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INVESTIGATIN OF *M. agalactiae* AND STUDY ITS EFFECTS ON SOME HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN SHEEP AND GOATS INFECTED WITH MASTITIS

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ABSTRACT

This study was performed to investigate *M. agalactiae* as a cause of mastitis in sheep and goat and study some hematological and biochemical parameters, for this aims (102) milk samples and (102) blood samples from mastitic sheep and goat were collected . our results showed that detection of *M. agalactiae* in ratio of (10.87 %) and (16.6 %) by culture and PCR technique. Regarding biochemical parameters we noted that zinc decrease significantly ($P \leq 0.05$) in infected group, while copper, Total protein, Albumin and Globulin levels not affected. As far as hematological parameters we noted no significant differences between Infected and Non Infected group in the level of WBC and Hb while significant decrease in the level of RBC and PCV in infected group in compare with control group . we conclude from this study *M. agalactiae* consider as one of mastitis cause in Salahaldeen province and it affects on hematological and biochemical parameters in studied group.

INTRODUCTION

Mastitis defines as inflammation of parenchymal tissue of mammary glands by microorganism leads to chemical, physical and microbial changes, its consider an important economic and public healthy disease (1,2). Mastitis may be clinical or subclinical and according to its causes either contagious mastitis like *Staphylococcus aureus,*

Streptococcus agalactiae and *mycoplasma spp*. or Environmental mastitis like coliform bacteria (3). Mycoplasma is smallest free living prokaryotics , loss of cell wall so that its highly polymorphic shape and it has fried egg appearance colony, many species of them causing mastitis in sheep like *M. capricolum subsp capricolum* and *M. mycoides* subsp. *mycoides* but main of them *M. agalactiae* (4). *Mycoplasma agalactiae* cause many economic enzootic diseases and main complication are septicemia, mastitis, arthritis, pleurisy, pneumonia, and kerato conjunctivitis (5).

regarding tissues infection with *Mycoplasma agalactiae* are mostly associated with exudations and cellular infiltration which alter blood cellular counts, Serum biochemical changes are of paramount importance in accessing the level of cellular and systemic responses of tissues and organs to damage (6) . Nelson and others (7) reported serum zinc levels of (4.86-6.08 µmol/l) in a herd which suffered from cases of anorexia, wool eating, alopecia, hyperkeratosis and parakeratosis, Çamaļ and others (8) have reported a level of (3.80-7.30 µmol/l) for similar symptoms. In ruminants, normal serum zinc levels are between (11 and 18 µmol/l) and animals with levels below (10.5 µmol/l) are considered deficient (9). Normal serum copper levels for sheep as reported by various researcher (8) are (9.26-15.86 µmol/l).

MATERIALS AND METHODS

This study performed in Salahaldeen province

Milk sample: 64 milk samples were collected from mastitic ewes (43 from clinical cases and 21 sub clinical cases which positive to CMT). And 38 milk samples were collected from mastitic goats (26 from clinical cases and 12 sub clinical cases which positive to CMT). After disinfected of teats and removing first drops, 10 ml of milk were collected in septic tube.

blood samples: 102 blood samples were collected from same mastitic ewes and goats that milk collected. Each sample divided in to two parts, first for hematological and second for biochemical tests.

Culture methods : 0.1ml of homogenized milk sample were culturing in Freyś agar medium (Himedia- India) with additives 150ml horse serum (collected from jugular vein than centrifuged and filtrated by using 0.22µM filter), 100ml of yeast extract (10). 5ml of Cystin hydrochloroide (Himedia- India), 5ml of NAD (Himedia- India(0.1g:5ml), 20mlof Thalium acetate (1g:100ml), 5 ml of Penicillin solution (1000000I.U.Lml) (Segmi), dextrose (50g:100ml) and 1% phenol red (1g:100ml). final pH 8.9 (10).

Genetic methods:

DNA extraction: 500µl of samples were centrifuged at 13,000 rpm for 20 mints then 100µl of lyses buffer was added and inculpated at 56°C 4h. 200µl of phenol added and centrifuged at 13,000 rpm for 20 min. supernatant were taken and mixed with Phenol/ Chloroform (1:1). centrifuged supernatant mixed with pure Chloroform then recentrifuged at 13,000 rpm for 5 min. supernatant mixed with 1/10 volume of acetate sodium then kept at -20°C refrigerator with 2 fold volume of cool and pure ethanol (20 min), then the tube was centrifuged at 13,000 rpm for 15 min. 200µl of ethanol (70%) was added and the tube centrifuged at 13,000 rpm for 5 min, DNA was dried and redissolve in distal water (11) .

- The Compounds used in preparation of PCR reaction mixture as in table (1).

Table (1):. Compounds used in preparation of reaction Mixture

Compounds used in preparation of reaction mixture	Volume (microliters)	Reference
Taq PCR Master Mix KIT: Which contain Taq DNA Polymerase (2.5 Unit), PCR Buffer with 3mM MgCL2, 200µMdNTP	25	(Qiagen, Germany).
Forward primer S1:5/-AAAGGTGCTTGAGAAATGCC-3/	2 from 100pM Solution	Santos *et al.,* 2018 (12)
Reverse Primer /-GTTGCAGAAGAAAGTCCAATCA-3/	2 from 100pM Solution	
DNA Template	2	(Qiagen, Germany)
DNA free water	19	(Qiagen, Germany)
Total	50	

Table (2): Thermo cycler programs

Stage	Temperature (c)	Time	No.of cycles
First Denaturation	95	60second	1
Denaturation step	95	60second	35
Primer-annealing step	50	60second	
DNA extension step	72	60second	
Final DNA extension	72	5 mint	1
End Temperature	4		

Serum copper and zinc measurements were carried out with a Shimadzu Atomic absorption Spectrometry model AA-680 in accordance with the technique described in the references (13,14). In order to prevent contamination from glassware, plastic materials were used during the measurements of trace elements. Total protein and Albumen were measured by using special kit supplied by (RANDOX) company, while globulin value was obtained by

this equation (Total protein – Albumen = globulin) (15). Blood parameters were measured by using Vet. Hematological coulter apparatus .

Statistical analysis

The results were analyzed using the SPSS program for values representing the rate and the standard error; the data were analyzing using ANOVA Analysis of variance One Way. The differences between the groups were determined using the Duncan multiple range test, at a probability level ($P \leq 0.05$).

RESULTS

According to colony morphology, *M. mycoides* isolated from 11 milk samples with ratio 10.78% (11:102). As in table (3). In figure (1) shows morphology of mycoplasma colony.

Table (3): isolation ratio of *M. mycoides* from milk samples

Animal	Case	No. of sample	No. of positive sampled	Ratio
Sheep	Clinical	43	5	11.6%
	Sub clinical	21	1	4.7%
Goats	Clinical	26	4	15.3%
	Sub clinical	12	1	8.3%
Total		102	11	10.78%

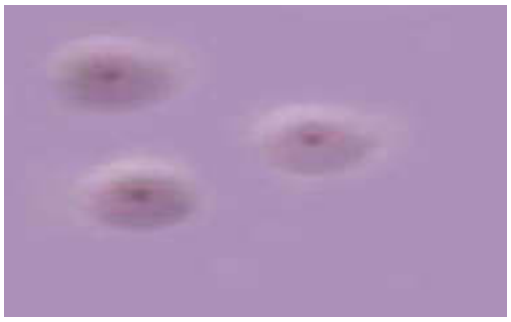
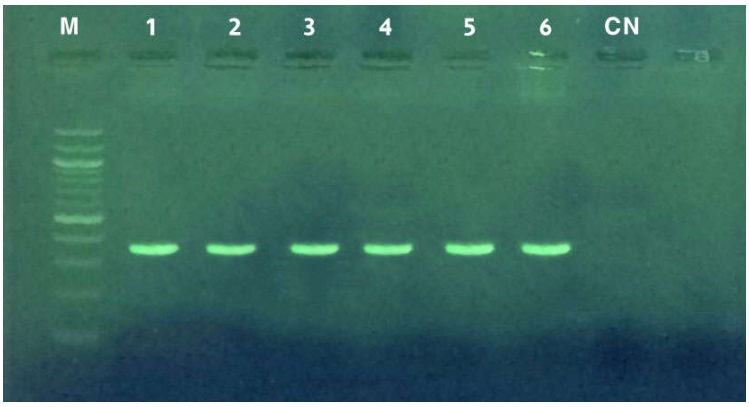


Figure (1): shows colony morphology of *M. agalactiae*

By using of PCR technique, *M. mycoides* detected in rate of 16.6% (17:102) from mastitic sheep and goat milk. As in table (4). Figure (2) show positive result of PCR test.

Table (4) *M. mycoides* detection ratio from mastitic sheep and goat milk

Animal	Case	No. of sample	No. of positive sampled	Ratio
Sheep	Clinical	43	8	18.6%
	Sub clinical	21	2	9.5%
Goats	Clinical	26	6	23.0%
	Sub clinical	12	1	8.3%
		102	17	16.6%



Figure(2): Electrophoresis on 2 % agarose gel and ethidium bromide staining, showing the results of PCR procedures. M: DNA marker, , wells 1-6 positive samples band in size 375 bp. (*M. agalactiae*), CN: control negative

From table (5) noted no significant differences between Infected and Non Infected group in the level of copper, Total protein, Albumin and Globulin, while significant decrease in the level of zinc in infected group in compare with control group

Table (5) : Biochemical parameters of studied cases

Groups / Parameters	Infected group	Non Infected group
	Mean ± S.D	Mean ± S.D
Copper (µmol/l)	13.22 ±1.43 a	12.54 ±2.12 a
Zinc (µmol/l)	8.92 ±1.76 a	10.86 ±1.54 b
Total protein (g/dl)	72.2 ±9.60 a	75.0 ±10.22 a
Albumin (g/dl)	33.0 ±3.50 a	32.6 ±4.10 a
Globulin (g/dl)	39.2 ±9.28 a	42.4 ±12.04 a

From table (6) noted no significant differences between Infected and Non Infected group in the level of WBC and Hb while significant decrease in the level of RBC and PCV in infected group in compare with control group

Table (6) : Blood parameters of studied cases

Groups Parameters	Infected group	Non Infected group
	Mean ± S.D	Mean ± S.D
WBC($x10^3$/µl)	10.90 ±1.64 a	9.59 ±1.56 a
RBC($x10^6$/µl)	13.29 ±2.15 b	15.27 ±2.60 a
Hb(g/dl)	10.70 ±1.77 a	12.24 ±2.41 a
PCV (%)	28.67±4.13 b	33.25 ±7.11 a

DISCUSSION

In current study *Mycoplasma agalactiae* detected in rate of 16.6% , that ratio is less than ratio recorded by (16).which is 57%, and less than result recorded by (17), which 44% in Iran , and more than ratio recorded by (18) which 8.26% in Syria. In Jordin mycoplasma isolate in milk with rate of 13%(19) that's due to deference in geographic area and using techniques. In our study *Mycoplasma agalactiae* we detected high rate in goat milk in compare with sheep milk , that's agreed with (20). In compare between results of culture and PCR techniques we found that PCR techniques more efficiency than culture methods, this results agreed with result of (16,21). In the other hand there was a considerable decrease in the level of zinc and our findings agreed with (22) who reported that decreasing in the level of zinc in case with mastitis infection due to disturbance in the immune system . and we find in this study there is no significant differences in the level of copper between studied groups. In the other hand we noted no differences observed in the concentration of total protein, albumin and globulin in the serum (23). There is no significant differences between the infected group in compare with control group in the values of total WBC. We find In the current study, there were significant decreases in the values of packed cell volume and RBC count in the infected group with mastitis in compare with control group. These results were similar to the findings of (24) . who observed a decrease in total erythrocyte count and attributed his results may be due to that RBC important determinants for accessing responses in systemic tissue injury depending on the stage and type of inflammation present (25) .

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الكشف عن المايكوبلازما القاطعة للحليب وتاثيرها على بعض المعايير الفسلجية والكيموحيوية في الاغنام والماعز المصابة بالتهاب الضرع

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الخلاصة

أجريت هذه الدراسة للتحري عن المايكوبلازما القاطعة للحليب M. agalactiae كاحد مسببات لالتهاب الضرع في الأغنام والماعز في محافظة صلاح الدين. اذ تم جمع (١٠٢) عينة من الحليب و (١٠٢) عينة دم من الأغنام والماعز. أظهرت النتائج أن الكشف عن المايكوبلازما القاطعة للحليب M. agalactiae كان بنسبة (١٠.٨٧ ٪) بواسطة الزرع الجرثومي و (١٦.٦ ٪) بواسطة تقنية البولمرات المتسلسلة . وقياس بعض المعايير الدمية والكيموحيوية، اذ لوحظ ان هنالك انخفاضا معنويا ($P \leq 0.05$) في عنصر الزنك في المجموعة المصابة في حين أن مستويات النحاس والبروتين الكلي والألبومين والجلوبيولين لم تسجل اي تغيير معنوي. وفيما يتعلق بالمعايير الدمية لوحظ عدم وجود فروقا معنوية في مستوى خلايا الدم البيض وخضاب الدم بينما لوحظ انخفاضا معنويا في مستوى كريات الدم الحمر وحجم الخلايا المرصوصة في المجموعة المصابة بالمقارنة مع المجموعة السليمة. نستنتج من هذه الدراسة ان المايكوبلازما القاطعة للحليب تعد احد اسباب التهاب الضرع في محافظة صلاح الدين ويؤثر على المعايير الدمية والكيموحيوية في الحيوانات المصابة .

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