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SOME STUDIES ON CLINICAL AND SUBCLINICAL MASTITIC CASES IN DAIRY SHE-GOATS IN RELATION TO CAUSATIVE PATHOGENIC ISOLATES AND THEIR SENSITIVITY PATTERN

(With 5 Tables)

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بعض الدراسات على التهاب الضرع الظاهرى والتحت اكلينيكى بالماعز: العلاقه مقرونة بالعزلات المرضية المسببة واختبارات الحساسية

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قد جمعت عينات اللبن من ٦٠ ماعز حلاب ببلدة اوسيم تعانى من مرض إلتهاب الضرع وتم اجراء اختبار تجمع اللبن الحلقى لاستبيان مرض البروسيلا فى هذه العينات التى اختيرت واظهرت انها سالبة لهذا المرض وقد اختيرت هذه العينات باختبارى الكاليفورنيا والهوايت ساييد ووجدت النسبة ٤ر٨٪ و ٣ر١٨٪ لمرض اللتهاب الضرع الخفى والتحت اكلينيكى ثم عمل فحص بكتريولوجى للعينات التى اظهرت ايجابيتها لمرض اللتهاب الضرع بالاختبارات الحلقية المنوه عليها اعلاه وتم عزل الميكروبات التالية الميكروب العنقودى والسبحى والقولونى والكورينيبيوجين وتم عمل اختبارات الحساسية للميكروبات المعزوله واظهرت النتائج ان المضاد تتراسيكلين وكلورامفينكول احسن المضادات الحيويه فى علاج التهاب الضرع بالماعز الحلاب.

SUMMARY

Milk samples from 60 dairy she-goats were collected from Oseim locality. Among these she-goats some showed clinical mastitis and the others were clinically sound. Investigation by Ring test (A.B.R.T.) for brucellosis was done on all milk samples collected and showed negative results. California mastitis test (CMT) and white side test (W.S.T.) were carried out to detect the subclinical mastitic cases were 10 (8.3%) and 22 (18.3%) owing to the

infection being single or double and ended with triple variably. Isolation and identification of the bacterial aetiological agents was carried out on mastitic samples. The isolated organisms were *Staph.aureus, Strept.agalactiae, Strept.dysgalactiae, Strept.uberis, E.coli* and *C.pyogenes.* These microorganisms were single or double and triple causative agents. Antibiotic sensitivity tests showed variable potency between high, moderate and slight effect by measuring the inhibition zones. Finally from these studies, the recommended products to use for treatment of these mastitic cases were tetracycline and chloramphenicol.

Key words: *She goats-Mastitis-Causes-sensitivity.*

INTRODUCTION

Milk is considered a complete food, it is digestible when consumed by either human (old and young)or suckling animals.These sucklings have simple and incapable digestive system to digestthe Stuff food (Kesler, 1981).

Caprine mastitis reduces the milk yield and shortens the productive life of affected dairy animals. Due to the numerous causative agents and the variety of predisposing factors, the disease may appear in different names peracute, acute, chronic or subclinical. California mastitis and white side tests are now commonly used in supporting mastitis control as an aid for the earlier detection of subclinical mastitis in she-goats (Shouman et al., 1986; Siddique et al. (1988) and Vihan (1989).

Kids of the infected mastitis she-goats had a poorer growth than kids of she-goats have sound mammary glands. Kids mortality was significantly higher in dairy she-goats's milk with a positive CMT (Blood and Handerson, 1974).

Mastitis is the result of interplay between infection of the gland by specific microorganisms and stresses placed upon the glands by different factors other than bacteriological agents.

This work was done to distinguish clinical and subclinical mastitis by C.M.T. and W.S.T. as well as to identify the different pathogens responsible for this problem. On the other hand, to detect the perfect antibiotic for each isolate, through each sensitivity test to follow up the treatment.

MATERIALS and METHODS

Milk samples were collected from 60 she-goats in Oseim locality-Giza Governorate suffered from clinical and subclinical mastitis. Two samples from both halves were collected as 15 ml. amount in sterile Mac. Cartney bottles for bacteriological examination.

Investigation of all milk samples for brucellosis by MRT was done according to Alton and Jones (1967) using blue haematoxyl in stained brucella Ag. Obtained from Burroughs Wellcome Research Laboratories, Beckenhan, London, England.

Results of Abortus Bang Ring test showed absence of brucellosis in all milk samples.

About I mI. of freshly prepared reagent (C.M.T. and W.S.T.). Results were read after 10 seconds as follows:

Reaction	Grade
* Negative No precepitate	-ve
* Slight precepitate developed	+ve
* Definite precepitate	+ve
* Developed of visceous mass (Gell) then return to flakes	++ve
* Very definite and persistant mass (Gell)	+++ve

In the laboratory, samples were cultivated after through mixing on the following media, Edwards, Blood agar McConky's and Looffler's serum media.

Pure suspected colonies were subcultured on nutrient agar plates for microscopic examination and biochemical reactions. Streptococci were typed using sodium hippurate, argenine, methylene blue reduction test, litmus milk and sorbitol. Staphylococci were examined for mannitol fermentation and gelatine liquefaction. Other suspected colonies as Actinomyces pyogens were also examined for gelatin liquification, litmus milk and lactose while *E.Coli* were examined for urea, indole and also lactose. Isolates were preserved on dorset egg media. Such isolates were typed as single, double or triple and registered as: *Staphylococci haemolytic , Streptococci (agalactia, dysgalactia* and *uberis) Corynaebacterium pyogenes* and *E.coli.*

Antibiotic sensitivity disks:

Name of antibiotic disc	Disc content	Diameter of inhibition zone(mm)		
		Rest	Int.	Sens.
a) Ampicillin	(10 mg)	11 or less	11-13	14 or more
b) Chloramphenicol	(30mg)	12 or less	13-17	18 or more
c) Etythromycin	(15 mg)	13 or less	14-17	18 or more
d) Neomycin	(30 mg)	13 or less	14-16	17 or more
e) Streptomycin	(10 mg)	11 or less	12-14	15 or more
f) Tetracycline	(30 mg)	14 or less	15-18	19 or more

Schalm reagent (3% alkylaryl sulphate plus bromocresol purple 0.09%) according to Schalm et al. (1957). Sensitivity to antibiotics according to Finegold and Martin (1982). The disk diffusion technique was done to each isolate alone performing the same for the all 6 isolates. Each antibiotic measures sensitivity of the 6 isolates individually, measuring the easily visible zones of inhibition of growth produced by diffusion of the antibiotics from the discs onto the surrounding medium.

RESULTS and DISCUSSION

Regarding clinical and subclinical by California mastitis test and white side test, the present findings (Table 1) showed that the percentage of clinical mastitis in she-goats was 8.3%, while in subclicial mastitis was 18.3% In this respect, Shouman et al. (1986) revealed that in she-goats reach 14.1%, while Siddique et al. (1988) reported higher percentage of subclinical mastitis (22.7%) among she-goats.

The results of ARBT investigated that all the selected she-goat was free from Brucellosis by applying the same technique (Alton and Jones 1967).

From the stated data, it was found that the main aetiological bacterial agents responsible for clinical and subclinical mastitis in she-goats were Staphylococci, Streptococci, Corynebacteria and *E.coli* with regard to *E.coli*, it was isolated from one case (Table 2).

In she-goats affected with clinical and subclinical mastitis the percent of pure culture of Staph. Aureus represented (19%) as a single isolate (Table 2). In this respect Nag (1975) and El-yas and Nashed (1988) reported higher

percentage (33.3%) and (26.67%) respectively. While Vihan (1989) gave nearly similar results (20.3%). Strept. agalactiae as a cause of mastitis in she-goats constitute 3%, our results agreed with that of Vihan (1989) who reported 2%, while El-yas and Nashed (1988) claimed higher results (20%.).

Strept. dysgalactiae was isolated single with an incidence of 3% causing mastitis, this agreed with Vihan (1989) who recorded that incidence was (2%). In addition from the table (2), *Strept. uberis* (6%) and *C.pyogenes* (3%), while Shouman et al. (1986) showed that *C. pyogenes* incidence reached 20.5%. With respect to table (2) revealed that the incidence of *Staph.aureuse* and *Strept.agalactiae* was (19%), this results agreed nearly with Siddique et al. (1988) who reported that it was 22%. In addition to *Strept.dysagalactias* and *C.pyogenes* incidence was 23%.

With regard to bacterial triple isolates *(Staph.aureus, Strept.agalactiae* and *Strept.uberis)* with an incidence of 18.3% and other isolates *(Strept. agalactiae, Strept.dysagalactiae* and *C.pyogenes)*. The incidence was 6% in comparison to these results (last triple isolates). Shouman et al. (1986) reported nearly similar percentage (8.7%).

From table (4), it revealed that *Staph.aureus* was 50% followed by *Strept.agalactiae* was 34-3%. Meanwhile the incidence of both *Strept.dysagalactiae* and *C.pyogenes* was 31.2%. The lower incidence was 3.15% in relation to *E.coli* isolates followed by *Strept.uberis* isolates (6.2%).

In regard to clinical and subclinical mastitis on CMT and W.S.T. bacteriological work had done. So the Staph. aureus recovered as preserved strains out of mastitis she-goats as shown in Table (4) in a range of incidence 20-50%. In this respect Nag (1975) somewhat agreeds to this work yet the incidence was 33.3%.

Beside the same organism Vihan (1989) gave an incidence 20.3%. While in relation to the incidence of the isolates (*Strept.agalactiae, Strept.dysgalactiae* and *Strept.uberis)* in mastitis cases among she-goats in range of 6.2% to 34.3% as shown in table (4).

Meanwhile in relation to the isolated Strept. isolates, El-yas and Nashed (1988) and Dautz et al. (1990) showed a range of 11.38% to 21.27% among she-goats for mastitic cases. Hence the pathogenic *E.coli* among mastitis she-goats (Table. 4) showed the percentage of 3.1%.

With regard to sensitivity test which was done onto bacterial isolates out of mastitic cases in she-goat as shown in Table (5). The Table showed that tetracycline was the most effective antimicrobial agent against the most isolated strains while the chloramphenicol comes in the second degree of efficiency. Then followed by erythromycin, the other antibiotics were variable

in their potency between moderate to slight effect, Parenteral treatment is advisable in all cases of mastitis by standard doses of antibiotic (Tetracycline 30 mg as well as dose of chloramphenicol 30 mg, 3 successive days) and intrammary antibiotic infusion treatment was applied under strict hygienic condition to avoid introduction of bacteria into treated quarters. Results of sensitivity test in relation to inhibition zone with pathogenic bacterial isolated strains were recorded according to Bailey and Scott (1978). On the other hand Blair and Nader (1989) recorded that the highly effective antibiotics were tetracycline for treatment of she-goat mastitis. Meanwhile our work agreeds with Korukov (1968) who illustrated that ampicillin had resistent on Staph. isolates of such mastitic cases, while Singh and Baxi (1982) made studies on sensitivity to antibiotics on bacteria isolated (strains of Staphylococci, Streptococci, *E.coli,* Corynacterium sp.) out of mastitic milk samples from she-goats who found that chloramphenicol was the most effective which agreed to our work. Rahman and Baxi (1982) agreed to our work.

Other types of antibiotics had been intermediated sensitive effective while some of them such ampicillin had been resistant effect as shown in Table (5), as well as Vasil et al. (1984) stated that samples from cows treated with chloramphenicol showed a reduction of infection reached 75% through strains of *E.coli,* Staph.*aureus* and Streptococcal infections. Hernandez et al. (1991) agreed to our work that the isolates of *Strept.agalactiae, Strept.uberis, Corynebacterium, E.coli* and *Staph.aureus* out of mastitic cases had 6 high resistance to streptomycin, penicillin and ampecillin, while Kotowski (1991) disagreed to this article that the isolates out mastitic cases. Thus, the following pathogens *Strept.agalactiae, E.coli, Staph.aureus, Strept.uberis* were sensitive to penicillin, neomycin, Streptomycine and erythromycin. Also Sharma et al. (1993) to our article who reported that *Staph.aureus,* corynebacterium Spp., *Strept.pyogenes, E.coli* showed multiple resistance to Streptomycin, ampicillin but sensitive to chloramphenicol.

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Table 1: Incidence of clinical and subclinical mastitis in she-goats.

No. animals	No. of examined animals	No.of halves	Clinical mastitis		Subclinical mastitis	
			No.of halves	%	No.of halves	%
32 she-goats	60	120	10	8.3	22	18.5

Table 2: From clinical and subclinical mastitic cases in she-goats.

No. of mastitic halves	Single isolates			Double isolates			Triple isolates		
	Organism	No.	%	Organism	No.	%	Organism	No.	%
32	Streptococci	6	19	Staphylcococci	15	19	Staphylococci		
	Strept.agalactiae	1	3	Strept.agalactiae			Strept.agalactiae	5	18.3
	Strept.dysagalactiae	1	3	Strept.daysagalactiae	17	23.3	Strept. uberis		
	Strept. uberis	2	6	C. pyogenes			Strept.agalactiae	2	6
	E. coli	1	3				Strept.dysagalactiae		
	C. pyogenes	1	3				C. pyogenes		

Table 3: Field tests on milk samples after treatment with the most effective antibiotic (she-goats).

Field test	She-goats No. of mastitic halves Grade	Reaction
1. California mastitis test (CMT)	-	No APPT*
2. White side test (W.S.T.)	+	Slight PPT**

*: APPT = Agglutination pericipitation.
**: PPT = Pericipition.

Table 4: Microorganisms recovered from clinical mastitis in she-goat by CMT.

No.of haves cases	Isolates	No.of cases	%
32	Staph. aerus	16	50
	Strept.agalactiae	11	34.3
	Strept.dysagalactiae	10	31.2
	Strept. uberis	2	6.2
	E. coli	1	3.1
	C. pyogenes	10	31.2
32		50	100

Table 5: Results of antibiotics sensitivity tests.

Antibiotics	Bacterial isolates					
	Staph. haemolytic	Strept. agalactiae	Strept. dysgalactiae	Strept. uberis	C. pyogenes	E. coli
Ampicillin	+	+	+	+	+	+
Chloramphenicol	+++	+++	+++	++	+++	+++
Erythromycin	+	++	++	+	++	++
Neomycin	+	++	++	++	+	++
Streptomycin	++	++	++	++	++	++
Tetracycline	+++	+++	+++	+++	+++	+++

+++ Means sensitive.
++ Means moderate.
+ Means resistant.