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Evaluation of some biochemical indices in "milk whey" for diagnosis of subclinical mastitis caused by some *Enterobacteriaceae* family

Mustafa Salah Hassan[1] and Afaf Abdulrahman Yousif [1]*

[1] Dept. of Veterinary Internal and Preventive Medicine / College of Veterinary Medicine/ University of Baghdad, Iraq

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***Corresponding author:**
Email address:
afaf_a.rahman@yahoo.com

Abstract

A total of 310 milk samples collected from the udder halves of 161 dairy ewes at mid period of lactation. This study designed to evaluate the variation in milk "California mastitis test" (CMT), "white side test" (WST), chloride test and pH test, in relation to some *Enterobacteriacae* family infection. The activities of whey "lactate dehydrogenase" (LDH), "alkaline phosphatase" (ALP) and "aspartate aminotransferase" (AST) were also estimated. The percentage of infection with subclinical mastitis caused by *Proteus mirabilis* and *E. coli* mastitis was 8.88% and 3.33% respectively. All samples were subjected to bacteriological examination. The whey samples were divided into three groups: normal animals (non-infected group), subclinical infected group with *Proteus mirabilis* and subclinical infected group with *E. coli* for estimation of enzymes. Activities of "LDH" "ALP" and "AST" were significantly higher in milk from the subclinical mastitis groups for *Proteus mirabilis* and *E. coli* (AST: 270.33±93.54; 268.25±114.37; ALP: 789.03±123.95; 838.12±92.50; LDH: 316.83±32.02; 407.12±38.82) respectively, than in non-infected group (AST: 38.84±2.71; ALP: 187.91±5.54; LDH: 142.59± 5.67). In conclusions, the measurement of AST, LDH and ALP activities in milk samples can be used as appropriateness and dependable method for detection of subclinical mastitis in ewes.

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Introduction

Mastitis is inflammation of the parenchyma of the mammary gland regardless of the cause. Mastitis characterized by a range of physical and chemical changes in the milk and pathological changes in the glandular tissue (Radostits *et al*., 2007). Coliform

mastitis seems to be less common among small ruminants than bovine mastitis. *E. coli* and *Klebsiella* species are the most common coliform bacteria implicated in mastitis. Coliform mastitis is more common in the post-parturient period, and it associated with severe systemic disease. It can be either a persistent or transient infection (Pugh, 2002). The high incidence of *E. coli* and other environmental pathogens suggests that poor air and litter hygiene, as a consequence of high stocking densities and prolonged feces accumulation in sheep houses (Albenzio *et al*., 2002). Many researcher isolated *Proteus mirabilis* from cases of mastitis (Adwan *et al*., 2005; Kwanashie *et al*., 2012). The prevalence of infection dairy sheep with mastitis, as determined by positive California and somatic cell count tests and a positive bacterial culture which revealed that 87.7% was a minor pathogen and 12.3% as major pathogens (Riggio *et al*., 2013). Kitchen *et al*., (1970) report that determination of enzymes activity might serve as a possible method for detection of subclinical mastitis and other udder diseases. Batavani *et al*., (2003) conclude that activity of LDH and ALP were higher in milk from subclinical udders than in milk from healthy udders.

This study was designed to evaluate some biochemical indices in "milk whey" for diagnosis of Enterobacteriaceae subclinical mastitis in ewes.

Materials and Methods

One hundred sixty-one lactating ewes at 2-6 years of age, from Al-Anbar province were used in this study. The ewes were examined clinically to confirm infection with mastitis or apparently normal. The study was carried out over a 6 months period starting from October 2012 to March 2013. Systemic vital signs (temperature, pulse and respiratory rate) and local signs on the udder (hotness, redness and swelling) were recorded. Three hundred ten (310) milk samples collected aseptically from the udder. The udder and teats were washed with water, and then the teat ends were disinfected with cotton soaked in 70% alcohol solution. The first three stripped milk were discarded, and 20 ml of milk was collected. These samples transported immediately to the laboratory by cooling box (Radostits *et al*., 2007). Milk samples were subjected to Color, odor and consistency, the physical Examination. White Side Test, California Mastitis Test, Chloride test and pH test, were also performed on the normally apparent milk samples (Schalm *et al*., 1971).

Bacteriological examination (included isolation and identification of bacteria from milk samples) were performed according to Quinn *et al*., (2004). All milk samples were cultured on blood, MacConkey and nutrient agar and incubated at 37 C° for 24 hrs. Microbiological characterization of the bacteria was depended on morphological character such as (shape, color and size of the colony), lactose fermentation and swarming on MacConkey agar. Colonies were subjected to Gram stain. The suspected isolates sub cultured on Chrome agar medium (specific for *E.coli*), and biochemical tests (catalase, oxidase, Gelatin liquefaction, Urease, O/F test, Sugar fermentation, Indole, Methyl red, Voges–Proskauer, citrate, Motility and TSI) were used for identification of *E. coli* and *Proteus mirabilis* isolates.

Biochemical analysis (Enzymes) in milk whey was done as followed: Ten milliliter of milk were centrifuged in cooled centrifuge high speed to separate whey of milk. Then AST, ALP, LDH was measured by spectrophotometer by using commercial kits

(Bio- Merieux, Laboratory reagents and Products, Marcy-I' Etoile, France).

Statistical analysis

All data are represented as means ± SE. One-way analysis of variance (One-way ANOVA) by using SPSS program, followed by Least Significant Difference (LSD) test to determine differences among means of investigated groups. The statistical significant was set at ($P< 0.05$) (Snedecor and Cochran, 1989).

Results

Bacterial isolation

The percentage of some Enterobacteriaceae family isolated and recognized from subclinical mastitic milk samples are presented in Table 1. The most common pathogens detected from 105 subclinical cases of mastitis, were 9 and 4 strains of *E.coli* and *Proteus mirabilis* respectively.

Table (1) Distribution of isolated bacteria in subclinical mastitis

Bacteria	No. of subclinical cases	% of subclinical cases
Proteus mirabilis	9	8.57
E. coli	4	3.8
Total No of Subclinical cases from 310 normal milk	105	33.87

Relation between CMT and bacteriological investigation

The percentage of *P. mirabilis* was 8.88% in a 90 +ve samples for CMT, and 6.66% from 15 –ve samples. While, *E. coli* percentage was 3.33% and 6.66% from 15 –ve samples (Table 2). The distribution of *P. mirabilis* and *E. coli* isolates at different scores of CMT {±, +, ++, +++} were showed (Table 3).

Relation of subclinical mastitis with enzymes activities in "milk whey".

After examination for subclinical mastitis, the animals were divided into three groups: normal animals (non-infected group), animals infected with subclinical mastitis due to *P. mirabilis* and *E. coli*. Estimation of biochemical analysis in "milk whey" showed that LDH, ALP and AST were significantly higher at ($P<0.05$) in the subclinical mastitis due to *P. mirabilis* and *E. coli* than normal animals (Table 4).
The efficacy of chemical tests and enzymatic activities used for detection of subclinical mastitis due to *P. mirabilis* and *E. coli*, revealed that enzymatic activities

were a higher percentage 100% than other chemical tests for detection of subclinical mastitis in relation with isolation of bacteria as showed in Table 5 (Figure 1).

Discussion

Ovine subclinical mastitis is an important disease of sheep which confirmed by many researchers. Beheshti *et al*., (2010) found that the periodic prevalence rate of SCM was 9.23%, and a similar result obtained by Contreras *et al*., (2007) who noticed the prevalence of Subclinical mastitis 5-30% in goats. The present investigation revealed isolation of *E. coli* in 3.5%.

Table (2) shows the relation between CMT and bacteriology

No of Milk samples examined	+ve *P. Mirabilis* from all +ve CMT	+ve *P.mirabilis* from -ve CMT	+ve *E. coli* from all +ve for CMT	+ve for *E. coli* from all -ve CMT
300	8	1	3	1
%	8.88%	6.66%	3.33%	6.66%

Table (3) shows the relation between CMT scores *P. mirabilis* and *E. coli* isolation

CMT scores	No of samples +ve to CMT	+ve for *P. mirabilis* mastitis	+ve for *E. coli* mastitis
±	21	1	0
+	30	4	2
++	38	3	2
+++	1	0	0
Total	12	8	4

Table (4) Shows the relation of subclinical mastitis due to P. mirabilis & E. coli with enzymes.

Groups of animals	Biochemical indices (Enzymes)		
	AST	ALP	LDH
	Means ±SE*	Means ±SE*	Means ±SE*
Normal animals (Non infected with mastitis)	38.84±2.71 B	187.91±5.54 B	142.59±5.67 B
Animals infected with subclinical mastitis due to *P. mirabilis*	270.33±93.54 A	789.03±123.95A	316.83±32.02A
Animals infected with subclinical mastitis due to *E. coli*	268.25±114.37 A	838.12±92.50 A	407.12±38.82 A

The different capital letters refer significant variations between groups at (P<0.05)

Table (5) Efficacy of chemical tests and enzymes activities in relation with isolated bacteria

No. of bacteria isolated	Chemical tests				+ve for enzymes activities
	+ve samples for CMT	+ve samples for WST	+ve samples for Chloride test	+ve for pH test	
P.mirabilis (9)	8 88.9%	7 77.8%	5 55.56%	4 44.4%	9 100%
E.coli (4)	3 75%	2 50%	1 25%	1 25%	4 100%

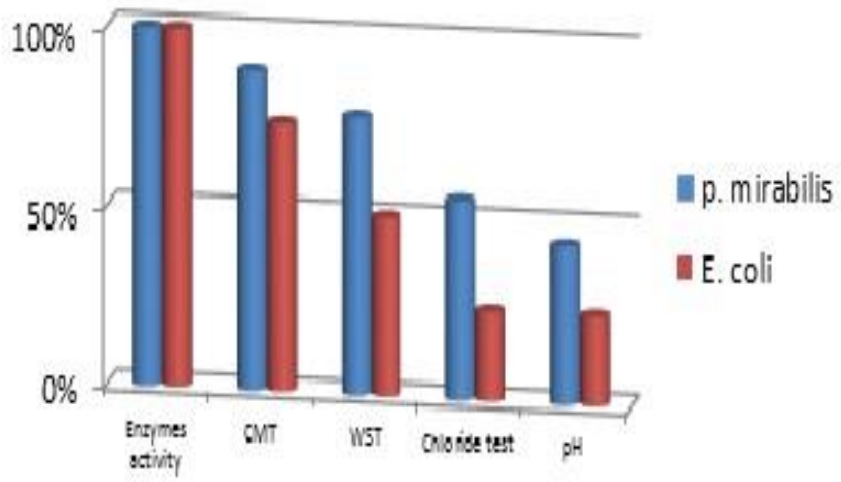

Figure (1): Chemical tests and enzymes activities in relation with isolated bacteria

This percentage compatible with the results of Vasil, (2007), who reported that percentage of *E. coli* was 0.0 %–7.8 %. The results of this study also compatible with a result of Kwanashie, (2012), who found that isolation percentage of *E. coli* was 4.5%. The percentage of Proteus mirabilis was 8.7%, this result in agreement with a result of Adwan *et al.,* (2005), who isolated Proteus mirabilis at 9.4%. The results of CMT test showed higher prevalence rate of subclinical mastitis than other tests (WST, chloride & pH tests). California mastitis test detects the increased number of leukocytes in mammary secretion indirectly detect. It can be considered as a good test and more accurate diagnostic technique for detection of subclinical mastitis (Schalm *et al.*, 1971). The CMT scores values of this study is compatible with those obtained by other authors (De la Cruz *et al.*, 1994; Fthenakis, 1994). The predictive value of a positive result is mainly influenced by the prevalence of mammary infections in the flocks. The results of this study showed also scores +1 and +2 of CMT, which is in agreement with (Fthenakis, 1994), who recorded that score +2 of CMT was appropriate threshold value for detection of subclinical mastitis.
E. coli was isolated in 3.5%, in this study. This result is in agreement with previous results (Vasil, 2007) who, reported 0.0 %–7.8 % of *E. coli* isolation percent. *E. coli*

considered as the important etiologic agents of SCM in goats and sheep (Islam *et al*., 2012). *Proteus mirabilis* isolated in this study at 8.7% and this result is in agreement with a result of Adwan *et al*., (2005) and Housawi *et al*., (2008).

The enzymes (AST, ALP, and LDH) are secreted by the epithelial cells of mastitic mammary gland. In mastitis, muscle and tissues of mammary gland are damaged which may lead to increase in the level of these enzymes (Khodke *et al*., 2009). Babaei *et al*., (2007) reports that the blood–milk barriers are damaged with infection, it is also possible that LDH or ALP was transferred from blood to the milk. The results of our study showed that the means of AST, ALP & LDH activities in milks from ewes with subclinical mastitis were significantly ($P< 0.05$) higher than those from healthy normal ewes. This indicates that determination of enzymes activities in serum milk is a sensitive and dependable method for detection of ovine subclinical mastitis. The results are in agreement with Batavani *et al*., (2007). They found that, the increased in milk enzymes (LDH, AST and ALP) in mastitic animals, might be linked with mammary tissue damage. It is also in agreement with the result of Katsoulos *et al*., (2009). They approved the sensitivity and reliability of determination method of LDH activity in milk serum for detection of subclinical IMI in dairy sheep and goats. It is also compatible with a result of Kalantari *et al*., (2013), who found that the measurement of LDH has high clinical accuracy, sensitivity and specificity in the detection of subclinical mastitis and could be used as a reliable method in dairy cows. Bogin and Ziv, (1973) found approximately a six-fold increase in the level of ALP after infusion of *E. coli* endotoxin into the bovine udder. Batavani *et al*., (2003) showed that the increment LDH and ALP in milk of udders shows the presence of tissue damage, these parameters might be suitable for use in the early diagnosis of subclinical mastitis in ewes. The results of this study that showed the reliability of enzymatic activity in diagnosis of subclinical mastitis in ewes, is also compatible with Hassan and Yousif, (2013). They study the alteration of some enzymatic activities in whey of ewe's milk Suffered from *Staphylococcal* mastitis. They found that LDH, ALP and AST activities were significantly higher in milk from the subclinical and clinical mastitis groups for S. aureus and coagulase negative *Staphylococcus* (CNS). Moreover, Fruganti *et al*., (1986) found that the increase in LDH and ALP activities were associated with clinical mastitis and to a lesser extent with subclinical mastitis. In contrast, the results of this study disagree with a study of Yang *et al*., (2011). They found non-significant different in AST activity of milk between normal and subclinical infected udders. In conclusion, alteration in enzymatic activity can be used as a reliable method for detection of subclinical mastitis in dairy ewes. Early diagnosis of subclinical mastitis in dairy animals may be important in reducing production losses and enhancing prospects of recovery herds. This procedure will help to avoid the development of clinical mastitis.

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