M.J. Paape, J.W. Lee, X. Zhao, J.C. Hope and P. Rainard. 2004. *Escherichia coli* and *Staphylococcus aureus* elicit differential innate immune responses following intramammary infection. *Clin. Diagn. Lab. Immun*., **11**(3): 463-472. DOI: 10.1128/CDLI.11.3.463-472.2004

Bansal, B.K. and D.K. Gupta. 2009. Economic analysis of bovine mastitis in India and Punjab. *Indian J. Dairy Sci*., **62**(5): 337-345.

Bansal, B.K., D.K. Gupta, T.A. Shafi and S. Sharma. 2015. Comparative antibiogram of coagulase-negative staphylococci (CNS) associated with subclinical and clinical mastitis in dairy cows. *Vet. World*, **8**(3): 421-426. DOI: 10.14202/vetworld.2015.421-426

Bansal, B.K., J. Hamamm, O. Lind, S.T. Singh and P.S. Dhaliwal. 2007. Somatic cell count and biochemical components of milk related to udder health in buffaloes. *Italian J. Anim. Sci*., **6**(2): 1035-1038. DOI: 10.4081/ijas.2007.s2.1035

Bansal, B.K., J. Hamann, N.T. Grabowski and K.B. Singh. 2005. Variation in the composition of selected milk fraction samples from healthy and mastitic quarters, and its significance for mastitis diagnosis. *J. Dairy. Res*., **72**(2):

144-152. DOI: 10.1017/s0022029905000798

Bansal, B.K., K.B. Singh, R. Rohan, D.V. Joshi, D.C. Nauriyal and M. Rajesh. 1995. Incidence of sub-clinical mastitis in some cow and buffalo herds in Punjab. *Journal of research: Punjab Agricultural University*, **32**: 79-81.

Berglund, I., G. Pettersson, K. Ostensson and K. Svennersten-Sjaunja. 2007. Quarter milking for improved detection of increased SCC. *Reprod. Domest. Anim*., **42**: 427-432. DOI: 10.1111/j.1439-0531.2006.00803.x

Bhutia, P.S., B.K. Bansal, D.K. Gupta, R.S. Singh and S.K. Uppal. 2019. Bacterial isolation of milk samples submitted from clinical mastitis buffaloes during 2007 to 2016. *Trop. Anim. Health Pro*., **51**(6): 1551-1557. DOI: 10.1007/s11250-019-01846-w

Bytyqi, H., U. Zaugg, K. Sherifi, A. Hamidi, M. Gjonbalaj, S. Muji and H. Mehmeti. 2010. Influence of management and physiological factors on somatic cell count in raw cow milk in Kosova. *Vet. Arhiv*., **80**(2): 173-183. Available on: http://vetarhiv.vef.unizg.hr/papers/2010-80-2-2.pdf

Chavoshi, M. and J. Husaini. 2012. Buffalo subclinical mastitis bacterial pathogens in Iran. *In The 2nd International Conference on Biomedical Engineering and Technology*, IACSIT Press, Singapore. **34**(2012): 143-146. Available on: http://www.ipcbee.com/vol34/029-ICBET2012-FB029.pdf

Dhakal, I.P. 2006. Normal somatic cell count and subclinical mastitis in Murrah buffaloes. *J. Vet. Med. B*., **53**(2): 81-86. DOI: 10.1111/j.1439-0450.2006.00918.x

Fahmid, S., E. Hassan, H. Naeem, S. Barrech, S. Lodhiand and S. Latif .2016. Determination of mastitis by measuring milk electrical conductivity. *International Journal of Advanced Research in Biological Sciences*, **3**(10): 1-4. DOI: 10.22192/ijarbs

Fosgate, G.T., I.M. Petzer and J. Karzis. 2013. Sensitivity and specificity of a hand-held milk electrical conductivity meter compared to the California mastitis test for mastitis in dairy cattle. *Vet. J*., **196**(1): 98-102. DOI: 10.1016/j.tvjl.2012.07.026

Hamann, J. 2002. Relationships between Somatic cell counts and milk composition. *Bulletin of the International Dairy Federation*, **372**: 56-59.

Hogan, J., R. Gonzales, R. Harmon, S. Oliver, S. Nickerson, J. Pankey and K. Smith. 1999. *Laboratory Handbook on Bovine Mastitis*. National Mastitis Council, Madison, Wisconsin, USA.

Holdaway, R.J., C.W. Holmes and I.J. Steffert. 1996. A comparison of indirect methods for diagnosis of subclinical intramammary infection in lactating dairy cows. *Aust. J. Dairy Technol*., **51**(2): 64-82.

Joshi, S. and S. Gokhale. 2006. Status of mastitis as an emerging disease in improved and periurban dairy farms in India. *Ann. N.Y. Acad. Sci*., **1081**: 74-83. DOI: 10.1196/annals.1373.007

Kandeel, S.A., A.A. Megahed, M.H. Ebeid and P.D. Constable. 2019. Ability of milk pH to predict subclinical mastitis and intramammary infection in quarters from lactating dairy cattle. *J. Dairy. Sci*., **102**(2): 1417-1427. DOI: 10.3168/jds.2018-14993

Kaur, G., B.K. Bansal, R.S. Singh, N. Kashyap and S. Sharma. 2018. Associations of teat morphometric parameters and subclinical mastitis in riverine buffaloes. *J. Dairy Res.*, **85**(3): 303-308. DOI: 10.1017/

S0022029918000444

Kumar, M., M.L.V. Rao and P.C. Shukla. 2012. Incidence of latent mastitis in buffaloes of Jabalpur region of Madhya Pradesh. *International Journal of Livestock Research*, **2**: 138-139. DOI: 10.5455/ijlr.20120930051522

Medrano-Galarza, C., J. Gibbons, S. Wagner, A.M. de Passille and J. Rushen. 2012. Behavioral changes in dairy cows with mastitis. *J. Dairy. Sci*., **95**: 6994-7002. DOI: 10.3168/jds.2011-5247

Mitchell, G.E., S.A. Rogers, D.B. Houlihan, V.C. Tucker and B.J. Kitchen. 1986. The relationship between somatic cell count, composition and manufacturing properties of bulk milk. I. Composition of farm bulk milk. *Aust. J. Dairy. Technol*., **41**(1): 9-12.

Mitra, M., D. Ghosh, K. Ali, C. Guha and A.K. Pramanik. 1995. Prevalence of subclinical mastitis in an organised buffalo farm at Haringhata. *Indian. Vet. J*., **72**(12): 1310-1311.

Mustafa, Y.S., N.A. Farhat and Z. Tooba. 2013. Prevalence and antibacterial susceptibility in mastitis in buffalo and cow in district Lahore-Pakistan. *Buffalo. Bull*., **32**(4): 307-314. Available on: http://ibic.lib.ku.ac.th/e-Bulletin/IBBU201304010.pdf

Oliveira, A.A.F. 2004. *Evaluation of aspiration and expression cytology in the diagnosis of buffalo mastitis and the search for toxigenic and beta-lactamase-producing strains of Staphylococcus aureus* and *Escherichia coli.* Thesis, Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista, Botucatu, Brazil.

Pankaj, A.S., R. Chhabra and N. Sindhu. 2013. Sub-clinical mastitis in Murrah buffaloes with special reference to prevalence, etiology and antibiogram. *Buffalo. Bull*., **32**(2): 107-115. Available on: http://ibic.lib.ku.ac.th/e-Bulletin/IBBU201302006.pdf

Patbandha, T.K., K. Ravikala, B.R. Maharana, R. Pathak, S. Marandi, P.U. Gajbhiye and R. Malhotra. 2016. Receiver operating characteristic analysis of milk lactose for identification of mastitis in buffaloes. *Indian J. Anim. Res*., **50**(6): 969-973. DOI: 10.18805/ijar.11176

Paura, L.D. Kairisha and D. Jonkus. 2002. Repeatability of milk productivity traits. *Vet. Zootec*., **19**(41): 90-93. Available on: https://vetzoo.lsmuni.lt/data/vols/2002/19/pdf/paura.pdf

Pyörälä, S. 2003. Indicators of inflammation in the diagnosis of mastitis. *Vet. Res*., **34**(5): 565-578. DOI: 10.1051/vetres:2003026

Rawdat, A.M and N.M. Omaima. 2000. Some biochemical changes in serum and milk of mastitic buffaloes. *Egyptian Journal of Agricultural Research*, **78**(4): 1737-1747.

Reddy, B.S.S., K.N. Kumari, Y.R. Reddy, M.V. B. Reddy and B.S. Reddy. 2014. Comparison of different diagnostic tests in subclinical mastitis in dairy cattle. *International Journal of Veterinary Science*, **3**(4): 224-228.

Riollet, C., P. Rainard and B. Poutrel. 2000. Differential induction of complement fragment C5a and inflammatory cytokines during intramammary infections with *Escherichia coli* and *Staphylococcus aureus*. *Clin. Diagn. Lab. Immunol*.,**7**(2): 161-167. DOI: 10.1128/cdli.7.2.161-167.2000

Rogers, S.A., G.E. Mitchell and J.P. Bartley. 1989. The relationship between somatic cell count, composition and manufacturing properties

of bulk milk 4 - non-protein constituents. *Aust. J. Dairy Technol*., **44**(2): 53-56.

Ruegg., P.L., R.J. Erskine and D.E. Morin. 2015. *Large Animal Internal Medicine*, 5th ed. Elsevier Mosby, 3251 Riverport Lane St.Louis, Missouri, USA. p. 1023-1025.

Sawa, A. and D. Piwczynski. 2002. Somatic cell count and milk yield and composition in Black and White x Holstein- Friesian cows. *Med. Weter.*, **58**(8): 636-640.

Schultz, L.H. 1977. Somatic cell in milk-physiological aspects and relationship to amount and composition of milk. *J. Food Proctect*., **40**(2): 125-131. DOI: 10.4315/0362-028X-40.2.125

Sharma, N. 2007. Alternative approach to control intramammary infection in dairy cows- A review. *Asian J. Anim. Vet. Adv*., **2**(2): 50-62. DOI: 10.3923/ajava.2007.50.62

Sharma, N., T.Y. Kang, S.J., Less, J.N. Kim, C.H. Hur, J.C. Ha, V. Vohra and D.K. Jeong. 2013. Status of bovine mastitis and associated risk factors in subtropical Jeju Island, South Korea. *Trop. Anim. Health Pro*., **45**(8): 1829-1832. DOI: 10.1007/s11250-013-0422-3

Singh, A., B.K. Bansal, R.S. Singh, D.K. Gupta, S. Singh and S. Sharma. 2018. Prevalence and diagnosis of subclinical mastitis in sahiwal dairy cows. *International Journal of Livestock Research*, **8**(6): 337-345. DOI: 10.5455/ijlr.20170621112044

Supriya, V.S. and D. Lather. 2010. Prevalence of sub-clinical mastitis in an organized cow herd. *Haryana Veterinarian*, **49**: 64-65. Available on: https://www.luvas.edu.in/haryana-veterinarian/download/haryana-veterinarian/20.pdf

Thakur, S., M. Singh, G.K. Aseri, A. Verma and S.S. Khan. 2018. Isolation and characterization of mastitis pathogens and milk composition changes in Murrah buffaloes (*Bubalus bubalis*) during winter season. *Indian J. Anim. Res*., **52**(2): 276-280. DOI: 10.18805/ijar.11320

Tiwari S., T.K. Mohanty, T.K. Patbandha, A. Kumaresan, M. Bhakat, N. Kumar and R.K. Baithalu. 2018. Critical thresholds of milk SCC, EC and pH for detection of sub-clinical mastitis in crossbred cows reared under subtropical agroclimatic condition. *International Journal of Livestock Research*, **8**(6): 152-159. DOI: 10.5455/ijlr.20170620120712

Uallah, S., T. Ahmad, M.Q. Bilal, Z.U. Rahman, G. Muhammad and S.U. Rahman. 2005. The effect of severity of mastitis on protein and fat contents of buffalo milk. *Pak. Vet. J*., **25**(1): 148-152. Available on: http://www.pvj.com.pk/pdf-files/25_1/1-4.pdf

Uppal, S.K., K.B. Singh, K.S. Roy, D.S. Nauriyal and B.K. Bansal. 1994. Natural defense mechanism against mastitis: A comparative histo-morphology of buffalo and cow teat canal. *Buffalo J*., **2**: 125-131

Yang, W., H. Zerbe, W. Petzl, R.M. Brunner, J. Günther, C. Draing, S.V. von Aulock, H.J. Schuberth and H.M. Seyfert. 2008. Bovine TLR2 and TLR4 properly transduce signals from *Staphylococcus aureus* and *E. coli*, but *S. aureus* fails to both activate NF-kappaB in mammary epithelial cells and to quickly induce TNF-alfa and interleukin-8 (CXCL8) expression in the udder. *Mol. Immunol*., **45**(5): 1385-1397. DOI: 10.1016/j.molimm.2007.09.004