Studies on Mastitis in Cows at Kaliobia Governorate and the Public Health Importance of Their Milk

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Abstract

A total numbers of 395dairy cows at Kaliobia Governorate were clinically examined and milk samples of them were examined visually and by California mastitis test. The results were 12.9% clinical mastitic cows and 27.1% subclinical mastitic ones. The bacteriological examination of 89 mastitic milk samples proved that 85.4% were positive and 31.6% of them gave pure single cultures and 68.4% mixed ones .They yielded 175 isolates where Staphylococci (34.9%), mainly Coagulase positive *Staph.aureus* (28.6%), and Streptococci (33.1%), mainly *Strept.agalactiae* (17.1%), were the most isolated strains. While from 127 subclinical mastitic samples, 64.1% were positive with 29.1% containing pure single cultures and 70.9% mixed ones. They yielded 241 bacterial isolates where Staphylococci (33.6%), mainly Coagulase positive *Staph.aureus* (23.2%), Streptococci (30.7%), mainly *Strept. agalactiae* (15.4%) and *Strept.dysagalactiae* (11.2%),and *E.coli* (20.3%) were the main pathogens while other pathogens isolated with low incidence.Coagulase activity of 142 isolated Staphylococci showed 106 Coagulase positive *Staph.aureus* ; 10 Coagulase negative *Staph.aureus* and 26 Coagulase negative *Staph.epidermidis* .Haemolysis and CAMP test for 132 isolated Streptococci showed 67 *Strept.agalactiae* were positive while 48 *Strept.dysagalactiae* and 17 *Strept.uberis* were negative.Eight different serotypes of 80 *E.coli* isolated strains were obtained and *E.coli* O111 was the most predominant (18.7%).Also, 7 different serogroups of 36 isolated *Pseudomoneus aeruginosa* and serogroup K was the most one (25.0%). Most isolated strains were positive for mouse pathogenicity test. Enrofloxacin, lincomycine and gentamycin were the proper antibiotics with the highest *in-vitro* efficiency against most bacterial isolates. The chemical analysis of milk samples revealed a significant decrease in lactose and casein content .The haematological and biochemical analysis of blood samples showed a significant increase in total leukocyte counts; AST; ALT; AP; urea and creatinine. Meanwhile, a significant decrease in erythrocyte counts; haemoglobin contents; calcium; phosphorous and iron. Treatment of mastitic cows by intramuscular injection of enrofloxacin and intramammary infusion of lincomycine or gentamycin results in complete relief. The public health of isolated strains and controlled measured were also discussed.

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Introduction

Mastitis is the most prevalent infectious disease affecting dairy cattle which have many adverse economic implications world wide represented by decrease in quantity and quality of milk components and shorten the reproductive life of affected animals ,also feeding of the younger animals by mastitic milk leads to retention of the growth ; fall of immunity and pathologic states ,sometimes with fatal end **(2 and 18)** .Moreover , mastitis is considered of vital importance due to its association with many zoonotic diseases in which milk acts as a source of infection **(58).** Clinically, the disease occurs as per acute, acute or sub acute which is easily recognized by visual examination of the milk and udder. The subclinical mastitis is serous as it occurs without obvious signs and the secreted milk acts as an invisible potential source of spreading infection and may turn into the clinical form **(3 and 22).** Therefore, screening tests, bacteriological and biochemical examinations are necessary for identification of affected quarters **(34 and 50).** Bovine mastitis is due to different factors but the most important is the invading microorganisms that mostly found in mixed infection. The predominant organisms are ***Staphylococcus aureus;*** **Streptococci** and ***E.coli*** **(3, 11, 16, 18, 26, 55 and 59).**

Mastitis is characterized by marked changes occur in the level of nearly all major and minor constituents of the milk .These compositional reflect the degree of damage caused to the cells and to the complex array of blood capillaries by the pathogen **(5, 9 and 34).** Some of these compositional changes are increase of sodium ,chloride and phosphorous concentration, while, the casein content, lactose ,fat, potassium ,calcium and iron were lowered compared with normal ones **(9, 15, 26 and 41).** Moreover, there is ultration in the haematological and biochemical parameters in mastitic cows viz: Significant increase in total leukocytic counts; AST; ALT; AP; urea and creatinine and a significant decrease in total red blood cells and haemoglobin concentration **(2, 51 and 56).** As mastitis has sever adverse economic and public health effects, so, this study was carried out to estimate the prevalence of both clinical and subclinical mastitis in cows at Kaliobia Governorate ; isolation and identification of the causative agents with bacteriological studies on pure isolates and antibiogram for them to

decide the available and specific treatment of mastitic cows .Moreover, some milk constituents; haematological and biochemical parameters associated with mastitic cows were estimated to be helpful in the diagnosis of mastitis. Also, public health importance of mastitic milk was discussed.

Materials and Methods

A total 398 dairy cows from two private farms and different veterinary clinics at Kaliobia Governorate ,were examined for mastitis .The animals were subjected to clinical examination by visual inspection; palpation of the udder for swelling , redness and pain; beside the physical changes in the milk secreted from such udders. California mastitis test was used for detection of the subclinical mastitis according to **(52)**. With strict aseptic precautions and after discarding the fore milk, milk sample from each quarter was collected in sterile MaCartney bottles and transported to the laboratory in ice container for bacteriological examination and biochemical analysis. For bacteriological examination , a total of 89 clinically mastitic milk samples and 198 subclinically ones were activated by incubation for 12 hours at 37c° then centrifuged at 3000 rpm for 20 minutes .A loopful from sediment was streaked on the surface of Nutrient agar, MacConkey's agar , blood agar ,Mannitol salt agar; Baird Parker agar and modified Edward's media. All plates were incubated aerobically at 37c° for 24-72 hr. The developed colonies were picked up and subcultured for purification. The pure colonies were morphologically identified by Gram-stain and biochemically according to **(32 and 46).**

Further bacteriological studies on pure isolates were done:

a- Coagulase activity of **Staphylococci** to different types of plasma (rabbit, sheep, horse and human) using slide coagulase test and confirmed by tube coagulase test following **(32).**

b- Haemolysis and Christie-Atkins-Munoh-Peterson (CAMP) test for isolated **Streptococci** was carried out following **(8 and 10).**

c- Serotyping of isolated ***E.coli*** strains following **(14).**

d- Serotyping of ***Pseudomoneus aerugenosa*** following **(27).**

N.B: Antisera of ***E.coli*** and ***P.aerugenosa*** were obtained from Denka Seiken Co.Ltd., Tokyo, Japan.

e- Mouse pathogenicity test for isolated strains was carried out following **(25 and 32).**

f- ***In-vitro*** sensitivity test for isolated strains against different antibiotic discs (Oxoid standerized discs) using the agar diffusion method following **(32)**

For chemical analysis of milk, milk gross composition (fat and lactose contents) was measured following **(44);** casein content was determined by standard method according to **(39).**

For haematological and biochemical analysis, blood samples with anticoagulant (heparin) were collected from all diseased and healthy cows for determination of total erythrocyte counts ,total leukocyte counts and haemoglobin concentration according to **(29)** .Also, whole blood samples without anticoagulant were collected for determination of Aspartate aminotransferase and Alanine amino-transferase **(49),** urea **(20),** creatinine **(21),** calcium **(23),** phosphorous **(17)** and iron **(43).**

Data obtained were analyzed according to **(57).**

For trails of treatment, according sensitivity bases 11 clinically mastitic cows and 15 subclinically ones were treated with the most sensitive and effective antibiotics. Enrofloxacin 10% was used intramuscularly for each cow in a dose of 5mg/kg.body weight for 5 successive days and intramammary infusion of either lincocin forte (10ml syringe contain 330mg lincomycine + 100mg neomycin + 10mg predinsolin anhydrous) or Gentamast (7.5 ml syringe contain 100 mg gentamycin as sulphate) once daily for mastitic teat for 7 days in clinical cases and 5 days for subclinically ones. After treatment, clinical; California mastitis test, bacteriological and biochemical examination were applied as described above.

Results

The results of clinical examination and California mastitis test (Table, 1) appeared that 51 (12.9%) were clinically mastitic cows. Affected udder was warm, swollen, doughy to firm and painful. The milk was watery, purulent or with thick clots and seven samples were tanged with blood. Most cases showed only one or two quarters affected. The California mastitis test revealed that 107 (27.1%) apparently healthy cows were affected with sub clinical mastitis.

The results of bacteriological examination of 89 mastitic milk samples and 198 subclinically ones (Table,2) showed that 76(85.4%) of clinically mastitic milk samples were positive with 24 (31.6%) containing pure single cultures (***13 Staph.aureus;7 Strept.agalactiae; 1 Strept. dysagalactiae; 1 E.coli and 1 Ps. aerugenosa***) and 52 (68.4%) mixed ones .While, 127 subclinical mastitic milk samples (64.1%) were positive with 37 (29.1%) containing pure single cultures (***11 Staph. aureus; 4 Staph.epidermidis; 6 Strept.agalactia; 5 Strept. dysagalactiae; 6 E.coli and 5 Ps. aerugenosa***) and 90 (70.9%) mixed ones . Table (3) revealed that a total of 416 bacterial species were isolated from positive mastitic milk samples and both **Staphylococci** (142) 34.1% and **Streptococci** 132 (31.7%) were the most predominant pathogens, followed by ***E.coli*** 80(19.2%); ***Pseudomoneus aerugenosa 36 (8.7%); Actinomyces pyogenes 19 (4.6%) and Klebsilla pneumonae*** 7(1.7%). For clinical mastitic samples, 175 bacterial species were isolated and **Staphylococci** 61(34.9%), mainly Coagulase positive ***Staph. aureus*** 50 (28.6%) and **Streptococci** 58(33.1%), mainly ***Strept. agalactiae*** 30 (17.1%) and ***Strept. dysagalactiae*** 21(12.0%) were the most isolated strains, followed by ***E.coli*** 31(17.7%); ***Ps. aerugenosa*** 12 (6.9%); ***A. pyogenes*** 11(6.3%) and ***kl. pneumonae*** 2 (1.1%). Meanwhile, the positive milk samples of subclinical cases yielded 241 bacterial isolates where **Staphylococci** was the most predominant 81(33.6%), mainly Coagulase positive ***Staph. aureus*** 56 (23.2%) followed by **Streptococci** 74 (30.7%) , mainly ***Strept. agalactiae*** 37 (15.4%) and ***Strept. dysagalactiae*** 27 (11.2%) ; ***E.coli*** 49 (20.3%) ; ***Ps. aerugenosa***24 (10.0%) ; ***A.pyogenes*** 8 (3.3%) and ***kl. pneumonae*** 5 (2.1%) .

The results of Coagulase activity of 142 isolated **Staphylococci** (Table, 3) revealed that, 106 isolates were Coagulase positive ***Staph. aureus;*** 10 Coagulase negative ***Staph.aureus*** and 26 Coagulase negative ***Staph.epidermidis***.

The results of haemolysis and CAMP test for 132 isolated **Streptococci** proved that, 67 ***Strept.agalactiae*** showed large zone of β haemolysis on blood agar and were CAMP positive with large zone of β haemolysis at the junction of tested strains and ***Staph. aureus*** strains .while, 48 **Strept.** ***dysagalactiae*** and 17 ***Strept.uberis*** strains were negative for both haemolysis and CAMP test.Table (4) showed that a total of 80 isolated ***E.coli*** strains were serotyped and **O111** was the most isolated (18.7%) followed by **O86 ; O44 ; O119; O126; O128; O125** and **O55** (11.3% ;10.0%;10.0%;10.0%;8.8%;7.5% and 5.0% respectively). Beside 15 strains (18.7%) were untyped. Also, 36 strains of ***Ps. aerugenosa*** were serotyped and serogroup **K** was the most isolated (25.0%) followed by **H** (19.4%); **M** (13.9%); **A, G** (11.1%for each); **C** (5.6%) and **B** (2.8%). Beside 4 strains (11.1%) were untyped.

The results of pathogenicity test (Table, 5) showed that the most isolated strains were positive for mouse pathogenicity test where injected mice were either dead or showed lower physical activities and injected strains were re-isolated from all mice. Mortality rates vary in between different strains. Coagulase positive ***Staph. aureus***; ***Strept. agalactiae***; ***A. pyogenes*** and ***Ps. aerugenosa*** H and K were the most virulent strains with mortality rate 100% followed by ***E.coli*** **O55** and **O111** (80% mortality rate) while other strains with 20% -60% mortality rates. The results of antibiotic sensitivity tests of 416 isolated strains (Table,6) revealed that enrofloxacin ; lincomycine and gentamycin were the most proper antibiotics with the highest in vitro sensitivity against most bacterial isolates .Mean while ,most isolated strains were resistant to tetracycline ; streptomycin and penicillin .

The results of chemical analysis of milk (Table, 7) appeared that, there was a significant decreased in lactose and casein concentration and non significant increase in fat concentration.

Haematological results (Table, 8) showed a significant increase in total leukocyte counts and a significant decrease in both erythrocyte counts and

haemoglobin concentration in mastitic cows when compared with normal ones. The results of serum biochemical analysis (Table, 9) showed a significant increase in AST; ALT; AP; Urea, and creatinine. Meanwhile, there were a significant decrease in calcium; phosphorous and iron in mastitic cows when compared with normal control ones.

The results of treatment showed that all cows were clinically normal; milk samples were negative for both California mastitis test and bacteriological examination and the biochemical parameters were ameliorated.

Table (1): Results of clinical examination and California mastitis test.

No. of cows	Clinical mastitis		Subclinical mastitis		Healthy cows	
	No.	%	No.	%	No.	%
395	51	12.9	107	27.1	237	60.0

Table (2): Bacteriological examination of both clinical and subclinical mastitic milk of cows.

Animal status	No. of milk samples	Negative samples		Positive samples		Prevalence of single and mixed cultures			
						Single		Mixed	
		No.	%	No.	%	No.	%	No.	%
Clinical mastitis cows	89	13	14.6	76	85.4	24	31.6	52	68.4
Subclinical mastitic cows	198	71	35.9	127	64.1	37	29.1	90	70.9

Table (3): Bacterial species isolated from positive milk samples of both clinical and subclinical mastitic cows.

Bacterial isolates	Clinical mastitis (76)		Subclinical mastitis (127)		Total (203)	
	No.	%*	No.	%*	No.	%**
Total Staphylococci:	61	34.9	81	33.6	142	34.1
Coag. Post.*Staph.aureus.*	50	28.6	56	23.2	106	25.5
Coag. Neg. *Staph.Aureus*	3	1.7	7	2.9	10	2.4
Coag. Neg. *staph.epidermidis*	8	4.6	18	7.5	26	6.2
Total Streptococci:	58	33.1	74	30.7	132	31.7
Strept. agalactiae	30	17.1	37	15.4	67	16.1
Strept. dysagalactiae	21	12.0	27	11.2	48	11.5
Strept. uberis	7	4.0	10	4.1	17	4.1
Actinomyces pyogenes	11	6.3	8	3.3	19	4.6
E. coli	31	17.7	49	20.3	80	19.2
Kelbsiella pneumoniae	2	1.1	5	2.1	7	1.7
Pesudomoneus aerugenosa	12	6.9	24	10.0	36	8.7
Total	175	100.0	241	100.0	416	100.0

* Percentage in relation to total No. of isolates in each case alone.
** Percentage in relation to total No. of isolates in both mastitic milk

Table (4): Serological identification of *E.coli* and *Ps. aeruginosa* recovered from mastitic cows.

E. coli			*Ps. aeruginosa*		
Serogroup	No.	%*	Serogroup	No.	%*
O44	8	10.0	A	4	11.1
O55	4	5.0	B	1	2.8
O86	9	11.3	C	2	5.6
O111	15	18.7	G	4	11.1
O119	8	10.0	H	7	19.4
O125	6	7.5	K	9	25.0
O126	8	10.0	M	5	13.9
O128	7	8.8	Untyped	4	11.1
Untyped	15	18.7			
Total	80	100.0		36	100.0

- Percentage in relation to total No. of each isolate.

Table (5): Pathogenicity of bacteria isolated from mastitic cow's milk.

Bacteria	No. of infected mice	Total mortality		Bacteria	No. of infected mice	Total mortality	
		No.	%			No.	%
Total Staphylococci:				*E.coli* O44	5	2	40.0
Coag. Post.*Staph.aureus.*	5	5	100.0	O55	5	4	80.0
Coag. Neg.*Staph.aureus*	5	1	20.0	O86	5	3	60.0
Coag.Neg.StaphEpidermidis	5	2	40.0	O111	5	4	80.0
Total Streptococci:				O119	5	2	40.0
Strept. agalactiae	5	5	100.0	O125	5	2	40.0
Strept. Dysagalactiae	5	3	60.0	O126	5	1	20.0
Strept. uberis	5	2	40.0	O128	5	2	40.0
Actinomyces pyogenes	5	5	100.0	*Ps. aeruginosa A*	5	1	20.0
Klebsilla pneumonae	5	2	40.0	B	5	2	40.0
				C	5	2	40.0
				G	5	3	60.0
				H	5	5	100.0
				K	5	5	100.0
				M	5	3	60.0

Table (6): In-vitro antibiotic sensitivity test of 416 isolated strains from clinical and subclinical mastitic cow's milk samples.

B	Amox.		Amp.		Chloromph.		Enroflo.		Genta.		Linco.		Penc.		Strept.		Tetra.	
	No	%	No	%	No.	%	No	%	No	%	No	%	No	%	No	%	No	%
Staph.aureus. (106)	85	80.2	81	76.4	65	61.3	90	84.9	87	82.1	91	85.8	58	54.7	44	41.5	32	30.2
Staph. aureus (10)	7	70.0	5	50.0	4	40.0	7	70.0	6	60.0	8	80.0	2	20.0	1	10.0	1	10.0
Staph.epiderm. (26)	14	53.8	12	46.2	9	34.6	15	57.7	13	50.0	15	57.7	8	30.8	4	15.4	3	11.5
Strept. Aglact. (67)	53	79.1	49	73.1	32	47.8	58	86.6	48	71.6	58	86.6	29	43.3	12	17.9	8	11.9
Strept.dysag.. (48)	37	77.1	33	68.8	25	52.1	40	83.3	37	77.1	39	81.3	17	35.4	9	18.8	7	14.6
Strept. uberis (17)	11	64.7	10	58.8	7	41.2	13	76.5	9	52.9	12	70.1	7	41.2	3	17.6	3	17.6
A. pyogenes (19)	15	78.9	13	68.4	10	52.6	15	78.9	16	84.2	16	84.2	8	42.1	5	26.3	3	15.8
E. coli (80)	49	61.3	50	62.5	56	70.0	71	88.8	73	91.3	65	81.3	22	27.5	28	35.0	27	33.8
Kl. .pneumoniae(7)	2	28.6	3	42.9	4	57.1	5	71.4	6	85.7	4	57.1	1	14.3	2	28.6	2	28.6
Ps. aerugenosa(36)	19	52.8	19	52.8	20	55.6	28	77.8	29	80.6	25	69.4	10	27.8	11	30.6	11	30.6

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Table (7): Chemical analysis of milk in mastitic cows (subclinical and clinical) before and after treatment cows.

Groups / Parameters	Control N=9	Subclinical Mastitic cows N=15	Clinical mastitic cows N=11	After treatment N=26	LSD P≤0.05
Fat gl^{-1}	30.46^{ab} ±0.676	31.22^{b} ±0.793	31.10^{b} ±o.810	28.68^{ab} ±0.659	2.539*
Casein gl^{-1}	26.31^{a} ±0.716	23.23^{b} ±0.412	24.22^{c} ±0.321	26.52^{a} ±0.635	3.081*
Lactose gl^{-1}	144.22^{b} ±0.741	123.90^{a} ±1.015	132.70^{c} ±0.980	140.80^{b} ±2.416	16.900*

Values with different litters within the same raw differed significantly at P≤0.05

Table (8): Haematological studies in mastitic cows (subclinical and clinical) before and after treatment cows.

Group / Parameters	Control N=9	Subclinical mastitic cows N=15	Clinical mastitic cows N=11	After treatment N=26	LSD P≤0.05
Total leucocytes counts (x10^{3}/ml)	8.322^{b} ±0.255	10.27^{a} ±0.180	10.89^{a} ±0.172	7.77^{b} ±0.442	1.500 *
Total erythrocytes counts (x10^{3}/ml)	8.088^{b} ±0.221	5.430^{a} ±0.240	6.20^{a} ±0.235	7.86^{b} ±0.334	2.430 *
Hb gm/dl	11.691^{b} ±0.344	8.530^{a} ±0.242	9.23^{a} 0.189	10.923^{b} ±0.394	2.393 *

Values with different litters within the same raw differed significantly at P≤0.05

Table (9): Some biochemical changes of serum of mastitic cows (subclinical and clinical) before and after treatment cows.

Group / *Parameters*	*Normal Control*	*Subclinical Mastitic cows N=15*	*Clinical mastitic cows N=11*	*After treatment N=26*	*LSD P≤0.05*
AST u/ml	52.017 [a] ±5.793	92.710 [b] ±0.670	90.320 [b] ±0.760	59.13 [a] ±3.390	33.58 *
ALT u/ml	16.362 [a] ±0.327	29.400 [b] ±0.573	28.56 [b] ±0.653	16.230 [a] ±0.409	13.037*
AP u/ml	12.150 [a] ±0.414	15.54 [b] ±0.462	16.120 [b] ±0.435	11.88 [a] ±0.476	3.390 *
Urea mg/dl	15.557 [a] ±0.233	20.332 [b] ±0.536	19.673 [b] ±0.464	15.420 [a] ±0.390	4.774 *
Creatinine mg/dl	1.358 [a] ±0.183	2.101 [b] ±0.193	1.998 [b] ±0.187	1.464 [a] ±0.132	0.637 *
Calcium mg/100ml	9.090 [b] ±0.288	7.680 [a] ±0.298	8..218 [a] ±0.253	8.940 [b] ±0.380	1.260 *
Phosphorous mg/100ml	6.487 [b] ±0.244	5.070 [a] ±0.250	5.086 [a] ±0.268	6.550 [b] ±0.285	1.417 *
Iron mg/100ml	1.593 [b] ±0.070	1.096 [a] ±0.069	1.087 [a] ±0.087	1.483 [b] ±0.094	0.387 *

Values with different litters within the same raw differed significantly at P≤0.05

Discussion

Mastitis is one of the most persistent and widely spread disease conditions of importance to milk hygiene and quality among dairy cattle and associated with many zoonotic diseases for human beings **(7, 9 and 58).**

The recorded results of clinical examination and California mastitis test (Table, 1) revealed that 237(60.0%) were normal cows; 51 (12.9%) clinical mastitis and 107 (27.1%) subclinically mastitic ones. These results are closed to that recorded by **(3, 11, 22, 42 and 55).**

The results of bacteriological examination of 89 clinically mastitic milk samples and 198 subclinically ones (Table,2) showed that 76 of clinical mastitic samples (85.4%) were positive with 31.6% containing pure single cultures and 68.4% mixed ones. Meanwhile, for subclinical mastitic samples, 127 (64.1%) were positive 29.1% containing pure single cultures and 70.9% mixed ones. Similar results were obtained by **(1, 11, 16, 28, 55 and 59).** A total of 416 bacterial isolates were recovered from positive mastitic milk samples (Table, 3). **Staphylococci** (34.1%) and **Streptococci** (31.7) were the most predominant pathogens followed by ***E.coli*** (19.2%); ***Ps.aerugenosa*** (8.7%); ***A.pyogenes*** (4.6%) and ***Kl. pneumonae*** (1.7%). Nearly similar results were obtained by **(16, 18, 22, 26, 28 and 59).** For clinical mastitic samples, 175 bacterial species were isolated and **Staphylococci** was the most predominant pathogen (34.9%) mainly Coagulase positive ***Staph.aureus*** (28.6%) followed by **Streptococci** (33.1%) mainly ***Strept.agalactiae*** (17.1%) and ***Strept. dysagalactiae*** (12.0%), then ***E.coli*** (17.7%); ***Ps.aerugenosa*** (6.9%); ***A.pyogenes*** (6.3%) and ***Kl. pneumonae*** (1.1%). Similar results were recorded by **(6, 11, 18, 22, 38, 47, 55 and 59).** Meanwhile, the positive subclinical milk samples yielded 241 bacterial isolates where **Staphylococci** was the most predominant one (33.6%), mainly Coagulase positive ***Staph.aureus*** (23.2%) followed by **Streptococci** (30.7%), mainly ***Strept.agalactiae*** (15.4%) and ***Strept. dysagalactiae*** (11.2%), then ***E.coli ; Ps.aerugenosa; A.pyogenes***

and ***Kl. pneumonae*** (20.3%; 10.0%; 3.3% and 2.1% respectively). Similar results were obtained by **(1, 11, 22, 28 and 38).** As shown in Table (3), 106 out of 142 isolated **Staphylococci** from both clinical and subclinical mastitic milk samples were identified as Coagulase positive ***Staph.aureus***; 10 Coagulase negative ***Staph.aureus*** and 26 Coagulase negative ***Staph.epidermidis***. These results came in harmony with **(30).** The results of haemolysis and CAMP test showed that all isolated ***Strept.agalactiae*** strains (67) were positive while, ***Strept.dysagalactiae*** (48) and 17 ***Strept.uberis*** strains were negative. The same results were obtained by **(13, 25 and 48).** This due to the co haemolysis by the concerted action of β-toxin from the ***Staph.aureus*** strains and CAMP factor (Protein B) from ***Strept.agalactiae*** strains **(25).** The serotyping of 80 isolated ***E.coli*** strains (Table, 4) showed that ***E.coli*** O111 was the most isolated (18.7%) followed by O86; O44; O119; O126; O128 and O55. These results came in accordance to some extent with that of **(4, 19 and 28).** Moreover, serotyping of 36 isolated ***Ps.aerugenosa*** appeared that serogroup K was the most isolated (25.0%) followed by serogroups H; M; A and B. Similar results obtained by **(28).** The results of pathogenicity test (Table, 5) showed that most isolated strains were positive and injected mice were either dead or showed lower physical activities and injected strains were re-isolated from all mice. Coagulase positive ***Staph.aureus ; Strept.agalactiae; A.pyogenes und Ps.aerugenosa*** H and K were the most virulent strains with mortality rate 100% followed by ***E.coli*** O55 and O111(80% mortality rate) while other strains with mortality rates 20%-60%. These results coincided to some extent with those of **(19 and 28).** The results of antibiotic sensitivity tests of 416 bacterial isolates of both clinical and subclinical mastitic milk samples (Table,6) revealed that enrofloxacin, lincomycine and gentamycin were the most proper antibiotics with highest *in-vitro* efficiency against most isolated strains. Nearly similar results were obtained by **(1, 6, 19, 42, 47 and 55).**

The results of chemical analysis of milk (Table, 7) showed an insignificant change in fat concentration and significant decrease in casein and lactose concentrations in diseased (subclinical and clinical mastitis) cows when compared with normal control ones. This result came in

agreement with (9, 26, 35 and 36) .who cited that the significant decrease of casein and lactose of mastitic milk may be due to activation of plasmin system resulting in proteolysis of casein. Also, enzymatic hydrolysis of casein liberates peptides this peptides reduce the out put of lactose and other osmotic components from the alveoli into the gland lumen (54).

Haematological results revealed that a significant increase in total leucocyte counts and significant decrease in erythrocyte counts and haemoglobin concentration in diseased (subclinical and clinical mastitis) when compared with normal control ones (Table, 8).This results were parallel with (51 and 56). The increase of total leucocyte counts were mainly due to increase the number of neutrophile as a results of inflammation (31) .The significant decrease of erythrocyte counts and haemoglobin concentration may be attributed to the decrease of glutathione peroxidase in the erythrocytes of the mastitic cattle may lead to an increase in the reactive oxygen species levels and decreased antioxidant status resulted in denaturation of Hb and disruption of red cell membrane which result in decreased life span of the erythrocytes and a state of anemia (51). The results of some serum biochemical analysis (Table, 9) showed a significant increase in AST, ALT, AP, urea and creatinine. Similar results were recorded by (2 and 51) .The elevation of liver enzymes may be attributed to the inflammatory process and decrease of the immune activity (2). Meanwhile the level of urea and creatinine in mastitis sick cows blood serum were more than healthy ones this may be connect to proteases activation in affected udder and amine nitrogen enter to urea cycle (2). Moreover , there were a significant decrease in calcium , phosphorous and iron in diseased (subclinical and clinical mastitis) cows when compared with normal control ones .This results came in agreement with (9, 15, 26, 37, 41 and 42) . This results were probably linked to the reduced secretory activities of the mammary cells and increased the permeability of the mammary epithelium which can lead to transfer of calcium ,phosphorous and iron from blood to milk so, reduced their levels (26). The decrease in serum trace mineral concentration in response to bacterial infection are thought to act as a nonspecific hot defense mechanism sequestering micro

minerals , which are vital for normal bacterial growth (37). The results of biochemical analysis must be taken in consideration during treatment of mastitic cases.

Regarding to trail of treatment, all cows were clinically normal and milk samples were negative for both California mastitis test and bacterial examination and the biochemical parameters were ameliorated .Same results were recorded by (42).

Regarding to the public health importance of mastitic cow's milk, the pathogens isolated from milk in the current study is associated with many diseases for human beings which milk act as a vehicle of infection. ***Staphylococcus aureus*** strains have the ability to produce one or more enterotoxins in milk and milk products resulting in food poisoning **(7, 18 and 45)**.Also, it is responsible for endocarditis, osteomylities and septic arthritis **(33 and 40).** ***Strept.agalactiae*** remains one of the most prevalent cause of invasive neonatal infections with sever pneumonia ,sepsis and meningitis **(12 and 53)**.Moreover, Gram-negative bacteria in mastitic raw milk when consumed by human result in food poisoning and gasteroentric infection **(18 and 58).**

Finally, it could be concluded that, mastitis is a serious disease of cows with economic and public health importance at Kaliobia Governorate. **Staphylococci**, mainly Coagulase positive ***Staph .aureus;*** **Streptococci,** mainly ***Strept. agalactiae*** and ***Strept dysagalactiae***, and ***E.coli*** are the most common causes of both clinical and subclinical mastitis. Enrofloxacin, Lincomycine and gentamycin are drugs of choice for treatment of mastitis.

Mastitis causes alteration in milk components and haematological and biochemical parameters in mastitic cows. Mastitis and its adverse effects can be controlled by detection of diseased animal and its isolation for treatment; cleaning of teats and udder before milking and dipping the teats into a sutable disinfection after milking; Milkers must be healthy and their hands must be free from any lesions or wounds; excluding milk from diseased animals and the raw milk must be boiled or pasteurized before human consumption.

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دراسات علي إلتهاب الضرع في الأبقار بمحافظة القليوبية والأهمية الصحية لألبانها

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الملخص العربي

تم الفحص الإكلينيكي لعدد ٣٩٥ بقرة حلاب بمحافظة القليوبية وأخذت عينات من ألبانها حيث تم فحصها ظاهريا وبواسطة إختبار كاليفورنيا. أظهرت النتائج أن ١٢,٩% من الأبقار مصابة بالتهاب الضرع السريري و ٢٧,١% بالتهاب الضرع تحت السريري . أوضح الفحص البكتريولوجي ل٨٩ عينة لبن من أبقار مصابة بالتهاب الضرع السريري أن ٨٥,٤% كانت موجبة وتم عزل الميكروبات بصورة منفردة ٣١,٦% أو مختلطة ٦٨,٤% . ويم عزل ١٧٥ عترة وكانت أعلي نسبة للعزل هي المكورات العنقودية (٣٤,٩%) وبخاصة المكور العنقودي الذهبي الموجب للتجلط (٢٨,٦%) والمكورات السبحية (٣٣,١%) وبخاصة المكور السبحى أجالاكتيا (١٧,١%). بينما أظهر الفحص البكتريولوجي ل ١٢٧ عينة لبن من أبقار مصابة بالتهاب الضرع السريري أن ٦٤,١% كانت موجبة و ٢٩,١% منها تحتوي علي الميكروبات منفردة و٧٠,٩% مختلطة .وتم عزل ٢٤١ عترة وكانت المكورات العنقودية (٣٣,٦%) وبخاصة المكور العنقودي الذهبى الموجب للتجلط (٢٣,٢%) والمكورات السبحية (٣٠,٧%) وبخاصة المكور السبحى أجالكتيا (١٥,٤%) والديس أجالكتيا (١١,٢%) وكذلك الميكروب القولونى (٢٠,٣%) هم الأكثر عزلا بينما بقية الميكروبات تم عزلها بنسب بسيطة. أظهرت إختبارت التجمع للمكورات العنقودية المعزولة (١٤٢) أن ١٠٦ منهم كانت المكورات العنقودي الذهبي الموجب للتجلط و١٠ المكور العنقودي الذهبي السالب للتجلط و ٢٦ المكور العنقودي البشري السالب التجلط. أظهر إختبار كامب للمكورات السبحية المعزولة (١٣٢) أن ٦٧ مكور سبحى أجالاكتيا كانت موجبة بينما ٤٨ مكورسبحى ديس أجالكتيا و ١٧ مكور سبحى ابريس كانت سالبة. التصنيف السيرولوجى للميكروب القولونى المعزول (٢٠) أظهر وجود ٨ مجموعات سيرولوجية والمجموعة O111 الأعلي تواجدا (١٨,٧%). والتصنيف السيرولوجي لميكروبات سيدوموناس ايروجينوزا المعزولة (٣٦) أظهر وجود ٧ مجموعات سيرولوجية والمجموعة k هي الأعلى (٢٥,٠%). أظهرت اختبارت الضراوة في الفئران أن جميع العترات المعزولة كانت مميتة او ممرضة للفئران. أظهر التحليل الكيميائي لعينات اللبن وجود نقص معنوي في نسب اللاكتوز والكازين .كما أظهرت نتائج الفحص البيوكيميائي وصورة الدم وجود ارتفاع معنوي في عدد كرات الدم البيضاء وإنزيمات الكبد واليوريا والكرياتينين. بينما لوحظ انخفاض معنوي لعدد كرات الدم الحمراء ونسبة الهيموجلوبين والكالسيوم والفسفوروالحديد في الحيوانات المريضة بالمقارنة مع الحيوانات السليمة. وقد تم العلاج بحقن الاتروفلوكساسين عضليا مع اللينكومايسين موضوعيا (الأكثر كفاءة) نتج عنة شفاء تام للأبقار المصابة بالتهاب الضرع . وأخيرا تم مناقشة طرق السيطرة علي التهابات الضرع في الأبقار والأهمية الصحية للميكروبات المعزولة من ألبان هذه الأبقار بالنسبة للإنسان.

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