Haryana Vet. **49** (December, 2010), pp 19-21

Research Article

ASSESSMENT OF CHEMICAL AND ELECTROLYTE PROFILE AS AN INDICATOR OF SUBCLINICAL MASTITIS IN RIVERINE BUFFALO (BUBALUS BUBALIS)

ANIRBAN GUHA, SANDEEP GERA[1] and ANSHU SHARMA
Department of Veterinary Biochemistry, College of Veterinary Sciences
CCS Haryana Agricultural University, Hisar-125 004

ABSTRACT

The present study was carried out to investigate whether any of the chemical and electrolyte components of milk can be used as an indicator to detect bubaline subclinical mastitis (SCM). Milk samples were sorted on the basis of bacterial culture examination and somatic cell count (SCC) into SCM positive and negative samples. These samples were analyzed for electrical conductivity (EC) and pH, sodium (Na), potassium (K) and chloride (Cl-). Percent sensitivity, specificity and accuracy were found out for the components which showed significant changes, taking bacterial culture examination as the benchmark. Further inter-correlation between various components and with Log10SCC were calculated separately in healthy and infected milk samples to study the effect on correlation between the measured milk components due to SCM. The findings revealed a significant ($P<0.01$) increase in EC, Na and Cl- and a decrease in K in buffalo with SCM. The Na was found to be more sensitive, specific and accurate parameter in detecting SCM, followed by K, Cl- and EC. From correlation coefficient amongst the milk components, it was established that Na, Cl- and K may be the indicators of bubaline SCM.

Key words: Subclinical mastitis, buffalo, chemical, electrolyte and somatic cell count

Mastitis inflicts heavy economic losses on account of reduced milk production, treatment costs, increased labor, milk withheld from human consumption following treatment and premature culling. Early detection at subclinical stage is important for most dairy farmers to reduce production losses and to enhance prospects of recovery. Diagnosis of clinical mastitis is based on the local and systemic reactions and changes in milk (e.g. off colour, watery, bloody appearance and presence of flakes, clots and pus). The diagnosis of subclinical mastitis (SCM) is problematic since the milk appears normal but usually has an elevated somatic cell count (Forsback *et al.,* 2010). Diagnosis of mastitis at sub clinical stage is vital because changes in the udder tissue take place much earlier than they become apparent. SCM also goes unnoticed and remain a depot for spreading infection to the herd mates (Gera *et al.,* 2006).

Various methods, based on physical and chemical changes of milk and cultural isolation of organisms, are used for diagnosis of subclinical mastitis. The diagnosis of mastitis according to the International Dairy Federation (IDF) recommendations is based on the somatic cell count (SCC) and microbiological status of the quarter. Bacteriological culture of milk samples is the standard method for identifying mastitis. However, the logistic and financial considerations involved with sampling all fresh cows have precluded this technique from being widely adopted (Sharma *et al.,* 2010). For this reason alternative parameters are used to identify trends in the development of the udder health in a dairy herd, although these parameters indicate inflammation (Sood *et al.,* 2008). Pertaining to the present scenario, the study was undertaken to evaluate the role SCM on milk electrical conductivity (EC), pH and electrolytes viz. sodium (Na), potassium (K) and chloride (Cl-) as an indicator of bubaline SCM.

[1]Corresponding author

MATERIALS AND METHODS

Milk samples from 450 (1798 quarters) apparently healthy buffalo with no previous history of mastitis were collected under aseptic condition in sterile single use disposable plastic containers. The milk samples were cultured on 5% ovine blood agar plates. The resulting growth was identified on the basis of morphology, colony characteristics and Gram's reaction (Tuteja *et al.,* 2001). The somatic cells (SCC) were counted microscopically after staining with Newman - Lampert stain (Tuteja *et al.,* 2001). EC was estimated in fresh milk samples by Systronics conductivity meter 306. The pH was estimated by Systronics µpH system 361 pH meter in fresh milk samples. Na and K concentrations were estimated with Flame photometer ELICO CL 361 after digestion with di-acid mixture (nitric acid and per chloric acid at the ratio 4:1) by the method described in the instruction manual. The chloride content of the

milk was estimated by Argentometric method described in the Laboratory Manual of Milk Industry Foundation (Anon, 2005). The chloride per cent of skimmed milk was estimated by multiplying the volume of 0.1 N of silver nitrate used to titrate 10% potassium dichromate with 0.0355. Before estimations, the milk samples were defatted by the method described by Vishnoi and Dang (2007).

Percent sensitivity and specificity were calculated by the formulae of Thrusfield (2005). Percent accuracy was calculated by the method described by Reddy *et al.* (2001).

Comparison of means of estimated concentration of different parameters in healthy and SCM milk was done by t-test. Arithmetic means of each component were considered as cut off points. The SCC values were transformed to Log10. Pearson's correlation coefficients were used to investigate the relationship between the investigated milk components with each other and with Log10SCC. All statistical analysis was done with SPSS statistical software version 11.0 (Gade *et al.,* 2010).

RESULTS AND DISCUSSION

The SCM positive milk samples in the present study were those which showed growth in culture media and had SCC > 2 lacs cell / ml of milk, while those without bacterial growth on culture plates and SCC < 2 lacs cell /ml were considered as healthy milk samples (Sharma *et al.,* 2010). On this basis, 81 buffaloes comprising 128 quarters were SCM positive. *Staphylococcus* sp. was the major mastitis pathogen (41.98%) followed by *Streptococcus* sp. (34.56%) and *Escherichia coli* (18.52%). A few animals (4.94%) showed mixed infection. Mean SCC was 2.06 ± 0.07 lacs cell/ml, which was in accordance with the reports of Sharma *et al.* (2010).

In the present investigation, EC of infected milk samples increased significantly whereas pH did not showed significant alteration (Table 1). The increase in EC is attributed to the increase of electrolytes in SCM milk samples (Schalm *et al.,* 1971). This observation is in agreement to the finding of Ognean *et al.* (2007) in cow milk. The insignificant increase of pH is attributed to the fact that the inflammatory reaction and leukocyte population in SCM are insufficient to raise the alkalinity beyond the buffering capacity of milk buffers (Schalm *et al.,* 1971). Insignificant changes in pH might also be due to increase level of citrates and bicarbonates during SCM (Ogola *et al.,* 2007).

The electrolyte profile showed significant ($p<0.01$) variation. Sodium (Na) and chloride (Cl^-) concentrations increased, while potassium (K) decreased in milk of buffaloes affected with SCM (Table 1). The increase in the concentrations of Na and Cl^- and decrease of K concentration were in agreement with previous report in cow milk (Sood *et al.,* 2008). The alteration could be attributed to increased blood capillary permeability, destruction of tight junctions and active ion pumping systems.

Table 1
Mean±S.E. of somatic cell count, chemical and electrolyte components in milk from healthy and subclinical mastitis cases in buffalo

Parameter	Healthy (n = 128)	Infected (n = 128)
Somatic cell count (x 105 cells/ml)	0.78^a±0.15	2.06^b±0.07
Electrical conductivity (mScm-1)	2.72^a±0.05	3.82^b±0.12
pH	6.66±0.06	6.81±0.17
Sodium (mM)	23.81^a±0.70	35.42^b±1.04
Potassium (mM)	24.39^a±0.37	16.81b±0.74
Chloride (%)	0.106^a±0.003	0.139^b±0.010

Mean having different superscripts in a row for a parameter differs significantly ($p<0.01$)

The SCC is a common method for the detection of subclinical intramammary infections (IMI) but factors such as breed, parity, stage of lactation and estrus contribute to significant variations. In the present study, bacterial cultural examination was taken as the benchmark instead of commonly used SCC, which was considered as inflammatory marker only. The arithmetic mean for each significantly altered milk component was taken as the threshold limit (Katsoulos *et al.,* 2010). Percent sensitivity, specificity and accuracy were the highest for Na, followed by K, Cl^- and EC (Table 2). Our finding about EC concurs with the report of Atasever and Erdem (2009) in cow milk. To the best of our knowledge, this type of analysis for Na, K and Cl^- has not been reported earlier. So this can be considered as the pioneer work for bubaline SCM.

The correlation coefficient investigation among the milk components in healthy and infected milk samples is shown in Table 3. The reason behind calculating the correlation coefficient in healthy milk was to compare the effect of SCM on correlation coefficient in infected milk. Log10 SCC was found to be correlated with EC at $P<0.05$ and with the electrolytes at $P<0.01$ in infected milk samples only. Similar observation was reported by Sood *et al.* (2008) in crossbred dairy cows. EC was found to be correlated with Cl^- in infected milk samples. The electrolytes were found to be correlated with each other in both healthy and infected milk samples. It indicated that change in

Table 2

Evaluation of chemical and electrolyte components as an indicator for diagnosis of subclinical mastitis in buffalo

Parameter	Total samples examined	Test positive samples	Test reaction as compared to cultural examination				Per cent sensitivity	Per cent specificity	Per cent accuracy
			True positive (a)	False positive (b)	True negative (c)	False negative (d)			
EC	1798	128	104	24	1362	308	81.25	81.56	81.54
Na	1798	172	122	50	1620	6	95.31	97.01	96.89
K	1798	250	116	134	1536	12	90.63	91.98	91.88
Cl	1798	309	109	200	1470	19	85.16	88.02	87.82
Bacterial culture examination	1798	128	128	-	1670	-	100	100	100

Table 3

Correlation coefficient amongst milk components in healthy and subclinical mastitis affected animals (n=128)

	Log10 SCC		EC		pH		Na		K		Cl^-	
	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected
Log10 SCC	-	-	-0.046	0.198*	-0.048	0.054	-0.064	0.210**	-0.136	-0.393**	0.470**	-0.066
EC	-	-	-	-	0.034	0.041	-0.081	0.170*	-0.066	-0.215*	0.182*	0.024
pH	-	-	-	-	-	-	-0.075	0.056	-0.144	-0.147	0.029	-0.134
Na	-	-	-	-	-	-	-	-	0.178*	-0.385**	0.498**	0.467**
K	-	-	-	-	-	-	-	-	-	-	-0.478**	-0.181*
Cl^-	-	-	-	-	-	-	-	-	-	-	-	-

* indicate significant at $p<0.05$ and ** indicate significant at $p<0.01$

one will cause change in the other. To the best of our knowledge, no such report exists on cow or buffalo milk. There is no single indicator of SCM but the present study concludes that electrolyte concentration may be used as indicators of bubaline SCM along with bacterial culture and correlation with Log10SCC.

REFERENCES

Anonymous. (2005). Analysis of Milk and its Products, A Lab Manual. (2nd edn.), Milk Industry Foundation, Biotech Book, Delhi, pp. 140-142.

Atasever, S. and Erdem, H. (2009) Association between subclinical mastitis markers and body scores of Holstein cows in the Black Sea region. *Turkey J. Anim. Vet. Adv.* **8**: 476-480.

Forsback, L., Mansson, H.L., Andren, A. and Sjaunja S.K. (2010). Evaluation of quality changes in udder quarter milk from cows with low-to-moderate somatic cell count. *Animal* **4**: 617-626.

Gade, N.E., Singh, G., Pankaj, R., Sonawane and Mahapatra, R. K. (2010) Effect of ascorbic acid supplementation on plasma profile in buffaloes during heat stress. *Indian J. Vet. Res*. **19**: 56-62.

Gera, S., Sharma, A., Dabur, R.S., Jain,V. K. and Garg, S.L. (2006). Studies on changes in milk composition and chemotherapeutic sensitivity in camel *(Camelus dromedarius)* in sub clinical mastitis. In: Proc. International Sci. Conf. Camels, (Quassim University), **2**: 937-946.

Katsoulos, P.D., Christodoulopoulos, G., Minas, A., Karatzia, M. A., Pourliotis, K., Spyridon, K. and Kritas, S. K. (2010). The role of lactate dehydrogenase, alkaline phosphatase and aspartate aminotransferase in the diagnosis of subclinical intramammary infections in dairy sheep and goats. *J. Dairy Res.* **77**: 107-111.

Ognean, L., Dragan, L., Moga. P. amd Tripon, S. (2007). Electrical conductibility of milk an alternative for lactation and mammary health supervisions in cows. *Revista Romana de Medicina Veterinaria* **17**: 29-36.

Ogola, H., Shitandi, A. and Nanua, J. (2007). Effect of mastitis on raw milk compositional quality. *J. Vet. Sci*. **8**: 237-242.

Reddy, L. V., Choudhuri, P. C. and Hamza, P. A. (2001). Comparative efficacy of different tests in the diagnosis of subclinical mastitis in crossbred cows. *Indian Vet. J.* **78**: 903.

Schalm, O.W., Carrol, E. J., Jain, N. C. (1971). Bovine Mastitis. Lea Febiger, Philadelphia, USA.

Sharma, N., Pandey, V. and Sudhan, N. A. (2010). Comparison of some indirect screening tests for detection of SCM in dairy cows. *Bulgarian J. Vet. Med.* **13**: 98-103.

Sood, R., Pachauri, S.P. and Nauriyal, D.S. (2008). Evaluation of markers of sub clinical mastitis for its early detection in dairy animals. *Indian J. Vet. Med.* **28**: 17-19.

Thrusfield, M. (2005). Veterinary Epidemiology. (3rd edn.), Blackwell Science, United Kingdom. p.158.

Tuteja, F.C., Kapur, M.P., Sharma, A. and Manuja, B. (2001). Mastitis pathogens from apparently healthy buffaloes and their relationship to somatic cell count of milk. *Indian J. Comp. Microbiol.* **22**: 162-163.

Vishnoi, P.C. and Dang, A.K. (2007). Changes in blood and milk DLC and its effect on milk composition in Murrah buffaloes *(Bubalus bubalis)* suffering from clinical mastitis. *Indian J. Dairy Sci.* **60**: 286-292.