

Some biochemical Studies on Friesian Suffering from Subclinical Mastitis

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Abstract: The present study was conducted to investigate the effect of subclinical mastitis on clinicopathological changes in Mastitic friesian. A total of 400 individual milk samples from clinically normal udder quarters of 100 diary friesians 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 analysed for hemogram, cortisol, alanine aminotransferase, aspartate aminotransferase, total protein, inorganic phosphorous and calcium. Also L.DH in milk was detected. The results indicated that there is a significant elevation of cortisol, Sgot, p.cv, L.DH 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. [Nature and Science. 2010;8(4):143-146]. (ISSN: 1545-0740).

Key words: Microbiology of mastitis, Pathology of mastitis, Enzymes in mastitis, changes in blood

1. Introduction

Mastitis is the most frequent, disease responsible for early culling of milking animals, this culling sometimes takes place before the animal reaches the age of maximum production. The colonization of mammary gland by pathogenic microorganisms results in series events which lead to major alteration in the composition of milk, and on the disease set in inflammatory reactions takes place for several days (Elsagheer *et al*, 1992). Preacute coliform mastitis is of great economic importance in the dairy industry since the infection with coliform organisms and the following production of endotoxin leads to high mortality. Akira (1989) reported that when diagnosis of preacute coliform mastitis is given by clinical signs and hematological hematobiochemical findings only misdiagnosis on the prognosis is common. Thus the presence of endotoxin in blood plasma should be checked for the precise diagnosis of bovine preacute coliform mastitis. This is characterized by severe quarter Inflammation (Schalm *et al*, 2006). Stress in the form of muscular exertion causes alterations in the different blood constituents (Agarwal *et al*, 1984; Bhasrekar *et al*, 1984 and Cabona *et al*, 1990). This work was initiated so as to investigate clinicopathological changes among infected friesian. The aim of the present work was also to study the bacteriological incidence of subclinical mastitis among friesian and to find the relationship between

clinicopathological changes and mastitis in subclinical mastitic friesian.

2. Material and Methods

For conducting this work 400 milk samples were aseptically collected for clinically normal quarters of friesian 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.
- (B) Microbiological examinations which include cultivation of milk sediment. The milk sediment obtained by centrifugation of 10 ml 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 identification was carried out according to Ajello *et al.*

Blood samples from Friesian were collected by jugular venepuncture in test tubes with or without EDTA.

Serum was harvested by centrifugation at 3000 rpm. Calcium, Inorganic phosphorous, total protein Sgot, Sgpt were determined in serum using kits from Diamond Diagnostica 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 (1976). A complete blood picture was manually performed as outlined by Jain (1986).

LDH assay

L.D.H in milk was measured by special kits according to methods of Kachmar and Moss (1976).

The samples were processed by centrifugation to remove fat and pellet and intermediate layers obtained were further centrifuged at 30,000 Xg for

al. (1966) and El-Sagheer *et al.* (1992).

min essentially according to the method of Bagin *et al.* (1977). The supernatant obtained was filtered through filter paper and used as the enzyme source.

LD.H activity was assayed spectrophotometrically at 340nm by special kits according to Kachmar and Moss (1976).

3. Result

The results obtained from Table 1 revealed that out of 100 lactating Friesian 14% were infected with *E.coli* 6% infected with *S.agalactia* 3.5% *S. aureus* & 1.5% *Pseudomonas aeruginosa*.

From the Table 1 it was obvious that C.M.T. reaction is positive for Friesian with preacute coliform mastitis. Further prognostic diagnosis have commonly been done based on clinical symptoms and hematological & biochemical examinations.

ABLE 1. Bacteriological examination of infected Friesian.

100 lactating Friesian	14% infected with E. coli	6% S. Agalactia	3.5% Pseudomonas Aeruginosa	1.5% S. Aureus
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The Hemogram showed a significant decrease in R.B.Cs, P.C.V. and hemoglobin while there was a significant increase in E-SR & W.B.C.S ($p < 0.01$) (Table 2).

Cortisol levels

The present investigation (Table 3) indicated that mastitic cows had significant elevation of serum Cortisol levels as compared with non mastitic cows ($p < 0.01$).

Liver junctions

Enzymatic activity

Sgot values were significantly ($p < 0.01$) higher in mastitic cows while Sgpt values revealed no obvious changes as compared with control group.

Calcium and Inorganic phosphorous

Slight non significant decrease were recorded in calcium level while inorganic phosphorous revealed no obvious changes.

Total protein

T.P. values were significantly lower in mastitic cows ($p < 0.01$). **LDH in milk**

L. DH level in milk were significantly higher in mastitic cows ($p < 0.01$).

4. Discussion

Subclinical mastitis is of great economic importance in the dairy industry since the infection with coliform organism leads to high mortality. When diagnosis is given by clinical signs only Ederhart (2007) suggested further investigations including hematological and biochemical investigations for confirmation. It is clear that incidence of subclinical mastitis among examined dairy cows is relatively high with reduction in milk yield which causes a heavy economic losses. The colonization of mammary glands by pathogenic microorganisms results in a series of events which lead to major alterations of milk compositions secreted from cells. Therefore C.M.T is a suitable measure for use on large scale monitoring programs. El-Sagheer *et al.* (1992) suggest that application of C.M.T. leads to early detection of subclinical infected quarters and aids in the selection

of dairy animals for either segregation or therapy for less than costs of the disease including the large losses in milk production for cows with preacute

coliform mastitis. The level of LDH seems to increase in mastitic milk, (Kerumori *et al*, 1989).

Table 2. Effect of mastitis on Hemogram of infected cows.

E. coli	Hemoglobin g/dl	P.C.V%	R.B.C.S 10 ⁶ /wd	W.B.C.D 10 ³ /wd	E.SR mrn/2hrs
Control	9.10 ±0.23	31 ± 0.32	8.10 ±0.18	10.35 ±0.64	1.03 ±0.092
Infected	8.60 ±1.94*	27.5 ±0.62	6.33 ±0.90	12.27 ±0.053*	1.83 ± 0.045**
Streptococcus agalactiae					
Control	9.9 ±0.20	32 ±0.67	9.3 ±0.74	10.00 ±0.26	1.00 ±0.35
Infected	7.80 ±0.58**	31 ±0.070*	8.8±0.80*	13.00 ±0.63*	1.72 ±0.072**
S. aureus					
control	9.45 ±0.05	34 ±0.69	9.00 ±0.07	10.57 ±0.71	1.23 ±0.021
Infected	7.94 ± 0.08**	28.00 ± 0.23**	8.40.09*	14.00 ±0.53**	2.2 ± 0.052**
Pseudomonas Aeruginosa					
Control	9.3 ± 0.13	32 ±0.72	9.00 ±0.74	10.00 ±1.83	1.00 ±0.64
Infected	7.00 ± 0.69	30 ± 0.93*	8.03 ±0.33*	12.00 ±0.77*	2.0 ±0.0.82**

** p < 0.01

* p < 0.05

Table 3. Effects of subclinical mastitis on Biochemical changes and Cortisol hormone level of infected cows.

E. coli	Total protein	L.DH activity U/ml	Cortisol mg/dl	Sgot U/L	Sgpt U/L	Calcium mg/dl	phosphorous mg/dl
control	7.95 ±0.73	57.3 ±2.23	0.93 ±0.32	75.3 ±0.64	13.23 ±0.37	8.93 ±0.74	6.94 ±0.78
Infected	6.33 ±0.27	10.14 ±0.62**	1.34±0.23	16.3 ±68**	15.00 ±0.26*	7.10 ±1.09*	6.74 ±0.53
Streptococcus agalactia							
control	7.84 ±0.14	67.00 ±0.40	0.70 ±0.13	80 ±0.62	12.00 ±0.074	8.00 ±0.52	7.00 ±0.35
Infected	6.10 ±0.37*	127 ±17**	1.83±0.29**	1.94 ±54**	14.00 ±0.19**	7.33 ±0.34**	6.89 ±0.92
S. aureus							
Control	7.90 ±1.23	50 ±0.27	0.90 ±0.54	94 ±0.40	13.00 ±1.23	8.73 ±0.51	7.5 ±0.68
Infected	6.59 ±0.22*	134±16**	1.91±0.82**	158±13**	14.3 ±1.73*	7.10±0.14**	6.8±0.88
pseudomonas aeruginosa							
control	7.97 ±0.62	73 ±0.48	0.83 ±0.34	0.94 ±1.20	14.00 ±0.73	8.51 ± 0.27	7.00 ±0.23
Infected	6.83±0.33	178±50**	1.93 ±0.33**	139 ±1.00*	1.48 ±1.70*	7.85 ± 0.34*	7.79 ±0.40

** p < 0.01

* p < 0.05

Prognostic diagnosis have commonly been done on clinical symptoms and the hemotological and biochemical examinations blood. Biochemical analysis of mastitic animals may help in diagnosis of subchemical abnormalities and become a helpful means for practice under field conditions (Rose, 1987). The present results in mastitic cows fell in the range given by Jain (1986) and Koneko (1989).

As shown in Table 1 the significant changes in hemogram and other biochemical values are due to

infection with mastitis. The highly significant increases detected in Sgot values & Cortisol are in line with the results of Sloss & Dufty (1980), Symons *et al.* (1974). Agarawal *et al.* (1984) however attributed these changes to stressful conditions. In the present study we have shown that L.D.H activities were enhanced in mastitic milk. The enhancement can be at least partly explained by the participation of leucocytes which have L. DH activity at the 1,000 U/mg protien level in mastitic milk (Kasumori *et al.*, 1989). Protien concentration as well as somatic cell

count and L. DH is increased when compared to normal milk.

In the present investigation we have also measured L. D.H 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. agolacaltiae* & *Pseudomonas aeruginosa*. The enzyme activities were much higher in case of the infected udders as compared to the control.

We could not determine the pattern of mastitic udders because the udders contained large number of leucocytes by washing small pieces of udder tissue with mechanical shaking.

Conclusively in mastitic animals the application of C.M.T. leads to early detection of subclinically infected quarters and aids in the selection of dairy animals for either segregation or therapy. Also we conclude that mastitis causes anemia in cows detected by decrease of hemoglobin, R.B.C.S, and P.C.V. L.D.H activity in milk increases, as well as Cortisol, Sgot and calcium in serum.

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