

Some New Parameters Can Be Used In Diagnosis of Subclinical Mastitis in Buffaloes

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Abstract—A total of 326 milk samples were collected from 90 lactating buffaloes in the period between the 2nd and the 10th week after calving. They were classified by California mastitis test (CMT) as positive (37.42%) were deemed to have quarters with Subclinical mastitis (SCM), and as negative (62.58%) from healthy quarters. Estimation of somatic cell count (SCC) revealed significant ($p < 0.01$) increase in positive CMT samples compared to the negative ones. The most common bacterial isolates from SCM cases were *Staphylococcus aureus* 97(79.51%), *Staphylococcus epidermidis* 24 (19.67%) and *E. coli* 94 (77.05%). Statistical analysis on the milk lactose, chloride, enzymes and serum enzymes values revealed that mean level of milk lactose was significantly lower, while the mean level of milk chloride, Lactate dehydrogenase (LDH) and Alkaline phosphatase (ALP) activities in milk and blood serum were significantly higher in milk samples affected with SCM than the healthy samples. Therefore, this study concluded that milk lactose, chloride and enzymes from milk and blood serum may appear to be a suitable diagnostic method for identifying SCM in dairy buffaloes.

Keywords—Subclinical mastitis, Etiology, milk LDH & ALP, lactose, chloride.

I. INTRODUCTION

Buffalo's milk is characterized by higher solid content for being richer source of lipids, protein, lactose and minerals. Changes in milk composition (for example in lactose content, mineral content, enzymes or somatic cell count) can be attributed to disease onset, so these differences in milk composition can be useful for early detection of health problems and starting the treatment (1). The prevalence of mastitis ranges from 66% -70.32% in buffaloes (2), (3). The milk somatic cells include 75% leucocytes (neutrophils, macrophages and lymphocytes), and 25% epithelial cells. Increasing SCC in milk leads to changes in its composition, for example, in representation of protein fractions, minerals and lactose content. These changes have a negative impact on the further processing of milk. (4).

Subclinical mastitis not only leads to reduced milk quantity and quality but also increases the risk of transferring of the disease to healthy animals. If subclinical mastitis is not recognized on time, the disease would be spread in the herd leading to an outbreak and consequently increased therapeutic expenses (5), (6). In addition, the bacterial contamination from the affected milk render it unfit for human consumption and

provide a mechanism of spread of diseases like tuberculosis, sore-throat, Q-fever, brucellosis and leptospirosis etc. This is usually has zoonotic importance (7). So the only opportunity to avoid this catastrophe is diagnosis and treatment before the infection flares up, i.e. at the subclinical stage. (8).

So, the present research seeks: (i) to study the changes occurring in somatic cell count, milk lactose and chloride levels also activities of LDH and ALP in buffaloes' milk and blood serum of the same animals as a result of SCM (ii) To investigate the etiology of SCM in dairy farm.

II. MATERIALS AND METHODS

A. Animals

A total of 326 milk samples were collected from 90 multiparous apparently healthy lactating dairy buffaloes aged 4 to 6 years old in the period between the 3rd and 10th week after calving on the basis of quarter samples. All milk samples were collected from medium sized dairy farms in Ismailia, Egypt.

B. Sampling

The udder was washed with clean water and dried with clean clothes, the teat ends were swabbed with a cotton wool soaked in 70% ethyl alcohol. Milk samples were collected prior to the afternoon milking in sterile screw capped test tubes (10 ml) after discarding the first three squirts of milk. Two test tubes were taken from each quarter, one used for somatic cell count, chemical analysis for (lactose, chloride, lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) activities) and one for bacteriological examination. Blood samples were taken in parallel with the milk, for determination of LDH and ALP activities in blood serum.

C. California mastitis test (CMT)

It was conducted on individual quarter milk samples collected from each quarter to differentiate between healthy (negative CMT) and diseased (positive CMT) samples following the methods as described by (9). According to the visible reactions, the positive results were classified in four scores: \pm = trace, + = weak, ++ = distinct positive and +++ = strong positive.

D. Estimation of somatic cell count

Milk was examined automatically for SCC using somatic cell counter KT05 apparatus. The milk sample was warmed in water bath at 40 C° for 5 minutes according to (10).

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E. Bacteriological examination

The milk samples were cultured on specific media as Mannitol-salt agar and EMB agar for *staph.*, & *E. coli* count respectively (11). The isolated microorganisms were identified according to (12).

F. Measurement of milk lactose and chloride levels

Lactose and chloride levels were performed as described in the laboratory manual of milk industry foundation called (13).

G. Assays of enzyme activity

Healthy and subclinical mastitis milk samples were skimmed by centrifugation at 10,000 rpm for 20 min at 4°C.

Defatted milk was used for enzyme activity estimations. LDH and ALP activity were assayed by spectrophotometer according to (14), (15) respectively.

H. Statistical analysis

Data for mineral, lactose and enzymatic activities are expressed as mean \pm standard Error (SE). For statistical analysis we used Student's t-test according to (16).

III. RESULTS

TABLE (1): SCORING OF EXAMINED QUARTER'S MILK SAMPLES BASED ON CALIFORNIA MASTITIS TEST

Examined Quarter [samples No.]	positive +ve samples		negative -ve samples		Score of positive quarter samples							
	No	%	No	%	\pm		+		++		+++	
					No	%	No	%	No	%	No	%
326	122	37.42	204	62.58	11	9.02	53	43.44	33	27.05	25	20.49

TABLE (2): SOMATIC CELL COUNT (CELLS/ML) IN EXAMINED QUARTER'S MILK SAMPLES BASED ON CMT

Parameters	Samples no.	mean \pm SE
CMT positive	122	$2 \times 10^7 \pm 1.71 \times 10^{5**}$
CMT negative	204	$2 \times 10^5 \pm 1.41 \times 10^3$

** Significance at P<0.01

TABLE (3): STATISTICAL ANALYTICAL RESULTS OF ISOLATED BACTERIA FROM EXAMINED POSITIVE CMT SAMPLES

Isolated bacteria	No.	%
<i>E. coli</i> only	11	9.02
<i>E. coli</i> and <i>Staph. aureus</i>	59	48.36
<i>E. coli</i> , <i>Staph. aureus</i> and <i>Staph. epidermidis</i>	10	8.20
<i>E. coli</i> and <i>Staph. epidermidis</i>	14	11.47
<i>Staph. aureus</i> only	28	22.95
total	122	100

TABLE (4): STATISTICAL ANALYTICAL RESULTS OF BACTERIAL COUNT /ML OF EXAMINED QUARTER MILK SAMPLES BASED ON CMT

Isolated organisms	Positive samples		Min.	Max.	Mean \pm SE
	No.	%			
<i>Staph. aureus</i>	97	79.51	4.9×10^4	2.7×10^9	$6.2 \times 10^8 \pm 1.9 \times 10^7$
<i>Staph. epidermidis</i>	24	19.67	3.8×10^4	3.8×10^8	$4.3 \times 10^6 \pm 1.8 \times 10^5$
<i>E. coli</i>	94	77.05	3.1×10^3	5.1×10^8	$6.1 \times 10^6 \pm 0.9^3$

TABLE (5): STATISTICAL ANALYTICAL RESULTS OF MILK BIOCHEMICAL PARAMETERS VALUES BASED ON CMT IN THE EXAMINED QUARTERS' MILK SAMPLES

Biochemical parameters	CMT positive (mean \pm SE)	CMT negative (mean \pm SE)
Lactose %	3.07 \pm 0.01*	4.14 \pm 0.02
Chloride %	0.22 \pm 0.01*	0.08 \pm 0.00
LDH (IU/L)	400.90 \pm 23.33***	192.80 \pm 9.06
ALP (IU/L)	441.20 \pm 20.65***	178.50 \pm 12.93

*Significance at P<0.05

*** significance at P<0.001

TABLE (6): STATISTICAL ANALYTICAL RESULTS OF SERUM LDH AND ALP ACTIVITIES ON EXAMINED ANIMALS BASED ON CMT

parameters	Examined animals (No.)	%	LDH (IU/L)	ALP (IU/L)
CMT positive	39	43.33	1236.42 \pm 48.76***	144.70 \pm 12.91***
CMT negative	51	56.67	892.40 \pm 17.21	48.68 \pm 5.07
total	90	100	-	-

***significance at P<0.001

IV. DISCUSSION

Out of the 326 quarter milk samples were collected from clinically healthy lactating buffaloes 122 (37.42%) quarter samples were positive for CMT and 204 (62.58 %) quarter samples were positive for CMT (table, 1). With a total of 39 (43.33%) dairy buffaloes were positive for CMT and total of 51 (56.67 %) dairy buffaloes were negative for CMT (table, 6). these results were lower than that reported by (2), (3) in buffaloes milk samples. Among the positive quarter milk samples, the highest incidence was recorded in CMT score (+) as 53 (43.44%) and the lowest in CMT score (\pm) as 11 (9.02%), these results were higher than that obtained by (17) The CMT score is based on the number of leukocytes in milk (18). The rise in the leucocyte number in milk as a response to the assaulting pathogens or to their metabolites leads to an increase in (SCC). The positive reaction of CMT is due to alkalinity owing to the increase of inflammatory cells (SCC). Estimation of SCC (table, 2) revealed significance (p<0.01) increase in SCM milk samples. Our result come in accordance with (19), (20) who reported that mean SCC were significantly (p<0.01) high in SCM milk samples due to the inflammatory reactions. The results of bacteriological examination obtained in table (3) explained the number of samples and percent of isolated bacteria from positive CMT samples were *E.coli* only in 11(9.02%) samples, *E. coli* and *S. aureus* in 59 (48.36%) samples, *E. coli*, *S. aureus* and *S. epidermidis* 10 (8.20%), *E. coli* and *S. epidermidis* 14 (11.47%) and for *S. aureus* only 28

(22.95). Table (4) pointed out that the percent of isolated mastitis pathogens from examined quarter's milk samples were 79.51, 19.67 and 77.05 % for *S. aureus*, *S. epidermidis* and *E. coli* respectively. These findings are higher than that recorded by (21). The high prevalence of *S. aureus* is mainly attributed to the wide distribution of microorganism inside the mammary gland and on the skin of teat and udder (22). *S. aureus* is one of the most common mastitis pathogens (23). It can produce heat stable enterotoxins which are not inactivated during milk pasteurization or production of milk products which can provoke food intoxication (24). The existence of one of the environmental mastitis pathogens e.g /Coliform organisms in milk samples is an indication to unhygienic conditions during milking and handling processes also indication to the fecal contamination of milk samples (25). Isolation of *E. coli* may associate with the existence of other enteric pathogens in the examined milk samples (26). Presence of coliform in milk samples possess a public health concern and it is epidemiologically significant that not only for animals but also for humans.

Clinical or subclinical mastitis is an economically damaging disease of the dairy industry, which causes physical, chemical and bacteriological alternation in the milk and blood along with morph-pathological changes in the mammary gland (27).

Lactose is the major carbohydrate in milk, its content decrease in diseased cases (28) lactose normal content compensate osmotic pressure in the mammary gland and its decrease leads to increased transfer of sodium chloride from blood to milk (29); (30). Our results which as shown in (Table, 5) revealed that lactose content showed significant ($P < 0.05$) decrease while chloride content showed significant ($P < 0.05$) increase in positive CMT samples. The higher value of chloride in mastitic milk agreed with those reported by (31), (32). Chloride of buffaloes milk increased ($p < 0.001$) in positive bacterial culture (33). During mastitis, the increase of chloride content of milk is due to the altered permeability and increased somatic pressure which can lead to entry of chloride from blood to the milk (34), (35).

Concerning chemical analysis tables (5, 6) showed significance increase ($P < 0.001$), in the activities of LDH and ALP in milk and blood serum in subclinical mastitis samples. Elevated LDH and ALP activity was contributed to disintegration of leukocytes, mammary epithelial and interstitial cells damaged during inflammation (36). Also references (6), (37) declared that LDH activity is sensitive and reliable marker for detection of subclinical mastitis. Our findings agreed with those of (21); (38); (36) and (39). Measuring LDH and ALP activities in milk could be a useful diagnostic marker in detection of subclinical mastitis (40). Blood serum was not a significant source of these enzymes in the milk (38). LDH was not a sensitive marker for early detection of subclinical mastitis and only ALP had a high sensitivity (41). In this regard, reference (36) explained the significant elevation of ALP in SCM milk might be due to both mammary epithelial damage and a breach in the blood-milk barrier selectively damaged by bacterial toxins. Reference (42)

suggested that origin of LDH in SCM milk is attributed to the presence of leucocytes and epithelial cells from the udder.

Somatic cell counts (SCC) lacks enough sensitivity to be used as a screening test in detection of infected quarters because in the early stages of mastitis somatic cell count may not be highly elevated (43), (44). Also, bacteriological test are not suitable to be used as a routine test in the diagnosis of subclinical mastitis because of being costly and time consuming. The possibility of false negative results in quarters, are chronically infected (41). Therefore, early detection of subclinical mastitis in milk requires inflammatory markers, which are reliable and fast enough to be used routinely (45). The present study showed that measuring LDH and ALP activities in milk which is both easy and low cost compared to other methods could be used as a diagnostic test with acceptable sensitivity and specificity for detecting subclinical mastitis. Moreover, unlike the other methods for routine diagnosis of mastitis, measurement of the above-mentioned enzymes is also appropriate to be used during early lactation and the dry period in order to selective treatment (41).

V. CONCLUSION

It was concluded that, incidence of subclinical mastitis per quarters milk samples and per animal were 122 (37.42%) and 39 buffaloe (43.33%) respectively. Distributions of microbial isolates responsible for infection of milk samples were: *S. aureus* 97(79.51%), *S. epidermidis* 24 (19.67%) and *E. coli* 94 (77.05%). Moreover, SCM increased milk Chloride concentrations, LDH and ALP activities while it has decreasing effect on milk lactose content which make these measurements could be used as a reliable and sensitive methods for detection of subclinical mastitis.

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