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SOME BACTERIOLOGICAL, HEMATOLOGICAL AND BIOCHEMICAL CHANGES ON CLINICAL MASTITIS IN GOAT

(With 8 Tables and One Figure)

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بعض الدراسات البكتيريولوجية والدموية والبيوكيميائية علي حالات التهاب الضرع في الماعز

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شمل البحث الفحص الإكلينيكي لعدد 120 ماعز مرباة في المزارع الخاصة بمحافظة الدقهلية وكان 70% منها مصابة بالتهاب الضرع. وقد أظهر الفحص الميكروسكوبي لـ 168 عينة لبن مصاب بالتهاب الضرع أن 73.8% كانت موجبة للفحص وتم عزل الميكروبات بصورة منفردة (77.4%) أو مختلطة (22.6%). بالفحص البكتريولوجي للعينات الموجبة طبقا للتصنيف المورفولوجي والبيوكيميائي للعترات المعزولة. هذا وقد أوضحت النتائج أن هذه الميكروبات هي العنقودي الذهبي والمكور العنقودي من نوع إبيدرميدس وإيشيرشياكولاي والمكور السبحي المعوي من نوع أجالاكتيا وديس أجالاكتيا ويوبريس بنسبة (35.4%، 27.1%، 16.1%، 10.4%، 8.3% ، 2.1%) علي التوالي. بإجراء اختبار العدوى الصناعية في الفئران تبين أن الميكروب العنقودي الذهبي والميكروب القولونى مميت في الفئران، وبإجراء اختبار الحساسية للمعزولات تبين أنها كانت حساسة لكل من الإنروفلوكساسين، سيفتوفيور، سبيروفلوكساسين، جنتاميسن ولينكومايسين. أسفرت الدراسات البيوكيميائية لعينات مصل الدم في الماعز المصابة بالتهاب الضرع عن زيادة معنوية في مستوي البروتينات الكلية وكذلك في مستوي كل من ألفا- 2، جاما جلوبيولين ولكن في نفس الوقت حدث انخفاض معنوي ملحوظ بمستوي كل من الألبيومين ونسبه الألبيومين/ جلوبيولين. وأوضحت الدراسة الخاصة بالأملاح المعدنية والعناصر النادرة عن نقص معنوي بمستوي كلا من الكالسيوم، الفسفور، الماغنسيوم، البوتاسيوم والزنك والحديد، بجانب زيادة معنوية بمستوي كلا من الصوديوم والنحاس وبالنسبة لبعض الدراسات البيوكيميائية والمتمثلة في بعض وظائف الكبد والكلية فقط أوضحت النتائج عن زيادة معنوية في مستوي إنزيمات الكبد ومعدل كلا من الكرياتينين والبولينا بالدم. وبالنسبة للقياسات الدموية أوضحت النتائج عن حدوث انخفاض معنوي في نسبه الهيموجلوبين وعدد كريات الدم الحمراء وأيضا العد التصنيفى لكلا

من خلايا الليمفوسايت والنيتروفيل بالدم وفي نفس الوقت حدثت زيادة معنوية بمستوي خلايا المونوسايت، الأزينوفيل والبيزوفيل.

SUMMARY

This study included clinical examination of 120 goats bred in different localities of Dakahlia Governorate where 70% were clinically mastitic. The results of microscopical examination of 168 mastitic milk samples revealed that 73.8% were positive, 77.4% out of them gave pure single cultures and 22.6% yielded mixed ones. The bacteriological examination of positive samples yielded 124 isolates where *Staphylococcus aureus*, *Staph. epidermidis*, *E. coli*, *Streptococcus agalactiae*, *Strept. dysglactiae* and *Strept. uberis* were the main organisms. These strains were isolated at varying percentages 35.4%, 27.1%, 16.1%, 10.4%, 8.3% and 2.1% respectively. Pathogenicity test in mice revealed that *Staph. aureus* and *E. coli* were highly virulent strains. The results of the invitro antimicrobial sensitivity test showed that Enrofloxacin, Ceftiofur, Ciprofloxacin, Gentamycin and Lincomycin were the most effective antibiotics for successful treatment of mastitic goats. Biochemical studies of blood serum samples of mastitic does were discussed in details and revealed on increase in the levels of total protein, Alpha 2, gamma globulin, but the level of albumin and A/G ratio were decreased significantly in their values. Meanwhile studies of minerals profiles revealed that, the mean values of Ca, IP, Mg, K, Zn and Fe were decreased significantly, at the same time the level of Na and Cu values were increased significantly. Some biochemical liver and kidney functions in the present studies were discussed and revealed a significant increased in the liver enzymes AST, ALT and also in the level of blood urea nitrogen and serum creatinine. Hematological parameters of blood samples of mastitis does were also discussed in details and the studies revealed that, the mean values of Hb, TRBCs, TWBCs were decreased significantly. At the same time, the differential leucocytic count profiles revealed that the percentage of lymphocytes, neutropbils were decreased significantly, while the percentage of monocytes, eosinophils and basophiles were significantly increased.

Key words: *Goats, mastitis, udder, milk, blood, antibiogram.*

INTRODUCTION

Mastitis is still the main problem of dairy farms especially when hygienic measures and milking sanitation are often insufficient and

affecting the quality and quantity of milk, with growth retardation of kids and pre-weaning mortalities (Kennedy and Miller, 1993).

Caprine mastitis is considered to be the result of infection of the mammary glands by specific microorganisms and also malsanitary conditions of the environment (Costa *et al.,* 1998). Clinical caprine mastitis is a serious inflammatory disease manifested by swelling and pain of the udder and very frequently with serious general symptoms (Masisi and Rupinen, 1991). Bacteriological examination of mastitic dairy goats was done by many authors as Topolko and Benic (1997) and Contreras *et al.* (1999). The most important isolated bacteria are staphylococci, streptococci and *E. coli* (Raid, 2004; Erson *et al.,* 2005).

Mastitis can be detected by different methods, but an accurate detection could be done mainly by microbiological examination (Brown *et al.,* 1990; Hernandez *et al.,* 1991).

This study was planned to investigate the prevalence of clinical mastitis in goats in addition isolation and biochemical identification of the causative pathogens were considered and antibiogram for the isolated bacterial pathogen was done. Also the study was prompted by findings of hematological and blood serum biochemical changes for detection and diagnosis of mastitic goats.

MATERIALS and METHODS

Milk Samples:

A total of 120 she goat collected from a private farm in Dakahlia Governorate and 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 with strict aseptic precautions and after discarding the fore milk. Two milk samples from each affected quarter were collected in sterile McCartney bottles and transported to laboratory in ice container for bacteriological examination.

Blood samples:

Two blood samples were collected from each animals. The first sample was collected from the jugular vein in dry sterilized centrifuge tubes without anticoagulant from goats under study, and allowed to clot at room temperature, then centrifuged to separate serum. The collected sera were kept at -20°C till used for estimation of biochemical parameters. The second blood sample was collected in heparinized tubes for haematological picture.

Bacteriological examination:

Isolation of bacteria was carried out according to Quinn *et al.* (1994). Milk samples were incubated aerobically at 37°C for 24hr., and then centrifuged at 3000 r.p.m. for 20 minutes. The cream and supernatant fluid were discarded. A loopfull from the sediment was streaked onto the surface of Nutrient agar, MacConkey's agar, Blood agar, EMB and Mannitol salt agar. The inoculated plates were incubated at 37°C for 24-48 hr. and examined for bacterial growth. The developed colonies were picked up and subcultured for purification. The pure colonies were morphologically identified by Gram-stain and biochemical test (Carter and Cole 1990; Quinn *et al.*, 1994).

Further studies on pure isolates of staphylococci were done. Coagulase activity to different types of plasma (Rabbit, sheep, horse and human) using slide coagulase test were done and confirmed by tube coagulase test.

Pathogenicity:

Pathogenicity of the recovered bacterial isolates in mice was carried out according to Koneman *et al.* (1996) and Qunin *et al.* (2002). 30 white mice were used to investigate the pathogenicity of isolated strains. All mice were examined bacteriological to ensure their freedom from pathogens and were divided into 6 groups, each contained 5 mice injected I/P with 0.5ml. of 5×10^8 C.F.U/ ml. The injected mice were kept under observation for 7 days and the results of mortality and resolution were recorded according to Calvinho and Dodd (1994).

Antibiogram activity:

Antibiogram of the recovered bacterial isolates: this was done by using disc diffusion standard technique according to Finegold and Martin (1982) and Qunin *et al.* (2002) using the discs according to Oxoid (1996) of Enrofloxacin, Ciprofloxacin, Ceftiofur, Gentamycin, Lincomycin, Amoxicillin, Trimethoprim–sulfamethoxazole, Oxytetracycline, Streptomycin and Erythromycin.

Biochemical analysis:

1- Blood serum total proteins were determined according to Sonnenwirth and Jaret, (1980)

2- Albumin was measured spectrophotometrically as described by Drupt (1974). Globulins were calculated mathermatically by substracting the value of albumin from total proteins.

3- Electrophoretic analysis: Electrophoresis of serum samples were determined according to Lewis (1976).

4- Blood serum samples were used also for determination of transaminase enzymes AST, ALT, blood urea, serum creatinine, calcium, phosphorus and magnesium were determined

spectrophotometrically using standardized test- kits supplied from Bio-Merieux (Baines, France) according to the methods mentioned by McMurray *et al.* (1984).

Blood serum sodium and potassium were determined using flam photometer (Concerning model AVL 988-3, made in USA) according to method described by Hawk (1965).

Blood serum cupper, iron and zinc were determined using atomic absorption spectrophotometer according to Meret and Henkin, (1971).

The second blood samples were collected in heratinized tube for determination of erythrocytes, total and differential leucocytic counts, haemoglobin concentration according to the standared methods of haematology previously described by Coles (1986).

Statistical analysis:

The obtained data was simultaneously analyzed statistically using Student test according to Petrie and Wastson (1999).

RESULTS

Table 1: Prevalence of bacteria causing clinical mastitis among goats.

Number of examined goats	Number of mastitic goats	%*	Number of quarters of positive goats	Number of positive quarters	%**
120	84	70	168	124	73.8

*Percent was calculated according to total number of examined goats.
**Percent was calculated according to number of quarters of positive goats.

Table 2: Prevalence of isolated single and mixed strains in examined milk samples from mastitic goats

Single isolated bacteria (96)			Mixed isolated bacteria (28)		
Bacterial strains	No.	%	Bacterial strains	No.	%
S. aureus	34	35.4	*E. coli + S. aureus*	10	35.7
S. epidermidis	26	27.1	*E. coli + S. epidermidis*	5	17.9
E. coli	16	16.7	*E. coli + St. agalactiae*	7	25.0
St. agalactiae	10	10.4	*E. coli + St.*	4	14.3
St. dysgalactiae	8	8.3	*dysgalactiae*		7.1
St. uberis	2	2.1	*E. coli + St. uberis*	2	
Total	96	77.4		28	22.6

* Percent was calculated according to total number of isolated bacteria of each.

Table 3: Pathogenicity of bacteria isolated from clinical mastitic goats

Bacteria isolates	No of dead mice/ day							Total	
	1	2	3	4	5	6	7	No	%
S. aureus	0	1	1	1	0	1	1	4/5	80
E. coli	0	0	1	1	0	1	0	3/5	60
S. epidermidis	0	0	1	1	0	0	0	2/5	40
St. agalactiae	0	0	0	1	1	0	0	2/5	40
St. dysaglactiae	0	0	0	1	0	1	0	2/5	40
St. uberis	0	0	0	1	0	0	0	1/5	20

* The percent was calculated according to the total number of mice in each group.

Table 4: Antibiogram of the most isolated microorganism recovered from clinical mastitic goats

Organisms	S. aureus (No = 34)		S. epidermidis (No = 26)		E. coli (No= 16)		St. agalactiae (No = 10)		St. dysgalactiae (No = 8)		St. uberis (No= 2)	
Antibacterial agent and its potency	Sensitive isolates	%	Sensitive isolates	%	Sensitive isolates	%	Sensitive isolates	%	Sensitive isolates	%	Sensitive isolates	%
Enrofloxacin 5µg	28	82.4	20	76.9	14	87.5	8	80	7	87.5	2	100
Ciprofloxacin 20µg	30	88.2	22	84.6	11	68.8	6	60	5	62.5	1	50
Ceftiofur 30µg	31	91.2	21	80.8	13	81.3	7	70	5	62.5	2	100
Gentamycin 10µg	25	73.5	19	73.1	12	75.0	6	60	6	75	1	50
Amoxycillin 25µg	14	41.2	11	42.3	8	50.0	4	40	0	0.0	0	0.0
Lincomycin 15µg	24	70.6	18	69.2	13	81.3	7	70	6	75	1	50
Trimethoprim-sulpamethoxazol 25 µg + 23.75µg	0	0.0	0	0.0	10	62.5	5	50	4	50	0	0.0
Oxytetracycline 30µg	18	52.9	16	61.5	8	50.0	5	50	5	62.5	1	50
Streptomycin 10µg	16	47.1	14	53.8	11	68.8	4	40	4	50	0	0.0
Erythromycin 10µg	12	35.3	12	46.2	8	50.0	5	50	3	37.5	1	50

No = Number of isolates.

Table 5: Mean values of total serum protein and electrophoretic pattern (gm/dl) of control healthy and mastitic goats

Parameter \ Unit	Control group	Mastitic group
Total protein (gm/ dl)	6.15 ± 0.51	8.92 ± 0.60**
Albumin (gm/dl)	2.04 ± 0.13	1.55 ± 0.14*
α1- globulin (gm/dl)	1.35 ± 0.09	1.48 ± 0.11
α2-globulin (gm/dl)	0.52 ± 0.09	1.34 ± 0.12**
β-globulin (gm/dl)	1.20 ± 0.12	1.29 ±0.18
γ-globulin (gm/dl)	1.04 ± 0.09	3.26 ± 0.25**
Total globulin (gm/dl)	4.11 ± 0.29	7.37 ±0.47**
A/G ratio	0.50 ± 0.04	0.21 ± 0.025**

*Signifiant at p < 0.05
**Signifiant at p < 0.01

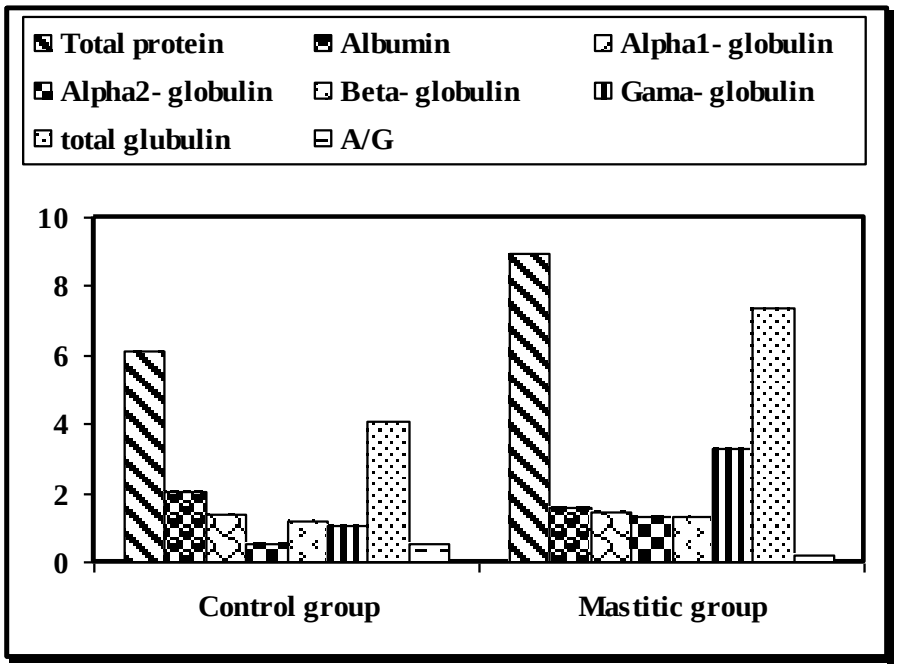

Fig. 1: Mean values of total serum protein and electrophoretic pattern (gm/dl) of control healthy and mastitic goats

Table 6: Mean values of minerals in serum of control healthy and mastitic goats

Parameters	Unit	Control group	Mastitic group
Ca	(mg%)	11.6 ± 0.28	8.5 ±0.30*
IP	(mg%)	5.8 ± 0.18	5.1±0.20*
Mg	(mg%)	2.6 ± 0.16	1.46 ± 0.30*
Na	(mEq/L)	129 ± 2.2	140± 3.0*
K	(mEq/L)	5.4 ± 0.93	3.8 ± 0.020*
Cu	(ug%)	60.4 ± 1.6	81.2 ± 2.4**
Zn	(ug%)	75.9 ±1.65	56.2±3.1**
Fe	(ug%)	156.7±4.1	130.4 ± 3.3**

*Significant at $p < 0.05$
**Significant at $p < 0.01$

Table 7: Mean values of some liver and kidney functions in control healthy and mastitic goats

Parameters	Unit	Control group	Mastitic group
AST	(iu/L)	40± 1.3	80.0± 3.1***
ALT	(iu/L)	38± 1.5	70.0 ± 2.9***
Urea	(mg%)	28±0.9	44.4 ± 1.8**
Creatinine	(mg%)	0.42± 0.03	1.3 ± 0.08**

**Significant at p. < 0.01
*** Significant at $p < 0.001$

Table 8: Mean values of the haemogram of control healthy and mastitic goats

Parameters	Unit	Control group	Mastitic group
Hb	(gm/dl)	9.9± 0.8	8.0 ± 0.6*
RBC_S	(T/ L)	8.1 ± 0.6	6.9 ± 0.4*
WBC_S	(G/ L)	9.2 ± 0.6	5.1 ± 0.1**
D.L.C %			
Lymphocytes	(%)	57.0± 2.07	49.4 ± 1.79*
Lymphocytes	(%)	35.6 ± 1.09	29.7 ± 0.91*
Monocytes	(%)	4.6 ± 0.39	10.8 ±0.90*
Eosinophils	(%)	2.5 ± 0.15	7.9 ± 046*
Basophils	(%)	0.30 ±0.01	2.2 ± 0.24**

*Significant at $p < 0.05$
** Significant at $p < 0.01$
T/L Terra /Liter
G /L Giga /Liter

DISCUSSION

Mastitis in goat is a problem that requires excessive studies. This necessitates recognizing the causative agent which is a complex object since many microorganisms were incriminated in the occurance of mastitis in goats which could act alone or may have synergistic effect (El-Shabiny *et al.*, 1996).

The date presented in Table 1 showed that the prevalence of bacteria causing mastitis in goats was 73.8%, from the total examined quarter milk samples (168), in this concern, El-Khodery and Hoedemaker (2005) recorded that the prevalence of bacteria causing mastitis was 69.7%.Meanwhile Assefa *et al.* (2006) recorded that the prevalence of bacteria yielded from lactating goats was 89.9%. Bacterial causes of mastitis in goats were also studied by some authors such as Topolko and Benic, (1997) and Machado *et al.* (1999). The results presented in Table 2 showed the prevalence of bacteria isolated from clinical mastitis in goats, where *S. aureus, S. epidermidis, E. coli, St. agalactiae, St. dysgalactiae and St. uberis* were isolated with different incidences, it was noticed that the highest incidences were for *S. aureus* (35.4%), then *S. epidermidis* (27.1%) and *E. coli* (16.7%). Many investigators studied the most prevalence of bacteria isolated from mastitis in goats as Ajuwape *et al.* (2005) who isolated *Staphylococcus epidermidis* (50.9%) and *E. coli* (15.1%) from mastitic goats. In addition, Moawad and Osman (2005) recovered *S. epidemidis* (50%) and *S. aureus* (30.65%) from dairy ewes. They also isolated *St. agalactiae* in (9.7%) and *E. coli* (4.84%), otherwise, Mohy and Abdel Fattah (2008) isolated *S. aureus* (40%) from 50 random goat milk samples. Meanwhile, Laila *et al.* (2000) isolated *S. aureus, St. agalactiae, St. dysgalactiae, St. uberis* and *E. coli* with a percentage 25%, 16.6 %, 16.6%, 8.3% and 8.3% respectively from mastitic goats.

The results presented in Table 2 showed the combination between bacterial isolates that induced mastitis in goats. This combination occurred mainly between *E. coli + S. aureus* (10 samples) followed by *E. coli + St. agalactiae* (7 samples), *E. coli S. epidermidis* (5 samples), *E. coli + St. dysgalactiae* (4 samples) and *St. uberis* (2 samples). In this concern, Hanaa *et al.* (2005) reported that 18.7% of milk samples had mixed infection with *S. aureus* and *St. agalaciae.*

It was noticed from Table 3 that the virulence of some bacteria isolated from mastitis in goats, as demonstrated by the sequence of mortality in mice. *S. aureus* and *E. coli* were virulent strains followed by *S. epidermidis, St. agalaciae, St. dysgalactiae* and *St. uberis*. These may

by attributed to pathogenic nature of the strain or the presence of virulence associated plasmid or production of endo. or exotoxins. These results coincided to some extent with those of Magda, (2007) who reported that *S. aureus* and *St. agalactiae* were virulent strains followed by *E. coli*, *S. epidermidis*, *St. dysgalactiae*.

Table 4 showed the antibiogram of 10 chemotherapeutic agents on 5 isolates from milk of mastitic goats. Most isolates were sensitive to enrofloxacin, ciprofloxacin, ceftiofur, gentamycin and lincomycin. Mohan *et al.* (2004) cited that *S. aureus*, *E. coli* and *Streptococcus* spp. were sensitive to Enrofloxacin, Gentamycin, Ciprofloxacin and Chlormphenical. In addition, Sharma *et al.* (2005) recorded that *Staphylococcus* spp., *Streptococcus* spp. and *Enterobacteriae spp.* were highly sensitive to Ciprofloxacin, Gentamycin, Chlormphenical and Tetracycline.

Concerning total serum protein and its fractions values, our results in Table 5 showed that a significant increase in total protein, α2- and γ globulin and a significant decrease in serum albumin and A/ G ratio were also detected but α-1 and β - globulin demonstrated non significant changes in their values in mastitic goat if compared with the healthy ones. Similar results were recorded by Rashed *et al.* (2002) and Fatma, Darwish *et al.* (2003) who reported that, a significant drop in blood serum albumin and A/G ratio accompanied by significant increase in α2, β and γ globulin were detected. Also, Beisel, (1976) noticed that a decrease in blood serum albumin level was detected and also characteristic for chronic inflammatory process.

Also, Pesce and Kaplan, (1987) observed an elevation in blood serum globulins which could be referred to an inflammatory reaction due to chronic infections of mammary tissues. On the contrary, Said, (1968) found that mastitis due to coliform bacteria did not changed serum proteins values from normal range. Our observation came in agreement with most finding reported by Rawdat *et al.* (1999) who mentioned that total protein and its fractions showed that there was a significant decrease in serum albumin and β- globulin. On the other side, observed a significant elevation in alpha and gamma globulins.

Our data concerning the level of minerals in Table 6 revealed that, blood serum calcium, phosphorus, magnesium, potassium, zinc and iron were showed a significant decrease in their values but the concentration of sodium and copper demonstrated a significant increase in their average. Similar findings were pastulated by Beisel, (1976) who mentioned that hypercopremia was associated with hypozincemia and

due to occuring infections. Roeser (1980) concluded that *hypoferraemia* is a mechanism that limits bacterial, multiplication by deprivation of Fe, these bacteria have the ability to cause generalized inflammatory changes, although hypoferraemia may limit the spreading of the organism from the gland to different organs. Also potassium level reported significant decrease in mastitic animals but sodium did not revealed any significant difference between healthy and mastitic animals.

As mentioned before in our study concerning calcium, phosphorus and magnesium levels were decreased significantly and these observed results came in agreement with the observation of Sandholm *el al.* (1995) who reported that, during mastitis the selective ability of the udder epithelium to concentrate ions is weakened and the passive permeability increase. As a consequence, the salts concentration in blood and milk balanced in such a way that sodium become more concentrated and the amount of calcium, phosphorus, magnesium and potassium decreased considerably.

Our results illustrated the variation in values of AST, ALT, area and creatinine in mastitic does when compared with control ones. Table 7 showed that parameters are altered in mastitic does by increasing significantly in their values. 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 mastitis and these results are in accordance with that reported by Bertoni *et al.* (1994)

Results concerning hematological profile of mastitic goats when compared with control ones were tabulated in Table 8 which revealed a significant statistical depression in total RBCs count (oligocythemia), haemoglobin value; marked leucopenia, total and differential leucocytic count especially lymphocytes, neutrophils percentages with exception of monocytes, eosinophils and basophils percentages which showed a significant increase in their values. Such increase in monocytes indicating the chronicity mean while increasing in esinophils and basophilis percentages reflect the condition of allergx. Our findings came in agreement with the finding of Radostits *et al.* (1994) who reported that in peracute picture of the disease, the total and differential leucocytic counts are characteristic and useful diagnostic aids, where there are a marked leucopenia, 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 different blood parameters was varied with the severity of mastitis.

In conclusion, high prevalence of environmental bacterial clinical mastitis in goat was mainly caused by *Staph. aureus*, *E. coli* and *Streptococcus* spp. and proved as an under pathogens. Enrofloxacin, Ceftiofur, Ciproflaxacin, Gentamycin and Lincomycin are the drugs of choice which could be successfully used for the treatment of mastitic goats. Obtained hematological and biochemical profiles and their alteration in mastitic goats may not be enough for diagnosis of mastitis in goats, but marked leucopenia and neutropenia were characteristic for diagnostic aid in early stage of clinical mastitis.

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