Some Biochemical And Bacteriological Studies On Mastitis In Ewes In Sharkia Governorate With Trial For Treatment With Cefotaxime And Levamisole

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ABSTRACT

This work was planned to evaluate the efficacy of cefotaxime alone or cefotaxime plus levamisol HCL in treatment of mastitis in ewes and effect of mastitis on some hematologyical and biochemical parametals. Twenty four ewes aged 2-3years,divided into three equall groups (8 each),1st group healthy ewes and kept as control group, 2nd and 3rd groups were suffering from clinical mastitis and treated with cefotaxime alone or Cefotaxime plus levamisol HCL respectively. Milk samples were obtained for bacteriological examination, two blood samples from jugular vein were taken before and after 7th and 14th days post treatment for hematological and biochemical analysis. Efficacy of cefotaxime alone or together with Levamisole HCL in treatment of mastitis were recorded.

Bacteriological findings indicated that E.coli,Corynebacterium pyogens, Klebsiella pneumonia,Staph.Aureus and strept.Uberis in precentage of (6) 31.25 % , (2) 12.50 % , (2) 12.50 % , (4) 25% and (2) 12.50% respectively

The activity of cefotaxime,ceftiofur sodium,amoxycillin,gentamycin,enrofloxacin and other antibiotic disc *in vitro* against previous isolates by disc diffusion test, showed that cefotaxime and Ceftiofur sodium was the highest effective on all isolated organisms than other tested drug followed by gentamycin, enrofloxacin but all isolated microorganisms moderately sensitive to erythromcin, oxytetracycline and amoxycillin and all isolated microorganisms not sensitive to nalidixic acid.

Erythrogram data for mastitic ewes revealed microcytic hypochromic anaemia represented by significant reduction in erythrocytic counts,haemoglobin content, packed cell volume percent associated with significant increase in leukocytic count.

Serum biochemical analysis showed elevation in total protein, globulin, amino-transferases (AST-ALT) alkaline phosphatase and copper, meanwhile albumin,A/G ratio, iron, zinc phosphorous and potasium significantly decreased and insignificant increase in serum concentration of calcium, sodium and magnesium in mastitic ewes. These biochemical alterations were returned to the normal levels 14th days post treatment.

Efficacy of cefotaxime together with levamisole HCL *in vivo* study was recorded, when compard with the cefotaxime , all mastitic ewes were cured in 100% cure rate at 6 day of treatment by cefotaxime alone and at 4 day by cefotaxime plus levamisole

It could be concluded that mastitis induce several adverse effects on erythrogram, liver and kidney functions in ewes which need about 14th days post treatment either by cefotaxime alone or with Levamisole HCL is to return to the normal levels and cefotaxime together with Levamisole HCL more potent in treatment of mastitis than cefotaxime alone.

INTRODUCTION

Milk is synthesized in specialized cells of the mammary gland and is virtually sterile when secreted into the alveoli of the udder *(1).*

Mastitis is one of the most important causes of loss in milk production, mortality and premature culling in many countries *(2)* .Mastitis is a disease complex having different cause, different degrees of intensity and variation in duration. Clinically , the disease occurs as peracute, acute or subacute which is easily recognized by visual examination of the milk and udder. Udder infection originates either from hematogenous , lymphatogenous,cutaneous or glactgenous routes *(3)* and influenced by different environmental factors including feeding, housing beding and mangement *(4).* Mastitis is of agreat economic importance in farm animals.It is considered to be world wide costlist production disease in dairy herds *(5).*

Mastitis caused by various bacterial agents mainly *Staphylococus areus,* followed by *Escherichia coli Strptococus species,Corynebacterium pyogens,* **(6).** Coliform bacteria such as E.Coli and klebsiella sp. are the most common pathogens isolated from mastitic milk *(7).*

Cephalosporins are a group of antibiotics derived from mould of cephalosporium species and are based on 7-aminocephalosporic acid which corresponds to 6-penicilanic acid in penicillins *(8).* Cefotaxime is a third generation semisynthetic cephalosporins with a broad bactericidal effect against G+ve and G-ve microorganisms *(9).*

Levamisole is an anthelmintic drug which also has immunomodulatory properties *(10).* levamisole enhance activity of the bovine mammary immune system and be of value for control of bovine mastitis *(11).*

This study was planed to investigate the effect of matitis on blood picture and some biochemical changes.Moreover, the isolation of the causative bacterial agents. Further more, arteriogram for the isolated strains was done and the efficiency of the treatment by concurrent administration of cefotaxime alone or with Levamisole HCL for treating mastitis in ewes.

MATERIAL AND METHODS

Drugs

A-cefotaxime(Cefotax)® Its a sterile, semisynthetic, broad - spectrum antibiotic from Epico Co It is available in the form of vails containing 500mg or 1000mg cefotaxime sodium.

B-Levamisole HC L7.5 (Levamisole HCL 0.075gm Pharmachim Bulgaria)

Animals

Twenty four ewes (16 infected with clinical mastitic and others 8 were clinically healthy) were used in this study.These animals belonged to diferent localities in Abo hamad City Sharkia Province and their age ranged from 2-3 years old.

Table 1. Experimental design

Group	Ewes No	Treatment	Dose
1	8	Non-infected and non treated.(control)	----
2	8	Infected treated with cefotaxime	(15mg/kgm b.wt.) 3day
3	8	Infected treated with cefotaxime and levamisole HCL	15mg/kgm b.wt (I/M)3day 7.5 ml/100kg.b.wt(S/C)

Milk samples

Milk samples were collected from affected quarters after and before treatment where the udder was thoroughly washed with running water, then dried with clean towel. The teats orifice was disinfected by ethyl alcohol 70%,after that the first few squirts of

milk was discarded then milk sample from each infected quarter were collected in sterile Macarteny bottles for bacteriological examination. All samples were cooled at 4^0C and transported to the laboratory

Bacteriological examination

Milk samples were activated by incubation for 12h.at 37°C then centrifugated at 3000 r.p.m.for 30 min. All samples were examined microbiologically by streaking loopful of centrifugated sediment from each sample on surface of nutrient agar, MacConkey agar and blood agar containing 7.5% defiberinated sheep blood.All plates were observed after incubation 24h at 37^0C and recorded any growth. Biochemical tests of the isolated strains were detemined *(12).*

Antibacterial seneitivity test

Isolated organisms were used to check their susceptibility for cefotaxime (75 ug),ceftiofur sodium(30ug),amoxycillin (30ug),enrofloxacin (10ug),erythromycin (15ug) and gentamycin (10ug).All sensitivity discs were supplied from bio Merieux Co,France. Adisc diffusion technique of antimicrobial sensitivity testing was done *(12).*

Blood samples

From healthy and mastitic ewes two blood samples from jugular vein were taken before treatment and after 7th and 14th days post treatment.

First blood sample was collected on tube contain EDTA as anticoagulant for haematological studies *(14).*

Second sample was collected in centrifuge tube to obtain clear serum for total protein determination *(15),* serum albumin *(16)* and serum globulin were calculated as difference between total proteins and albumin,serum AST and ALT *(17),* alkaline phosphatase *(18),* Calcium *(19)* phosphorus *(20)* copper *(21),*iron *(22)* , zinc *(23),* sodium and potassium were measured using flame photometer, magnesium was also estimated *(24).*

Statistical analysis

The obtained data were tabulated and statistically analysed *(25).*

RESULTS

The obtained results were tabulated in Tables 2-8

Table 2. Results of bacteriological examination of mastitic ewes at Sharkia Province

Types of samples	Number of milk sample	Positive samples		single isolates		Mixed isolates	
		No	%	No	%	No	%
Milk	16	16	100	16	100	-	-

Table 3. Bacteriological isolation causing mastitis in ewes at Sharkia Province (N=16)

No. of tested ewes	*E- coli*		*Cory. Pyogen*		*Klebsiella pneumone*		*Staph. aureus*		*Strep. uberis*	
	No	%	No.	%	No.	%	No.	%	No.	%
16	6	31.25	2	12.50	2	12.50	4	25	2	12.50

Table 4.Sensitivity tests of isolated organisms against different antimicrobial agent

Antibiotic disc	Disc conc.	Bacterial isolates				
		E. coli	*Cory pyogen*	*Klebsiella pneumone*	*Staph. aureus*	*Strep. uberis*
Cefotaxime	75ug	+++	+++	++	++++	+++
Ceftiofur sodium	30ug	+++	++	++	++++	+++
Amoxycillin	30ug	+	++	++	++	++
Enrofloxacin	10ug	++	+	++	++	++
Erythromycin	15ug	-	++	+	+	++
Gentamycin	10ug	+++	+++	+++	++	++
Colistin	10ug	-	-	-	-	-

Table 5. Haemogram of normal blood and mastitic ewes before and after treatment.

parameter	Healthy ewes (n=8)	Diseased ewes				
		Before treatment (n=16)	Post treatment			
			Cefotaxime alone (n=8)		Cefotaxime+Levamisole (n=8)	
			7days	14 days	7days	14 days
HB (g m %)	10.9±0.75	7.43±0.88**	9.03±0.73*	10.2±1.43	8.02±1.16*	10.29±1.26
PCV(%)	39.3±2.6	25.2±2.73**	33.3±0.84*	38.9±3.76	28.5±2.52*	37.83±2.54
RBCs (106/ul)	9.23±0.9	7.10±0.12**	7.59±0.09*	8.62±0.76	6.12±0.41*	8.88±0.82
WBCs (103/ul)	10.9±0.9	14.3±0.70**	7.14±0.89*	9.42±0.99	6.99±0.81*	9.222±0.86

* P < 0.05 ** P < 0.001

Table 6. Some serum biochemical parameter on healthy and mastitic ewes before and after treament

parameter		Healthy Ewes (n=8)	Diseased buffaloes				
			Before treatment (n=16)	Post treatment(days)			
				Cefotaxime alone (n= 8)		Cefotaxime+Levamisole (n =8)	
				7days	14 days	7days	14 days
Liver enzyem	AST (u/l)	72.2±4.7	95±8.21*	86.3±3.52*	77.8±2.9	84.7±3.88*	74.84±4.87
	ALT (IU.L)	51.7±2.8	61.9±3.5**	59.47±1.4*	56.9±2.9	60.54±3.02*	55.28±3.89
	Alk.ph.(IU.L)	43.9±2.2	51.3±2.8*	47.87±0.63*	42.3±3.9	47.29±1.08*	45.72±2.87
Protein picture	T.protein (gm/dl	8.20±0.8	11.2±0.5**	9.92±0.32*	8.65±0.9	9.06±0.12*	8.59±0.85
	Albumin (gm/dl)	4.80±0.3	3.55±0.2**	3.70±0.12*	4.38±0.7	3.81±0.19*	4.65±0.52
	Globulin (gm/dl)	3.40±0.9	7.66±1.2**	6.22±0.82*	4.27±0.8	5.85±0.87*	3.94±0.96
	A/G ratio	1.38±0.12	0.46±0.03*	0.59±0.06*	1.03±0.4	0.65±0.07	1.18±0.52

* P < 0.05 ** P < 0.001

Table 7. Mean value of some serum macro and micro elements on healthy and mastitic ewes before and after treament.

Parameter		Healthy ewes	Diseased buffaloes				
			Befor treatment (n=16)	Post treatment(days)			
				Cefotaxime alone (n= 8)		Cefotaxime+Levamisole (n =8)	
				7days	14 days	7days	14 days
Micro element	Copper µg%	117.5±5.6	85.0±5.87*	101.1±3.55*	109.1±4.4	102.4±4.62*	113.20±5.9
	Zinc µg%	109.2±5.6	92.2±3.19*	100.2±3.08*	107.7±4.4	102.3±1.51*	108.0±4.92
	Iron µg%	130.2±6.2	95.3±6.93*	107.6±3.22*	127.2±4.8	105.7±434*	126.2±9.4
Macro element	Calcium (mg/100ml	8.53±1.98	9.09±0.78	890±0.56	8.65±0.54	8.98±0.32	8.54±0.98
	Phosph. mg/100ml	7.20±0.89	5.12±0.20*	573±0.57*	7.13±5.76	5.62±0.06*	6.94±0.74
	Magesium mg%	3.88±0.69	4.08±0.94	3.91±0.85	3.80±0.86	3.99±0.67	3.91±0.89
	Sodium mEq/L	140±9.15	112.0±8.8*	138.0±15.22	126.9±4.1	130.3±3.54	127.4±4.3
	Potassium mEq/L	5.9±0.68	3.4±0.81*	4.2±0.19*	5.74±0.43	4.60±0.23*	5.42±0.34

* $P < 0.05$ * * $P < 0.001$

Table 8.Efficacy of cefotaxime alone or with Levamisole HCL in treatment of mastitis

Antibacterial agent	T.No. of ewes	3 days of treatment		4 days of treatment		6 days of treatment	
		No. of cured ewes	%	No.of cured ewes	%	No. of cured ewes	%
Cefotaxime alone	8	2	25	6	75	8	100
Cefotaxime with Levamisole	8	4	50	8	100	-	-

DISCUSSION

Mastitis is a serious disease affecting dairy animals. It causes enomrous losses for breeders and consequently influences the national income of the country ***(26).***

In the present study, it has been shown that mastitic ewes showed clinical signs manifested by general depresion,anorexia , fever and singes inflammation of the affected udder represented in enlargement of supramammary lymph nodes ,hotness and tenderness of affected udder. The systemic clinical signs that are often observed during bacterial mastitis include general depression, anorexia,fever,decrease in feed intake,in addition to hotness, enlargements, and pain of affected udder ***(27,28).***

Bacteriological results of this study showed that milk of mastitic ewes revealed that *E.coli,Corynebacterium pyogens, Klebsiella pneumoniae, Staph. aureus* and *Strept. uberis* were isolated from ewes showed clinical signs of mastitis in precentage of 31.25%,12.50%,12.50%,25% and 12.50% respectively. The predominant organisms caused mastitis are *Staph. aureus, Streptococci and E.coli* ***(29)***. Meanwhile in another study ***(30)*** it has been recorded that the main cause of mastitis are *Staph. aureus* and *E. coli* in percentage of 20% and 16.7%. *Staph. aureus* and *E.coli* were the most isolates in milk of both subclinical and clinical mastitis. Coliform bacteria such as *E.coli* and *klebsiells sp.* are the most common pathogens isolated from mastitic milk.

Disc diffusion test is widely used for antimicrobial sensitivity test for reasons of time,simplicity and cost ***(31).*** In present study using the disc diffusion test results showed

that the cefotaxime and Ceftiofur sodium was the highest effective on all isolated organisms than other tested drug followed by gentamycin enrofloxacin but all isolated microorganisms were moderately sensitive to erythromycin and amoxicillin and all isolated microorganisms not sensitive to colistin. Cefoperazone and cefotaxime are effective to pathogenic strains of *E.coli (32)*. About 500 isolates from cases of clinical mastitis, over 90% were sensitive to cefotaxime at the levels attainable in the milk of treated quarters for three to four milkings after treatment *(33)*. Twenty nine *(34)* staphylococcal and 27 streptococcal isolated were sensitive to the cefotaxime *(35)*. *E.coli* isolate from mastitic goats were sensetive to gentamycin *(35)*. Gentamycin had inhibitory effect on *E.coli*, *Strept uberis*, *Klebseilla* and *Corynebacterium*.

In the present study, mastitic ewes showed microcytic hypochromic anaemia represented by significant reduction in erythrocytic counts,haemoglobin content, packed cell volume percent associated with significant increase in leukocytic count . These results are in line with the previous findings *(36)*. The change in erythrogram may be attributed to the increased in reactive oxygen species especially H2O2 which causes oxidation of the sulphahydril groups *(37)*. It has been proved that the change in hematological parameter due to damage of erythrocytic membrane resulting in removal of the erythrocyte from circulation *(38)*. Also, the reduction in erythrocytic counts, hemoglobin content may be due to the effects of bacterial toxins where iron is diverted to tissues and is not available for hemoglobin *(39)*. An increase in leucocytic count in mastitic cows was recorded *(40)*.

It is evident from the present study that mastitis. induced significant increase in the activities of the liver enzymes (AST–ALT) and alkaline phosphatase. These are greatly similar to the previous findings *(41)*. Elevation in liver enzymes explained by damage of hepatic tissues in acute mastitis **(42)**.

Results of the present study revealed that mastitis in ewes induce a significant elevation in serum total protein, globulin meanwhile albumin and A/G ratio were significantly decreased. The significant decrease in serum albumin concentration in mastitic ewes may be attributed to the inflitration of albumin from the blood to milk due to the increase permeability of blood vessels as a consequence of inflamation *(6,43)* and the bacterial infection and its toxins which may affect worsely the hepatic parenchyma resulting in failure of liver to produce protein and albumin *(40)*.The increase in serum globulin level may referr to the inflammatory reactions of mammary tissues *(44)*.

The present study illustrated that the mastitic ewes revealed significant decrease in serum iron and zinc,on the other hand serum copper was significantly increased when compared with the healthy one. A significant decrease in serum iron and zinc in mastitic cattle *(27,45)*. The changes are attributed that to the effect of endotoxin release from pathogenic bacteria which stimulates the circulating endogenous mediators that cause trace minerals alteration and/or due to the anorexic condition which resulted in decreased intestinal absorption of such element *(46)*.The analytical findings of the serum constituents of mastitic ewes in this study revealed a significant decrease in phosphorous and potasium and insignificant increase in serum concentration of calcium,sodium and magnesium. A significant decrease of serum phosphorous and potassium in mastitic cowes was recorded previously *(47)*.These was due to the high demand of milking drainage for these elements and also the anorexic condition of diseased cattle*(46)*.

Treatment of mastitic ewes with either cefotaxime or cefotaxime together with levamisole HCL with the previously mentioned doses revealed that the all mastitic ewes were cured with 100 % cure rate, at 6^{th} day of treatment by cefotaxime alone and at 4^{th}day of treatment by cefotaxime plus levamisole HCL. Cefotaxime (250mg) were good in treatment of subclinical and clinical mastitis in cows *(48)*. The cure rate for clinical mastitis was 84.60% The treatment was very

effective against streptococcus and *Escherichia coli* ***(49)***. It has also been found that the cefotaxime being effective against many G+ve and several G-ve microrganisms. Cefotaxime is very suitable for treatment of *Staph. aureus* strains, *E.coli* and *Klebsiella pneumoniae* strains .

The noticed cure rate 90.93% in mastitic ewes treated with cefotaxime together with Levamisole HCL may be due to that the Levamisole increased the proportion B-lymphocytes of milk to the same as or more than that of similar cells in peripheral blood. This compound may enhance activity of the bovine mammary immune system and be of value for control of bovine mastitis ***(11)***. The application of Levamisole in treatment of bovine mastitis is considered, and levamisole may have rational application in mastitis prevention ***(10)***.

It could be concluded that the mastitis induce several adverse effects on haemogram,liver and kidney functions in ewes which need about 14 days post treatment either by cefotaxime alone or with levamisole HCL to retern to the normal levels and cefotaxime together with levamisole HCL are more potent in treatment mastitis than cefotaxime alone.

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الملخص العربى
الدراسات البيوكيميائية والبكتريولوجية على التهاب الضرع في النعاج بمحافظة الشرقية مع محاولة العلاج

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كان الغرض من هذه الدراسة هو عزل الميكروب المسبب لالتهاب الضرع في الأغنام و دراسة تاثير التهاب الضرع على صورة الدم ، بعض الوظائف البيوكيميائية وبعض المعادن النادرة وكذلك تقييم كفاءة السيفوتكسيم وحدة أو مع اليفاميزول في علاج التهاب الضرع فى الأغنام .

أجريت هذه التجربه على عدد ٢٤ من الأغنام (٨ منهم سليمة و١٦ مصابة بالتهاب الضرع) قسمت الأغنام إلي ثلاث مجموعات متساوية تحتوى كلا منها على ثمانية أغنام المجموعة الأولي غير مصابة بالتهاب الضرع استخدمت كمجموعة ضابطه(محكمة) أما الثانيه والثالثة مصابة بالتهاب الضرع وتم علاجهم باستخدام السيفوتكسيم وحدة بالجرعة العلاجية أو السيفوتكسيم مع اليفاميزول بالجرعة العلاجية على التوالي لمدة ثلاث أيام.تم أخذ عينة من اللبن قبل العلاج وذلك لعزل الميكروب المسبب لالتهاب الضرع وكذلك تم اخذ عينتين دم من كل حيوان الأولى على EDTA وذلك لدراسة تأثير التهاب الضرع على صورة الدم والأخرى لفصل المصل وذلك لقياس بعض المؤشرات البيوكيميائية قبل العلاج ,٧ , ١٤يوم بعد العلاج.

وبالفحص الميكروبيولوجى تم تحديد المسببات البكترية للالتهاب الضرع وكانت كالآتي ميكروب القولون العصوي، ميكروب الكورينى بكتريم الصديدى الكلبسيلا نمونى ، ميكروب العنقود الذهبي، ميكروب السبحي الأيضى فكان(٦) ٣١,٢٥%،(٢)١٢,٥٠% ،(٢) ١٢,٥ %، ٢٥(٤)% و(٢) ١٢,٥٠ %، على التوالي.وتم إجراء اختبار الحساسية للمعزولات البكتيرية وكانت أكثر حساسية لمركب السيفوتكسيم والسفتى فيور صوديوم هما الأكثر تاثيرا على المعزولات يليهما الجنتاميسين والانروفلوكساسين

يتبين لنا من تلك الدراسة أن التهاب الضرع أدى إلى حدوث نقص معنوى فى عدد كرات الدم الحمراء تركيز الهيموجلوبين ، حجم خلايا الدم المرصوصة مصحوبة بزيادة معنوية فى عدد كرات الدم البيضاء ،الخلايا المتعادلة و نقص معنوى فى عدد الخلايا اللمفاوية.

تشير النتائج أن التهاب الضرع أدى الى حدوث زيادة معنوية في البروتين الكلي، الجلوبيولين، الترانس أمينيزسس (AST–ALT)،الفوسفاتي القاعدي،الفسفور، البوتاسيوم والنحاس ولكن حدث نقص معنوى فى الزلال والنسبة بين الزلال والجلوبيولين الزنك،الحديد وزيادة غيرة معنوية فى الكالسيوم الصوديوم والماغنسيوم .كما تلاحظ أن أستخدام السيفوتكسيم منفردا أو مع اليفاميزول أديا إلي عودة هذه الوظائف إلي المستوى الطبيعي في مصل النعاج المصابة والمعالجة بعد العلاج ب ١٤ يوم

وبدراسة الكفاءة العلاجية لاستخدام السيفوتكسيم سوا منفردا أو مع الاليفاميزول حيث اختفت الأعراض الظاهرية للمرض بنسبة٧٥ % عند اليوم الرابع من العلاج و ١٠٠% عند اليوم السادس من العلاج باستخدام السيفوتكسيم منفردا بينما كانت نسبة الشفاء١٠٠% عند اليوم الرابع من العلاج باستخدام السيفوتكسيم مع اليفاميزول.

من مجموع ما تقدم من نتائج نستخلص أن التهاب الضرع أدى إلى حدوث تأثيرات عكسية على صورة الدم، بعض الوظائف البيوكيميائية وبعض المعادن النادرة. كذلك وجد أن السيفوتكسيم مع اليفاميزول لة تاثير أحسن فى علاج التهاب الضرع عن السيفوتكسيم منفردا.لذلك نوصى باستخدام اليفاميزول فى علاج التهابات الضرع مع المضادات الحيوية.