Vet. Med. J., Giza. Vol. 46, No.3. (1998):311-317.

SOME STUDIES ON SUBCLINICAL OVINE MASTITIS

A.A. BAHOUT * and M.A. BALATA **

* Deprt. of Food Control, Fac.of Vet. Med. Zagazig Univ., Egypt.
** Dept. of Animal Health, Desert Research Center, Mataria, Egypt.

Received:3/12/1997
Accepted :18/2/1998

SUMMARY

The occurrence of subclinical ovine mastitis among 56 barky breed ewes in part of the North Western coastal Desert, Egypt (Mariout Research Station & Bourg El-Arab farm) was studied by field tests (bromothymol blue indicator, whiteside test, California mastitis test, somatic cell count and catalase test) and also confirmed by the isolation of the causative microbial agents.

32 (28.57%) out of the 112 examined udder halves infected with subclinical mastitis . Out of 112 milk samples, 38 (33.9%) were positive to bromothymol blue indicator, 50 (44.64%) were positive to whiteside test and 42 (37.5%) were positive to California mastitis test.

Whiteeside test & California mastitis test scores of 2+ ve & 3 + ve were positively correlated with the results of microbial culture.

Staphylococcus aureus, *Streptococcus epidermidis*, *Streptococcus agalactia*, *Escherichia coli*, *Corynebacterium* spp., *Penicllium* spp., *Aspergillus flavus*, *Aspergillus fumigatus* and *Aspergillus terreus* could be isolated from such cases with incidence of 30%, 16%, 20%, 12%, 4%, 6%, 4%,4% and 4% respectively.

The in vitro sensitivity pattern of isolated organisms was tested against different antibiotic and antimycotic discs and the obtained data were presented.

Control measures for improvement of the hygiene quality of ewe's milk were suggested.

INTRODUCTION

Subclinical mastitis is considered to be a very serious form of disease in dairy animals as the udder looks apparently normal and acts as invisible source for the dissemination of many zoonotic pathogens (Bele, 1989).
A great variety of pathogenic microorganisms have been associated with subclinical ovine

mastitis which causes marked loss in milk production, increased somatic cell counts and growth retardation of lambs (Fthenakis and Jones, 1990).

Much ewe's milk in the North Western Coastal Desert, Egypt is drunk raw. Therefore, This work was planned to throw the light on the prevalence of subclinical ovine mastitis in part of this area (Mariout Research Station & Bourg El-Arab Farm) because of the usual use of raw milk. In addition, studying the correlation between the field tests for detection of subclinical ovine mastitis and the microbiological examination of milk samples, as well as the antimicrobial sensitivity pattern of the isolated microorganisms.

MATERIAL AND METHODS

Collection of samples:

One hundred and twelve milk samples from 56 apparently healthy barky breed dairy ewes (40 from Mariout Research Station and 16 from Bourg El-Arab Farm) were collected under hygienic precautions using sterile bottles after cleaning the teats with 70% ethyl alcohol. The samples were kept in an ice box and transferred to the laboratory without delay. Each sample was divided into two portions. One for field tests and the other for microbiological examination.

A.Field tests:

The California mastitis test (CMT), whiteside test (WST), direct microscopical somatic cell count (SCC), bromothymol blue indicator test (BTB) and catalase test were applied according to the technique recommended by American Public Health Association (A.P.H.A., 1985).

B. Microbiological examination:

Ten ml of each sample were centrifuged at 3000 rpm for 20min. Loopfuls were taken from the milk sediment and plated on Baird Parker's medium, Edward's medium , blood agar, MacConkey agar and Sabouraud's maltose agar. The inoculated plates were incubated at 37°C for 48h. except Sabouraud's maltose agar plates which were incubated at 25°C for 5 days. The bacterial isolates were purified and identified according to Krieg and Holt (1984), while the fungal isolates were identified according to Raper and Fennel (1965) and Al-Doory (1980).

Sensitivity Tests:

The antimicrobial sensitivity patterns of the bacterial isolates were done in vitro against* kanamycin 30 (k30), streptomycin 10 ug (S10), ampicillin 10 ug (Amp 10), nitrofurantoin 300ug (F300) gentamycin 30ug (GM30), oxytetracycline 30 ug (Ot 30), neomycin 30 ug (N30), colistin 10 ug (CL10) and trimethoprim sulfamethoxazole 1.25 + 23.75ug (SXT 1.25-23.75), while the fungal isolates were tested in vitro against** clotrimazole (Canesten) 0.1mg

(*) Antibiotic sensitivity discs: (Bio-Merieux 69260 charbonnieres, Les Bains, France).
(**) Antifungal sensitivity discs: (Janssen Pharmaceutica, Beerse, Belgica).

miconazole, (Ductarin) 0.12 mg and Ketoconazole (Nizoral) 0.2 mg according to Bauer et al. (1966).

RESULTS

Table (1) : Prevalence of subclinical mastitis in ewes according to microbiological isolation.

Soruce	Examined Halves	Infected Halves	
		No.	%
Mariout Station	80	27	33.75
Bourg El-Arab Farm	32	5	15.62
Total	112	32	28.57

Table (2) :Correlation between different results of field tests in subclinical ovine mastitis and the microbiological findings.

Test	Reaction	No. of samples	%	Microbiological findings				Prevalence of infected cases
				Positive samples		Negative samples		
				No.	%	No.	%	
(BTB)	(-)	74	66.10	0	00.00	74	66.07	-
	(+)	38	33.90	32	28.57	6	05.36	84.2
(WST)	(-)	62	55.36	0	00.00	62	55.36	-
	(+)	16	14.29	0	00.00	16	14.29	-
	(++)	11	10.71	9	08.93	2	01.78	81.82
	(+++)	23	19.64	23	19.64	0	00.00	100.00
(CMT)	(-)	70	62.50	0	00.00	70	62.50	-
	(+)	9	8.03	0	00.00	9	08.04	-
	(++)	6	5.36	5	04.46	1	00.89	3.33
	(+++)	27	24.11	27	24.11	0	00.00	100.00

(BTB) = Bromothymol blue indicator.
(WST) = Whiteside test.
(CMT) = California mastitis test.

Table (3): Results of somatic cell count and catalase test of subclinical ovine mastitis (infected milk or uninfected milk sample).

Test	Infected samples				Uninfected samples			
	Min.	Max.	Mean	S.E.M.±	Min.	Max.	Mean	S.E.M.±
Somatic cell count/ml	$1.3x10^6$	$3.0x10^6$	$2.0 x10^6$	$0.1 x10^6$	$3.3 x10^5$	$1.0 x10^6$	$6.2 x10^6$	$0.26 x10^6$
Catalase test (ml of O_2)	3.00	6.0	3.9	0.7	1.0	2.5	1.8	0.05

Table (4) : Number of isolates associated with subclinical ovine mastitis.

Isolates	No. of isolates	%
Bacterial		
Staph. aureus	15	30
Staph. epidermidis	8	16
Strept. agalactia	10	20
E. coli	6	12
Corynebacterium spp.	2	4
Fungal		
Penicillium spp.	3	6
Asp. flavus	2	4
Asp. fumigatus	2	4
Asp. terreus	2	4
Total	50	

Table (5): In vitro antibiotics sensitivity pattern of the bacterial isolates:

Isolates	No.	Antibiotic sensitivity %								
		Gm_{30}	K_{30}	Amp_{10}	Cl_{10}	S_{10}	F_{300}	OT_{30}	SXT 1.25+23.75	N_{30}
Staph. aureus	15	60.0	40.0	33.3	-	-	-	-	-	0.0
Staph. epidermidis	8	50.0	25.0	25.0	-	50.0	-	25.0	-	-
Stept. agalactica	10	70.0	80.0	30.0	50.0	30.0	-	50.0	-	0.0
E. coli	6	50.0	50.0	-	33.3	-	83.3	-	-	0.0
Corynebacterium spp.	2	100	50.0	50.0	-	-	-	-	-	-

Gm_{30} = Gentamycin 30µg, K_{30} = Kanamycin 30 µg, Amp_{10} = Ampicillin 10µg
Cl_{10} = Colistin 10 µg , S_{10} = Streptomycin 10µg , F_{300} : Nitrofurantion 300µg
OT_{30} Oxytetracycline 30µg , Sxt $_{1.25+23.75}$ = Trimethoprin sulphamethoxozole 1.25 + 23.75 µg
N_{30} = Neomycin 30µg

Table (6) : In vitro antimycotic sensitivity pattern of the fungal isolates.

Isolates	No.	Antimycotic sensitivity %		
		Coltrimazole 0.1 mg	Miconazole 0.2 mg	Ketoconazole 0.2 mg
Penicillium spp	3	66.6	66.6	33.3
Asp. flavus	2	50.0	50.0	0.0
Asp. fumigatus	2	50.0	50.0	0.0
Asp. terreus	2	0.0	100	0.0

DISCUSSION

Data recorded in Table (1) proved that milk samples from apparently healthy ewes and other infected halves were positive to some bacterial isolates. These results agree within the range recorded by Keisler et al. (1992) who mentioned that the incidence of subclinical ovine mastitis varied from 17% to 50%. On the other hand, these percentages are higher than those reported by Maisi et al. (1987), Watson et al. (1990) and Watkins et al. (1991).

The higher incidence of subclinical ovine mastitis in this study may be due to bad hygienic conditions resulting from high humidity , poor ventilation and over-crowding as well as contaminated environmental condition which predispose to mastitis.

Table (2) showed that out of 112 milk samples, 38 (33.9%) were positive to BTB test, 50 (44.64%) positive to WST and 42 (37.5%) positive to CMT.

The percentage of agreement between positive BTB test and the microbiological results was 84.2%, those for WST giving score 2+ve and 3+ve were 81.82% and 100% respectively, while in CMT they were 83.33% and 100% respectively. On the other hand, the positive samples with grade 1+ve were microbiologically negative (Table 2).

It is clear that, normal ewe's milk without subclinical mastitis gave score 1+ve CMT and WST reaction, while scores 2+ve and 3+ve harbor the pathogens.

These results are nearly in agreement with those revealed by Hueston et al. (1986) and Maisi et al. (1987).

It was noticed from Table (3) that the somatic cell count (SCC) in uninfected milk samples ranged from $3.3x10^5$ to $1.0x10^6$ with a mean value of 6.2×10^5 cells/ml, while in infected samples varied from $1.3x10^6$ to $3.0x10^6$ with a mean value of $2.0x10^6$ cells/ml. On the other hand, the amount of O_2 produced by catalase test in uninfected milk samples ranged from 1.0 to 2.5 with an average 1.8ml, while in infected samples varied from 3.0 to 6.0 with an average 3.9ml.

Nearly similar results were reported by Mackie and Rodgers (1986), Bele (1989) and Deutz et al. (1990).

From Table (4), it is clear that the percentage of different isolates were *Staph.aureus* (30%), *Strept. agalactia* (20%), *Staph, epidermidis* (16%), E.coli (12%), *Corynebacterium* spp. (4%), *Asp. fumigatus* (4%) and *Asp. terreus* (4%).

Similar microorganisms were isolated by Sherma (1983), Maisi et al. (1987), El-Yas and Nashed (1988), Karmy (1990), Watkins et al. (1991) and Keisler et al. (1992).

It is noteworthy that, *Staph aureus* was the most

common isolate from ewes milk samples and may originate from the skin of teat, streak canal or the milker's hand. (Maisi et al. 1987 and Deutz et al. 1990).

With regard to the sensitivity tests in vitro Tables (5,6) showed that *Staph. aureus* strains were sensitive to gentamycin ampicillin and kanamycin, *Staph. epidermidis* strains were sensitive to gentamycin, streptomycin, kanamycin, ampicillin and tetracycline, *strept. agalactia* strains were sensitive to kanamycin, gentamycin, oxytetracycline, colistin, ampicillin and streptomycin, E.coli strains were sensitive to nitrofurantion, gentamycin, kanamycin and colistin, Corynebacterium strains were sensitive to gentamycin, kanamycin and ampicillin. On the other hand, the *Penicillium* strains were sensitive to clotrimazole,miconazole and ketoconazole, *Asp. flavus* and *Asp. fumigatus* were sensitive to clotrimazole and miconazole, while *Asp. terreus* strains were sensitive to micronazole only. So gentamycin, kanamycin, amplicillin, clotrimazole and miconazole were found to be effective in controlling such microorganisms associated with subclinical ovine mastitis. The present results go hand to hand with those reported by Elyas and Nashed (1988) and Karmy (1990).

From the public health of view, *Staph aureus* and E.coli have been implicated in cases of food poisoning and gastroenteritis among consumers (Robinson, 1990).

Finally it appears that consumption of raw, insufficient heated or unpasteurized ewes milk can act as a source of human infection and lambs may die after suckling such infected milk. Therefore, attention must be paid towards feeding , housing , udder hygiene, segregation of ewes positive to the CMT and heat treatment of raw ewe's milk in order to reduce bacterial contamination of milk, improving milk quality and aids in the control of subclinical ovine mastitis.

REFERENCES

Al-Doory, Y. (1980): Udder pathogenic fungi. In: Laboratory Medical Mycology. Lea and Febiger Publ. Philadelphia. P. 249.

American Public Health Association (A.P.H.A) (1985): Standard Methods for the Examination of Dairy Products. 15th Ed. Washington, D.C.

Bauer, A.W.; Kirby, W.M. and Sherris, J.C. (1966): Antibiotic susceptibility testing by a standardized single, disc method. Am. J. Clin. Path. 45; 493-496.

Bele, S. (1989: Studies on subclinical mastitis in sheep and goats in veterinary practice. Buletini I Shkencave Zooteknike Veterinare 7 (2): 101-106.

Deutz, A.; Pernthaner, A.; Schlerca, G. and Baumgartner, W. (1990): Cell count of milk from sheep and goats and the occurrence of bacterial mastitis in lower Austria. Wiener Tierarztliche Monatsschrift. 77 (3): 70-77.

El-yas, A.H. and Nashed, S.M. (1988): Bacteriological studies on mastitis in ewe's and she-goats . Assiut Vet. Med. J., 20 (40): 38-42.

Fthenakis, G.C. and Jones, J.E.T. (1990): The effect of experimentally induced subclinical mastitis on milk yield of ewes on the growth of lambs Br. Vet. J. 146 (1), 43-49.

Hueston, W.D.; Hartwig, N.R. and Judy, J.K. (1986): Detection of ovine intramammary infection with the California mastitis test. JAVMA, 188 (5), 522-524.

Karmy, S.A. (1990): Bacteriological studies on mastitis in small ruminants and she-camel in Upper Egypt. J. Egypt. Vet. Med. Ass., 50 (1) 69-79.

Keisler, D.H.; Andrews, M.L. and Moffatt, R.J. (1992): Subclinical mastitis in ewes and its effect on lamb performance. J. Animal Sci., 70 (6), 1677-1681.

Krieg, N.R. and Holt, J.G. (1984): Bergey's Manual of Systematic Bacteriology, Vol.1, Wiliams and Wilines Co., Baltimore M.D. 2120 USA.

Mackie, D.P. and Rodgers, S.P. (1986): Mastitis and cell content in milk from Scottish blackface ewes. Vet. Rec. 118 (4), 20-21.

Maisi, P.P.; Junttila, J. and Seppanen, J. (1987): Detection of subclinical mastitis in ewes. British Vet. J. 143 (5), 402-409.

Raper, K.B. and Fennel, D.I. (1965): Etiology and Ecology of Aspergillus. In: The genus Aspergillus. 2nd Publ. by Baltimore, Williams and Wilkins Company Philadelphia, London, P.30

Robinson, R.K. (1990). : Dairy Microbiology 2nd Ed. Chapman and Hall, London, New York.

Sharma, S.D. (1983): Studies on bovine mastitis with special reference to mycotic infections of udder. Vet. Res. J. 6(1/2), 105-106. D.S.A., 1986, 48 (2).

Watkins, G.H.; Burriei, A.R. and Jones, J.E.T. (1991): A field investigation of subclinical mastitis in Sheep in Southern England. British Vet. J. 147 (4) : 413-420.

Watson, D.L.; Franklin, N.A.; Davies, H.I.; Kettwell, P. and Frost, A.J. (1990). Survey of intramammary infections in ewes on the New England Tabeland of New South Wales. Aust. Vet. J., 67 (1), 6-8.