

Effect of Subclinical Mastitis on Some Biochemical and Clinicopathological Parameters in Buffalo

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Abstract: The present study was conducted to investigate the effect of subclinical mastitis on some biochemical and clinicopathological changes in buffalo. A total of 400 individual milk samples from clinically normal udder quarters of 100 dairy buffalo were examined microbiologically as well as by using California mastitis test (C.M.T.) for detection of subclinical mastitis and designing rapid diagnostic tests for other infection. Blood samples were analyzed for hemogram, cortisol, alanine aminotransferase, aspartate aminotransferase, total protein, inorganic phosphorous and calcium, also L-Lactate dehydrogenase (LDH) in milk was evaluated. The results indicated that there is a significant elevation of cortisol, SGOT, PCV, LDH activity in milk while a notable decrease in total protein, serum calcium and Hemogram was observed. However, serum phosphorous level did not exhibit obvious changes. The application of CMT leads to early detection of sub-clinically infected quarters and aids in the selection of dairy animals for either segregation or therapy. We conclude that sub-clinical mastitis causes anemia in cows detected by decreasing their hemoglobin and RBCs count. An integrated approach to dairy extension should focus more on the creation of mastitis awareness among producers by improving their milking procedures and presenting good hygiene for their animal environment, checking their animals for mastitis using California Mastitis Test and all the positive cases should be treated in order to control and prevent the spreading of this disease to other animals.

Key words: Subclinical mastitis • Microbiology • L-Lactate dehydrogenase (LDH) • Biochemical and hematological changes • Buffalo

INTRODUCTION

Ruminants play an important role in the nutrition and income of people worldwide, these animals serve primarily as sources of meat, but also provide milk and skins [1].

It could be argued that one word should strike fear in the minds of consumers and producers of milk and other dairy products: mastitis. That's because mastitis, an udder inflammation caused by bacterial infection, leads to lower milk production, decreased the percentage of protein, lactose and butterfat [2], in addition to a shorter life for infected dairy cows. It's the single most costly dairy cow disease.

Bacteria invading a cow's mammary gland absorb milk nutrients while secreting poisonous endotoxins that destroy mammary tissue. If these toxins escape the gland

and spread throughout the cow's body, they eventually cause organ failure and death. These infections, usually, spread from cow to cow at milking if milking hygiene is not good enough [3].

Annual losses due to mastitis are estimated at more than \$2 billion in the United States, and about 3 million dairy cows each year show visible signs of acute infection. Of those 3 million, about 300,000 die from shock induced by the endotoxins or have to be culled.

The types of infection that can be routinely monitored with subclinical mastitis are most often the contagious types such as *Staphylococcus aureus*, *Streptococcus agalactiae* and the environmental mastitis pathogens. About 95% of all infections are caused by *Streptococcus agalactiae*, *Staphylococcus aureus*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, and *Escherichia coli*, the last two organisms are

environmental pollution organisms; they live in feces, polluted water, and bedding material [4, 5], the remaining 5% are caused by other organisms.

Antibiotics help, but resistant pathogens can make them ineffective [6]. Milk containing antibiotics is not allowed to be sold to consumers, so producers have to wait a few days before selling milk from treated cows.

Stress in the form of muscular exertion causes alterations in the different blood constituents [7]. This work was initiated so as to investigate clinopathological changes among infected cows. The aim of the present work was also to study the bacteriological incidence of subclinical mastitis among cows and to find the relationship between clinopathological changes and mastitis in subclinical mastitis buffalo.

MATERIALS AND METHODS

For conducting this work 400 milk samples were aseptically collected from clinically normal quarters of animals selected from Mounofia governorate. Samples were examined using the following tests:

- (A) California Mastitis test C.M.T. according to the procedure described by American Public Health (APH) [8].
- (B) Microbiological examinations which include cultivation of milk sediment. The milk sediment obtained by centrifugation of 10ml of the samples for 20m at 3000 rpm, was seeded on a plates of nutrient agar, blood agars, Edward medium, Mackonkey's agar and subarouds dextrose agar.

Examination of incubated milk: Loopfuls from the incubated samples over night at 37° were streaked on the same forementioned media and inoculated plates were incubated at 37° for 48hr except sabarouds agar plates which were incubated at 25° and checked daily for the growth of fungi for 3 weeks. Suspected colonies appearing on different media were examined microscopically and indentification was carried out according to Ajella *et al.* [9] and El-Sagheer *et al.* [10].

L-Lactate dehydrogenase (LDH) in milk was measured by specials kits according to methods of Kachmar and Moss [11]. The samples were processed by centrifugation to remove fat and pellet and intermediate layers obtained, were further centrifuged at 30.000 Xg for min. essentially according to the method of Bagin *et al.* [12]. The supernatant obtained was filtered through filter paper and used as the enzyme source. LDH activity was assayed

spectrophotometrically at 340 nm by special kits according to Kachmar and Moss [11].

Blood Analysis: Blood samples from buffalo were collected by jugular venepuncture in test tubes with and without EDTA.

Serum was harvested by centrifugation at 3000 rpm. Calcium, Inorganic phosphorous, total protein, SGOT and SGPT were determined in serum using kits from Diamond Diagnostic company, Egypt and measured by spectrophotometer in the UV range (240nm).

Cortisol was assayed by R.I assay technique using kits from Diagnostic Products Corporation, Los Angeles USA, according to method of Kowalaski and Paul [13].

A complete blood picture was manually performed as outlined by Jain [14].

Statistical Analysis: The obtained data were subjected to the student t-test according to Gad & Well [15].

RESULTS

The results obtained from Table 1 revealed that out of 100 lactating buffalo 14% were infected with *E. coli*, 5% infected with *S. agalactia* 3.5% *S. aureus* & 2.5% *Pseudomonas aeruginosa*.

The Hemogram presented in Table 2 showed a significant decrease in RBCs, PCV and hemoglobin while there was a significant increase in ESR & WBCs ($p < 0.01$).

The results obtained from Table 3 indicated significant elevation of serum cortisol levels in infected buffalo as compared with non infected buffalo ($p < 0.01$). SGOT values were significantly ($p < 0.01$) higher in mastitic buffallow while SGPT values revealed no obvious changes as compared with control groups. Calcium showed non significant decrease, while inorganic phosphorous revealed no obvious changes. LDH activity indicated significant elevations in infected milk, while total protein values revealed a notable decrease in infected buffalo.

DISCUSSION

Mastitis reduces milk yield and alters milk composition. The magnitude of these changes in individual cows varies with the severity and duration of the infection and the causative microorganisms. Mastitis is almost always caused by bacteria. These microorganisms produce toxins that can directly damage milk-producing tissue of the mammary gland, and the

Table 1: Bacteriological examination of buffalo's milk and their LDH activity

Milk Samples N=100	Types of Infecting Organism			
	<i>E. coli</i> 14%	<i>S. agalactia</i> 5%	<i>S. aureus</i> 3.5%	<i>Pseudomonas aeruginosa</i> 2.5%
LDH Activity U/ ml				
Control	57.3±2.24	46.0±0.40	50.0±0.27	73.0±0.48
Infected	100.4±0.63**	117.0±18**	133.0±16**	177.0±59**

* P< 0.01

Table 2: Effect of subclinical mastitis by different organisms on the Hemogram of the infected buffalo

Hemogram	Hemoglobin	PCV %	RBCs count	WBCs count	ESR
<i>Types of Organism</i>	mg/dl		10 ⁶ /mm ³	10 ³ / mm ³	mm/2hrs
<i>E. coli</i>					
Control	9.10±0.33	30.00±0.32	8.00±0.18	10.35±0.64	1.03±0.092
Infected	8.60±1.94*	27.50±0.62*	6.33±0.90*	11.37±0.053*	1.83±0.045**
<i>Streptococcus agalactiae</i>					
Control	9.80±0.20	33.00±0.67	9.40±0.74	10.50±0.26	1.10±0.35
Infected	7.80±0.58*	31.00±0.070*	8.80±0.80*	13.00±0.63*	1.7±0.072**
<i>S. aureus</i>					
Control	9.44±0.05	34.00±0.89	9.10±0.07	10.58±0.71	1.33±0.021
Infected	7.85±0.08**	29.00±0.13**	8.30±0.19*	13.00±0.63*	2.10±0.062**
<i>Pseudomonas aeruginosa</i>					
Control	9.50±0.13	32.00±0.82	9.00±0.73	10.00±1.84	1.00±0.68
Infected	7.10±9.59**	30.00±0.84*	8.03±0.24*	12.00±0.68*	2.00±0.093**

Table 3: Effect of subclinical mastitis by different organisms on some biochemical parameters and the level of cortisol hormone of the infected buffalo

Some Biochemical Parameters	Cortisol	Total protein	SGOT	SGPT	Calcium	Phosphorous
<i>Types of Organism</i>	Mg/dl	mg/dl	U/L	U/L	mg/dl	mg/dl
<i>E. coli</i>						
Control	0.93±1.33	7.95±0.74	75.30±0.65	13.23±0.38	8.93±0.75	6.94±0.79
Infected	1.24±0.24**	6.23±0.28*	153.00±68**	14.00±0.28	7.00±1.9*	6.64±0.63
<i>Streptococcus agalactiae</i>						
Control	0.70±0.13	7.84±0.14	80.00±0.62	12.00±0.074	8.00±0.52	7.00±0.35
Infected	1.73±0.39**	6.00±0.4*	184.00±64**	13.00±0.29	7.23±0.33*	6.84±0.93
<i>S. aureus</i>						
Control	0.90±0.54	7.90±1.23	94.00±0.40	13.00±1.23	8.73±0.51	7.50±0.68
Infected	1.90±0.83**	6.49±0.23*	157.00±14**	14.40±1.74	7.00±0.13*	6.99±0.89
<i>Pseudomonas aeruginosa</i>						
Control	0.83±0.34	7.97±0.62	0.94±1.20	14.00±0.73	8.51±0.27	7.00±0.23
Infected	1.80±0.34**	6.73±0.34*	1.29±1.90*	13.80±1.80	7.75±0.84	7.89±0.70

** p<0.01

* p < 0.05

presence of bacteria initiates inflammation within the mammary tissue in an attempt to eliminate the invading microorganisms, in general, compositional changes involve an increase in blood components present in milk and a decrease in normal milk constituents.

The diagnosis of clinical mastitis is straightforward. Something is obviously wrong and it is easy to see. With subclinical mastitis, the problem is detection. It is clear that incidence of subclinical mastitis among examined

dairy is relatively high. Elsagheer *et al.* [10] suggest that application of CMT leads to early detection of subclinical infected quarters, Kivaria [16] concluded that CMT is the best cut off to correctly identify subclinical mastitis especially by *S. aureus* in low yielding cows.

Table 1 showed that of the 100 milk samples, 25% were positive on bacteriological examination and the isolates were *E. coli* 15% *Staphylococcus agalactiae* 5%, *Staphylococcus aureus*.

3.5% and finally *Pseudomonas aeruginosa* 2.5%, so *E. coli* was the major pathogen in the aetiology of subclinical mastitis in the studied animals, which was classically considered to be an environmental pathogen, suggesting an adaptation of this pathogen to the udder environment as described by Passey *et al.* [17]. The *Staphylococcus* was the second major pathogen, and it causes clinical and subclinical intramammary infection as described by Kerro Deggo *et al.* [18].

In the present study we have shown that L-lactate dehydrogenase (LDH) activities were enhanced in mastitic milk. The enhancement can be at least partly explained by the participation of leucocytes which have LDH activity at the 1,000 U/mg protein level in mastitic milk. In the present study, we have measured LDH of 4 species of bacteria which were isolated from the mastitic milks used in the present study and the activity was detected in the extracts of *E. coli*, *S. aureus*, *S. agalactiae* & *Pseudomonas aeruginosa*. The enzyme activities were much higher in case of the infected udders as compared to the control. The level of LDH was increased in mastitic milk [19, 20].

The Hemogram presented in table 2 showed a significant decrease in RBCs, PCV and hemoglobin, while there was a significant increase in ESR & WBCs ($p < 0.01$). These findings are in agreement with Cebra *et al.* [21], while in contrary in relation to the total leucocytic count, there were no significant changes in PCV, hemoglobin concentration or red cell count seen by Griel *et al.* [22].

The changes in serum Ca and Phosphorus in the studied animals were in agreement with Griel *et al.* [22], and El-Zubeir *et al.* [23] in relation to Ca only, while Phosphorus in our study showed no obvious changes.

The results obtained from Table 3 indicated that the total protein values revealed a notable decrease in infected buffalo and this was in agreement with that of Cebra *et al.* [21]. The highly significant increases detected in SGOT values & cortisol, are in line with the results of Griel *et al.* [22], Symons *et al.* [24], Sloss & Dufty [25] and Las Haeras *et al.* [26]. Bayumi *et al.* [7] and Agarwal *et al.* [27] attributed these changes to stressful conditions.

Prognostic diagnosis has commonly been evaluated on the clinico pathological findings and the hematological and biochemical examinations of blood.

We could not determine the pattern of mastitic animals but the application of CMT leads to early detection of sub-clinically infected quarters and aids in the selection of dairy animals for either segregation or therapy. Also we conclude that sub-clinical mastitis causes anemia in cows detected by decreasing their

hemoglobin and RBCs count. An integrated approach to dairy extension should focus more on the creation of mastitis awareness among producers by improving their milking procedures and presenting good hygiene for their animal environment, checking their animals for mastitis using California Mastitis Test and all the positive cases should be treated in order to control and prevent the spreading of this disease to other animals.

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