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BACTERIAL AND BIOCHEMICAL STUDIES ON MASTITIS OF CATTLE IN SHARKIA GOVERNORATE

(With 5 Tables)

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دراسات بكتيرية وبيوكيميائية على التهاب الضرع للأبقار بمحافظة الشرقية

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شـــمل البحث عدد ٩٢ عينة من أبقار حلابة مصابة بالتهاب الضرع الاكلينيكى بأربعة مناطق مختلفة بمحافظة الشرقية، هذا وقد أتضح بعد الفحص اكتشاف المسببات البكتيرية وتحديد نسبة حدوثهـــا حيـــث تم عزل بكتيريا الميكروب القولوني بنسبة (٥١,١%) والميكروب العنقودي الذهبـــي (٢١,٧%) والميكروب السبحي (١٨,٥%) والكليبسيلا(٧,٦%) بالإضافة إلى وجود عـــدوى مشتركة لأكثر من نوع. كما تم إجراء اختبار حساسية للميكروبات المعزولة لعدد من المضـــادات الحـــيوية أتضـــح من خلالها أن الجنتاميسين، السيفالوسبورين، أنروفلوكساسين والأموكسيســـللين هم أكثر المضادات الحيوية تأثيرا علي الميكروبات المعزولة. وقد تم علاج الأبقـــار المصابة باستخدام هذة المضادات الحيوية حيث أظهرت هذه الأدوية كفاءة عالية في عـــلاج هـــذه الحـــالات. شمل البحث أيضا دراسة بعض التغيرات الخلوية والبيوكيميائية لدم الأبقـــار المصـــابة بالتهاب الضرع وإمكانية استخدامها للمساعدة في تشخيص التهاب الضرع الاكلينيكى للأبقار بين السلب والإيجاب.

SUMMARY

This research was carried on 92 samples from clinically mastitic cows collected from 4 different places (farms) at Sharkia governorate and tested for detection and determination of the incidence of pathogenic bacteria among cows. The prevalence of the major contagious pathogenic bacteria of mastitis were E. coli, Staphylococcus aureus, Streptococcus agalactia and Klebsiella pneumonae in a ratio of 51.1%, 21.7%,18.5%, 7.6%, 1.1% respectively. Mixed infection was observed in 21.37%, while bacteriologicaly negative samples were absent. Sensitivity test against isolated strains in vitro appeared that they were

susceptible to cephalosporin, gentamycin, enrofloxacin, and amoxicillin. This retrospective study was prompted by findings of haematological and biochemical examinations, and was adopted for detection and diagnosis of mastitic cows.

Key words: *Mastitis, cows, milk, biochemical parameters, antibacterial drugs*

INTRODUCTION

Mastitis as a widely health problem does not only causes economic disease-related losses in dairy herd farm, but it is also responsible for extended usage of antibiotics in these enterprises, Kromker and Grabowski, (2002). This disease considered as multifactorial where development of infection depends on presence of mastitic pathogens and a series of additional factors that act concomitantly (Smith, 2002).

This study was conducted to determine the prevalence of the major pathogenic bacteria of mastitis. The isolated strain were tested in vitro and in-vivo for antibiotic sensitivity tests and estimated of some hematological and blood biochemical constituents.

MATERIALS and METHODS

237 cows from four farms (3 cooperative farms and one familial farm), about 4-6 year of age at Sharkia governorate were monitored and sampled for clinical mastitis for one and a half year.

Procedures for collection and diagnosis of milk samples were performed as described by Brown *et al.* (1981). A total of 92 collected milk samples were positive for clinical mastitis based on any observable signs in the udder and/or milk and delivered for bacteriological examination.

Bacteriological examination:

This was done according to Brown *et al.* (1981)

In vitro-sensitivity test.

This was carried according to Bauer *et al.* (1966), where the isolated strains were tested against cephalosporin, gentamycin, enrofloxacin, ampicillin, penicillin G, oxytetracycline, amoxicillin and neomycin using the disk agar diffusion method in accordance with the instruction of the antibiotic disk supplier (Oxoid company).

Drugs and treatment:

Four groups of diseased cows were treated with Gentamycin (10%), Cephalosporin (1g.diluted in 20 cc dil.), Enrofloxacin (10%) and

Amoxicillin (15%) pharmaceutical preparations. Every cow received (4ml/100kg), (1ml/50kg), (1ml/40kg.), (1ml/15kg.) b.wt / 24 hr. by the intra-muscular route from the respective drug for 4 consecutive days.

Milk yield per day:

Daily milk yield from mastitic cattle were recorded before and after 1, 2 and 3 weeks of treatment

Haematological examination:

Haemoglobin content (HB), total and differential leucocytic counts were examined according to Benjamine (1978), and total erythrocytic count, according to Schalm (1979).

Biochemical examination:

AST, ALT, urea and creatinnine were determined calourimetrically using reagent kits purchased from Bio Merieux Chemicals (France).

Statistical:

The obtained data were statistically analysis using student's "t" test by Snedecor and Cochran (1989).

RESULTS

Clinically infected quarters often show moderate swelling, firmness, visible signs of chunks of milk, clots in milk and some cases milk become viscous.

Rate of clinical mastitis in dairy herds based on quarter levels.are shown in Table (1):

The most severe effect of clinical mastitis was found in our work, when it occurred (a) during the first 60 days of lactation; (b) after parturition during summer and (c) when recurrences appeared more than twice during the same lactation.

Table 1: Rate of clinical mastitis in dairy herds based on quarter levels.

variables	Number	%
rear quarters	151	63.7%
Front quarters	86	36.3%
Cows have one quarter affection	145	61.2%
Cows have two quarters affection	69	29.1%
Cows has three quarters affection	15	6.3%
Cows has four quarters affection	8	3.3%
Quarters with abnormal palpation	216	91.1%
Quarters with acute catarrhal mastitis	161	67.9%
Quarters with abnormal secretion	196	82.7%
Hyperkeratosis of teat	22	9.3%

Bacteriological finding:

All the mastitic milk samples were positive for isolation and identification of several bacterial microorganisms, (bacterial mastitis). The main bacteria distributed in Table (2).

Table 2: Distribution of bacteriological pathogens of clinical mastitis

Pathogenic Bacteria	Farm1 NS=22 NC=50	Farm2 NS=15 NC=44	Farm3 NS=23 NC=74	Farm4 NS=32 NC=69	Total NS=92 NC=237	%of observation
E.coli	12	11	9	15	47/92	51.1%
Stap. Aureus	5	-	6	9	19/92	20.7%
Strep.Agalactiae	2	2	8	5	17/92	18.5%
Kleb.pneumonae	3	1	0	3	6/92	6.5%
Others	0	1	0	0	3/92	3.3%
Mixed infection	7	7	2	3	21/92	22.2%

NS= number of samples, NC= number of cows.

Sensitivity tests on isolated microorganisms:

The result showed that most effective tested drugs act on the isolated microorganisms were gentamycin, enrofloxacin, cephalosporin, amoxicillin, streptomycin, and tetracycline, in a percentage of 71.32%, 66.23 % , 56.78 %, 34.54%, 19.89%, 10 9% respectively.

The Efficacy of antibiotic in vivo:

This study cleared that treatment with gentamycin as the best drug than cephalosporin and enrofloxacin where the total cure rate of gentamycin was 74.2%, while that of cephalosporin and enrofloxacin were 69.7 % and 67.1% respectively.

Milk yield per day (kg / day):

Table (3) showed that Milk yield per day was significantly decreased in clinical mastitic dairy cows up to 21 days.Post treatment (PT) by gentamycin, cephalosporin and enrofloxacin for 4 consecutive days milk yield were improved, yet it was still significantly less than the control till 14 days for all the examined 4 farms and at 21days PT milk yield decreased than control, but with insignificant statistical values

Table 3: Milk yield (kg/day) of dairy cattle suffering from mastitis:

Farm No.	Pre-treatment	7days post treatment	14days post treatment	21days post treatment
control	26.4 ±0.43	27.76 ±0.60	26.56 ±0.02	27.87 ±0.57
Farm 1	17.6 ±0.47***	18.45±0.85***	24.43±0.58*	26.43 ±0.87
Farm 2	16.8 ±0.24***	20.750±0.45***	23.93±0.41**	26.51 ±0.74
Farm 3	16.23±0.37***	21.50±0.45 **	24.54±0.24*	26.54±0.90
Farm 4	18.34±0.43***	19.54±0.56***	25.29±0.03	27.01±0.54

***P < 0.0001, **P < 0.001, *P < 0.01

Haematological and biochemical findings

Clinical mastitis caused changes in some hematological and biochemical parameters as shown in Tables (4 and 5).

Table 4: Mean values ±SD of the haemogram in cows with mastitis.

	HB G/dl	RBCS $\times 10^6$/UL	WBCS $\times 10^3$/UL	Absolute differential leucocytic counts$\times 10^3$/UL				
				neutrophils	lymphocytes	monocyte	eosinophils	basophils
Control	11.3±0.3	8.9±0.6	9.2±0.6***	2.8±0.1***	5.7±0.3	0.39±0.07	1.08±0.002	0.01±0.001
Farm (1)	10.6±0.9*	8.1±0.6	5.1±0.1***	0.7±0.2***	4.1±0.1*	0.31±0.01*	1.07±0.001	0.02±0.001*
Farm (2)	9.9±\0.6*	6.9±0.4**	5.5±0.3***	0.9±0.1***	4.3±0.4*	0.29±0.05*	0.08±0.003*	0.01±0.001
Farm (3)	9.1±0.7**	7.2±0.4*	4.9±0.5***	0.5±0.3***	3.9 ±0.3**	0.41±0.02	1.09±0.002	0.00±0.001*
Farm (4)	8.0±0.6**	7.3±0.3*	3.9±0.6***	0.6±0.1***	3.1±0.2***	0.34±0.01	1.05±0.001	0.01±0.001

Table 5: Mean of biochemical values in cows under study.

	AST iu/L	ALT iu/L	Urea mg%	Creatinine mg%
control	89.3±7.2	21.1±0.4	12.3±1.7	1.6±0.1
Farm(1)	71.9±9.2*	29.3±0.1*	8.1±0.68**	1.3± 0.2*
Farm(2)	92.4±6.4	18.5±02*	11.5±1.1	1.8±0.3
Farm(3)	87.3±3.2	22.4±0.5	13.6±0.7	1.9±0.1*
Farm(4)	82.4±7.8*	19.5±0.6	10.7±1.0*	1.5±0.2

DISCUSSION

Table (1) showed that mastitis was found more often in rear udder quarters than in front udder quarters (63.7%% versus 36.3%). In 145 (61.2%) animals, only one udder quarter was affected. Insignificant differences between the rate of mastitis in right and left quarters were observed. Dislike to that reported by Kikkers *et al.* (2004) could be also due to random distribution.

Most of clinical mastitis in cattle of our study originated from environmental bacteria; Escherichia coli were the most dominant bacteria following by Staph. aureus, Strept. agalactia and Klebsiella pneumonae, others and mixed infection was observed in a ratio of 51.1%, 20.7%, 18.5%, 6.5%, 3.3% and 22.2% respectively,while bacteriologically negative samples were absent (Table 1), agreed with that recorded by Gregory and Hoedemaker (2002).

Because Escherichia coli was the most frequently isolated pathogen from cases of clinical mastitis, it was expected that risk factors for overall rate of mastitis were mainly associated with rate of clinical mastitis caused by this pathogen (Table 2). Most of the pathogen-specific risk factors for mastitis associated with the other three pathogens did not contribute enough to overall rate of clinical mastitis to be included in that model. agreed with Barkema *et al.*, (1998).

This study revealed that treatment with gentamycin as the best drug than cephalosporin and enrofloxacin, where the total cure rate of gentamycin was 74.2%, while that of cephalosporin and enrofloxacin were 69.7%, 67.1% respectively, our results are similar to that reported by Ismail and Hatem, (1998) where the authors mentioned that gentamycin showed superior effect on the isolated bacteria causing mastitis in dairy cows. Another interesting feature in our study is the decrease in susceptibility to neomycin and oxytetracycline. While penicillin G was ineffective against Staph. aureus strains. It becomes difficult to treat successfully such infection because drugs are not able to penetrate to all infection sites and because the bacteria live inside the white blood cells. This is because Staph. aureus produces an enzyme that inactivates most penicillin-based treatments, resulting in ineffective antibiotics,. Nearly similar finding were reported by Erskine *et al.*, (2002).

Milk yield (kg / day) was significantly decreased in clinical mastitic dairy cattle in our study; the extent of the changes depends on the casual organism and the severity of the infection. This findings

agreed with that reported by Friedmn *et al.* (2004) where two weeks post treatment with injectable antibiotic the milk quality were improved Table (3).

Haematological picture associated with clinical mastitis illustrated in Table (4) showed that a marked leukopenia, with counts as low as3900-5500- /ul is present when clinical signs appear and persists up to 14 days.This agreed with Radostits *et al.* (1994), where the authors reported that in peracute form of the disease the total and differential leucocytic counts are characteristic and useful diagnostic aids, where there are a marked leukopenia, neutropenia and degenerative shift to left. This is due to the migration of large numbers of neutrophils into the affected udder. The extent of changes in blood parameters varied with the severity of mastitic incidence. These agreed with that reported by Bertoni *et al.* (1994). Our studies showed that if the leukopenia, neutropenia and degenerative shift to left became worse on the second day after the onset of clinical signs, the prognosis is unfavourable. These agreed with Radostits *et al.* (1994).

Our results illustrated the variation in values of AST, ALT, urea and creatinine in mastitic cows when compared with control ones. Table (5) showed that parameters are altered in mastitic cows than in normal one. But there was no correlation between serum concentration of these parameters and clinical mastitis. This may be attributed to the extent of changes in blood parameters which varied with the severity of mastitic incidence. These agreed with that reported by Bertoni *et al.* (1994).

In conclusion, high prevalence of environmental bacterial clinical mastitis was mainly caused by E. Coli Staph .aureus and Strep. agalactiae. Furthermore the obtained haematological and biochemical changes in affected mastitic cows may not be enough to be a routine diagnostic aid, except marked leukopenia with shift to left which were characteristic and useful diagnostic aid in early diagnostic stage of clinical mastitis

Finally this study showed that, in order to minimize the damage from clinical mastitis it is necessary to focus on preventive actions at the farm-medical level rather than to resort to syringe and antibiotics.

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