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Comparative evaluation of Interleukin-6, somatic cell count and electrical conductivity as predictive tools for subclinical mastitis in dairy Buffaloes

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Abstract

Due to the economic and public health importance of mastitis, the present study was designed to evaluate the use of new sensitive subclinical mastitis detection system in dairy buffaloes in comparison to well-established used methods. The new method involved the specific quantification of interleukin-6 (IL-6) in buffaloes' milk using ELISA specific for bovine IL-6. 36 out of 50 buffaloes' milk samples were found to be subclinically mastitic with a mean interleukin-6 value of 51.3 ± 4.9 ng/ml. IL-6 determination showed great superiority and sensitivity for detection of subclinical mastitis in comparison to somatic cell count and electrical conductivity. Milk IL-6 determination comprises a valuable candidate for prediction of subclinical mastitis in dairy buffaloes.

Key Word: Buffaloes' milk, interleukin-6, subclinical mastitis, somatic cell count.

Introduction

Mastitis is an inflammatory condition of the udder caused mainly by invasion of a mastitis-associated pathogen²³. Mastitis is fairly the main costly disease affecting dairy cattle, due to its frequent recurrence in addition to constituting a potential health risk for other cows. Moreover, mastitis constitutes a major source of economic losses for dairy industry due to its detrimental effects on both milk quality and yield. In turn, it lowers the quality of most manufactured milk products²⁰. Furthermore, mastitis additionally worsens the economic losses via extra costs of treatments⁶. Milk antibiotics' residues constitute other public health concerns that arise due to extensive use of mastitis therapeutics.

Thus, the cruciality of early detection

of mastitis cases can be easily inferred from the above-mentioned problems. Detection of both types of mastitis, clinical and subclinical, is of great importance to maintain the dairy herd at best production capacity. Early detection of subclinical mastitis would surely help in limiting associated cost and bad prognosis of mastitis cases.

Several diagnostic methods are being used to detect subclinical mastitis through milk quality, mammary gland status and detection of causative agents of mastitis. Nowadays, somatic cell count (SCC), in-line electrical conductivity (EC) sensors, and California mastitis test are being widely used¹². Recently, inflammatory indicators as interleukin 6 (IL-6) and tumor necrosis factors- α were proved to be one of the reliable techniques for the early detection of subclinical mastitis in cows²⁹. Whereas cows represent the largest sector of milk

production worldwide, those aforementioned tests had been developed mainly for detection of mastitis in dairy cows. However, at certain countries like India, Egypt, Italy, Pakistan and China, dairy buffaloes herds represent a large segment of milk production⁵⁾. Similar to cows, mastitis is also representing an eminent economic-costing disease to dairy buffaloes⁴⁾.

In the view of the unavailability of specific detection methods for subclinical mastitis in dairy Buffaloes, this study was designed to evaluate a newly developed method that allows the specific detection of IL-6 in buffalo milk samples for detection of subclinical mastitis. This was accomplished by using ELISA specific for bovine IL-6 to quantify the IL-6 in whey of raw dairy buffalo milk and to compare the new test's result with some established methods for detection of subclinical mastitis.

Materials and methods

Samples: Fifty raw buffalo (*Bubalus bubalis*) quarter's milk samples were collected from many buffaloes' dairy farms and from individual owners at different regions of Sharkia Governorate, Egypt. Samples were collected in 50 ml screw capped sterilized bottles. All possible precautions were taken to avoid external contamination at the time of samples collection and during processing. Buffalo-cows showed any clinical sign of mastitis (udder or teat swelling, redness, pain, hotness and/or curdled milk) was excluded from this study.

Routine subclinical mastitis detection tests: Milk samples were initially warmed to 35°C for 5 minutes and their somatic cell contents were counted automatically using Somatic cell counter (DeLaval® cell counter). While for electrical conductivity, milk samples were cooled to 18°C and measurement was done using LactoscanLA™ (Milkotronic). Results were expressed as millisimens/cm (mS/cm).

Interleukin-6 determination: IL-6 was determined in milk samples using IL-6 ELISA Reagent Kit, Bovine (Thermo Scientific). Briefly, milk samples

were centrifuged (2500 RCF for 20 min at 4 °C and the cell-free aqueous whey fractions were collected as described by Nakajima et al.¹⁶⁾. Whey samples were then frozen at -30 °C until use. A sandwich ELISA using bovine antibodies specific for IL-6 was used to determine the concentrations of IL-6 in the whey samples following the manufacturer protocol. *Statistical analysis:* Presented data are the means ± SEM of three independent trails. The statistical significance of differences in IL-6 concentrations in the whey samples was determined by Student's t-test and one-way ANOVA. Pearson correlation analysis was conducted between SCC, EC and IL-6 at a probability level of $p < 0.05$.

Results and Discussion

Many negative consequences are associated with mastitis. For instance, milk production losses and quality impairment, labor and veterinary expenses^{25,28)}. In addition, various mastitis milk-originated pathogens may find their way to persist at dairy environment, which will represent an imminent health threat¹⁹⁾.

Thus, detection systems for subclinical mastitis should be developed and incorporated in milking lines to facilitate early intervention. In addition, an effective subclinical mastitis detection system should be able to combine the acceptable sensitivity and accuracy. Currently, many methods are available for detection of subclinical mastitis in dairy cows. In contrast, approved detection systems of subclinical mastitis in dairy buffalo are much less in comparison to cows. In this study, we aimed to assess new testing technique and comparing its result against well-established method.

Table (1): Statistical analytical results of SCC, EC and IL-6 of examined samples.

Statistical parameter	SCC		EC		IL-6	
	Normal ($\leq 2.0 \times 10^5$ /ml.)	Mastitic ($> 2.0 \times 10^5$ /ml.)	Normal (≤ 5 mS/cm)	Mastitic (> 5 mS/cm)	Normal (≤ 3.6 ng/ml)*	Mastitic (> 3.6 ng/ml)*
No. (%)	19(38)	31(62)	25(50)	25(50)	14(28)	36(72)
Mean \pm SEM	$2.9 \times 10^4 \pm 0.4 \times 10^4$	$1.6 \times 10^7 \pm 0.08 \times 10^7$	3.92 ± 0.19	7.65 ± 0.45	2.5 ± 0.3	51.3 ± 4.9
Min.	3.8×10^3	7.1×10^5	3.56	5.97	1.92	8.5
Max.	8.7×10^4	1.5×10^8	4.83	9.12	3.44	99.5

* According to Sakemi et al., 2011.

Initially, the competency of using common bovine reagents in studying IL-6 in both buffalo and cattle species was proved previously (Premraj et al. ²¹⁾ noticed a great similarity between IL-6 gene of both cow and buffalo species). Accordingly, commercial bovine IL-6 ELISA kit was used in this study.

According to Sakemi et al. ²⁴⁾, a level of 3.6 ng/ml was used to be the threshold to discriminate mastitic from normal cases. Data presented in table (1) showed that 72% of examined samples were found to be mastitic according to IL-6 determination (with a mean value of 51.3 ± 4.9 ng/ml).

Intramammary infections induce different alterations in milk composition. Rather than chemical composition, inflammatory proteins like interleukins and acute phase proteins are significantly increased²⁾. Sakemi et al. ²⁴⁾ experimentally established that inflammatory cytokines such as IL-6 are immediately associated with inflamed mammary glands at the onset of mastitis. Besides, IL-6 concentrations positively correlate with the severity of inflammation. In another report, Nayan et al. ¹⁷⁾ reported that in subclinical mastitis, IL-1 β and IL-6 expression was higher compared to control samples. However, higher expression of IL-1 β and IL-6 was evident in clinical samples compared to sub-clinical samples.

While according to other established methods for detection of subclinical mastitis, 50 and 56 % of samples were found positive according

to EC and SCC. These figures of values evince the significant sensitivity of IL-6 determination in the detection of subclinical mastitis rather than SCC and EC. Furthermore, the detection of IL-6 in milk indicated subclinical mastitis earlier than the detection of elevated SCC did. Thus, the detection of IL-6 in milk could be a future prediction marker for subclinical mastitis.

Regarding SCC, it was found that the mean SCC of examined buffaloes' milk samples was 2.9×10^4 in case of normal milk samples, while in case of mastitis positive samples, the mean value was $1.6 \times 10^7 \pm 0.08 \times 10^7$ (Table 1). Milk SCC is now considered as a sensitive monitoring technique for udder health and milk quality in dairy buffaloes ²⁶⁾. Somatic cells consist typically of neutrophils, macrophages and lymphocytes¹¹⁾. During the early onset of mastitis, SCC increased steadily due to the high flow of neutrophils into milk²⁷⁾. Jones¹⁰⁾ reported an obvious correlation between SCC and probability of pathogens contamination of raw milk. Moreover, elevated SCC may demonstrate the unhygienic conditions under which raw milk is produced. In addition to the indicatory aspect of elevated SCC, it is also rendering the suitability of raw milk for subsequent dairy products manufacture. Milk from normal uninfected quarters generally contains below 200,000 somatic cells/ml. An elevation of SCC to 300,000 and above is an indication of inflammation in the udder.

Concerning EC, Data present in table (1) showed that the minimum EC % was 5.97; the

maximum was 9.12 with a mean value of 7.65 ± 0.45 mS/cm. Nearly similar findings were reported by El-barawy and Ali³). Higher findings were reported by Mansell and Seguya¹³).

The changes in the EC of milk from infected quarter may depend on the specific pathogen causing subclinical mastitis. Conductivity increased in cows subclinically infected with *Staphylococcus aureus* but it was not detectably increased in intramammary infections caused by *Streptococcus uberis*¹⁴).

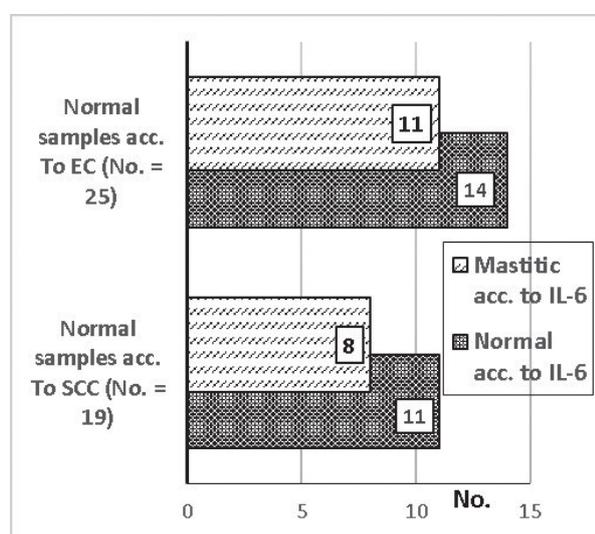


Figure (1): Distribution of examined samples according to IL-6 standard value among SCC and EC normal samples.

This certainly will have a great concerns about the credibility of using EC in detection of subclinical mastitis. EC of milk is controlled by sodium, potassium, calcium, magnesium, chlorine, and other ions^{1,8}). During mastitis, changes occur in these ions' concentration due to increased permeability of blood capillaries and impaired active transport system. However, data on the diagnostic value of this method is contradictory. According to some researchers^{7,9}), EC of healthy cow milk is 4.0-5.5 mS/cm. When EC increases to 6.0 mS/cm or more, pathological processes in the udder tissue may be suspected. Some authors point to a good correlation between EC and

bacteriological and cytological tests^{15,18}), while others consider this method to be insufficiently sensitive^{7,22}).

Further analysis of normal samples according to SCC and EC using IL-6 revealed that varying numbers among them were mastitic (above 3.6 ng/ml IL-6) (Figure 1). The mean level of IL-6 of mastitic samples according to IL-6 was 68.2 ng/ml among SCC normal buffalo milk samples, while it was 64.7 among EC normal samples.

Table (2): Correlation coefficients between IL-6 and SCC and EC of examined buffalo's milk samples.

	IL-6	SCC	EC
IL-6	1	0.917*	0.656
SCC	0.917*	1	0.842
EC	0.656	0.842	1

*: Significant ($P < 0.05$).

Pearson's correlations between IL-6 and SCC and EC of buffalo's milk samples are illustrated in table 2. Results of the cross-tabulation table showed that subclinical mastitis prediction accuracy based on IL-6 concentration was almost equal or superior to subclinical mastitis prediction accuracy based on SCC and significantly superior to that of EC.

Conclusion

From the above-mentioned results, the detection of IL-6 in buffaloes' milk could be a future prediction marker for subclinical mastitis in buffaloes. IL-6 detection in milk showed great sensitivity and superiority in comparison to well established SCC and EC. Further conformational studies are required for different buffaloes' breeds to ensure test applicability for all breeds.

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