

## **FURTHER STUDIES ON THE PREVALENCE OF SUBCLINICAL MASTITIS IN DAIRY COWS IN EL-GHARBIA AND KAFR EL-SHEIKH GOVERNORATES WITH SPECIAL OBSERVATION TO ANTIBIOTIC SENSITIVITY**

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### **ABSTRACT**

*A total of 1136-quarter cow's milk samples were collected from apparently healthy cow's from 2 dairy farms in El Gharbia and 3 dairy farms in Kafr El- Sheikh Governorates (Egypt). All samples were screened for subclinical mastitis using California Mastitis Test (CMT), Somatic Cell Count (SCC), lactose and chloride content. All Positive CMT samples were examined microbiologically for isolating the most causative microbial agents of subclinical mastitis. 136 (22.5%) samples collected from El-Gharbia Governorate were positive for CMT .Of them 100 samples (73.5%) scored 2+ and 36 (26.5%) scored 3+; 120 (20%) contained lactose below 4.7% and 140 (23.2%) had chloride content greater than 0.14%. While 65 (12.2%) quarter milk samples collected from Kafr El-Sheikh farms were CMT positive, of them 24 (36.9%) samples scored 2+ and 41(63.1%) samples scored 3+ ; 72 (13.5%) samples contained lactose less than 4.7% and 119 (22.4%) had chloride content greater than 0.14% .*

*Infectious organisms isolated from CMT positive samples were Coliforms (88.2%), Staphylococci (26.5%) and E-coli (14.7%) while both coagulase +ve Staph. aureus and Strept. agalactiae failed to be detected from El- Gharbia quarter samples. On the other hand Coliforms , Staphylococci, coagulase +v Staph. aureus , E-coli and Strept agalactiae were isolated with a percentages ranging from 1.5 to 52.3 % from Kafr El-Sheikh samples. Somatic cell count (SCC) ranged from >105 to <107  
ve in vitro sensitivity pattern of the*

*isolated organisms against 7 antibiotics was tested. Flumequin and Gentamycin gave the best results in inhibiting all the tested Staph. aureus , E-coli and Strept. agalactiae tested strains. It is concluded that subclinical mastitis in dairy cows is a serious problem in the surveyed area.*

## INTRODUCTION

Production of high quality milk in dairy farms depends essentially on minimizing bacteria and excluding chemical contaminants (**Pankey, 1989**). It also requires healthy dairy animals which are the result of many management factors including mastitis control and herd health programs (**Bodman et.al., 1988; Ibtisam, et al. 1995** ). Mastitis has a significant impact on the economics of milk production and consumer health specially in small private farms in developing countries where hygienic measures and milking sanitation are often insufficient(**Ramachandrainh, et. al., 1990; Zatoun and Manaa, 1992 and Omyma Saleh. 1999**).

Over 200 different organisms have been recorded in scientific literature as being causes of bovine mastitis the common pathogens were contagious bacteria which spread from infected quarters to other quarters as Streptococcus agalactiae and Staphylococcus aureus. Moreover, environmental bacteria that are commonly present in the cows environment may reach the teat orifices as E-coli and other groups of coliforms and Enterobacter species (**Hogan, et.al. 1986; Refai, 1988; Saeman et al., 1988 and Blowey & Edmondson, 1995**).

Due to the insufficient dosage in intra-mammary administration of antibiotics or non indicated application of dry cow therapy the development of antibiotic resistance strains of bacteria resembles a consumer risk as the responsibility of the veterinarian for consumers health is the major topic of importance (**Mahmoud, 1988**).

Therefore, this study was conducted to determine the subclinical mastitis in cows over a 12 weeks study period and to assess the resistance of the causative bacteria to selected antibiotic agents.

## MATERIALS & METHODS

This study was carried out in five semi- intensively arranged farms at El-Gharbia (i, ii) and Kafr El-Sheikh (iii, iv, v) Governorates. Each farm was visited once weekly for a 12-week period. A total of 1136 quarter milk samples (100 ml each) from cows in early and mid lactation period were selected and examined as follows: -

All quarter milk samples were subjected to California Mastitis Test (CMT) to detect subclinical mastitis quarters as described by *APHA*, (1993). Reaction of 2 + or higher were considered as evidence of subclinical mastitis.

Films were prepared from composite milk samples (25 ml of milk were pooled from the four quarters of each selected cow for somatic cells count (SCC) as described by *APHA* (1993). Chloride content was determined according to *Atherton and Newlander* (1977). Isolation and identification of coliforms, E.coli, *Streptococcus agalactiae* and *Staphylococcus aureus* were carried out according to *APHA*. (1993) and *Bailey and Scott*, (1994).

Antibiotic sensitivity tests were performed on *Staphylococcus aureus*, *E.coli* and *Streptococcus agalactiae* isolates. The following antimicrobial agents with respective disc potency (mg) were used: Gentamycin (10 mg), Neomycin (30 mg), Flumequine, Amoxicillin (25 mg), Oxytetracyclin (30 mg). Cloxacillin (5 mg) and Penicillin (10 I.U.) according to the method adopted by *Hirsh and Zee* (1999).

## RESULTS & DISCUSSION

**Table(1):** Chemical parameters of quarter milk samples collected from two Farms in El-Gharbia Governorate:

Source of samples	No. of examined samples	CMT +ve samples	California Mastitis Test (CMT)		Somatic cell count	Lactose <4.7%	Chlorine >0.14%
			2+	3+			
Farm i	204	49 (24%)*	33 (67.3%)**	16 (32.7%)	>10 <sup>5</sup> to <10 <sup>7</sup>	55 (27%)*	60 (29.4%)*
Farm ii	400	87 (21.8%)	67 (77%)	20 (23%)		65 (16.3%)	80 (20%)
<b>Total</b>	604	136 (22.5%)	100 (73.5%)	36 (26.5%)		120 (20%)	140 (23.2%)

from the total samples \*\*from the CMT +ve samples  
**Table(2):**Chemical parameters of quarter milk samples collected from three Farms in Kafr El-Sheikh Governorate:

Source of samples	No. of examined samples	CMT +ve samples	California Mastitis Test (CMT)		Somatic cell count	Lactose <4.7%	Chlorine >0.14%
			2+	3+			
Farm iii	152	12 (7.9%)*	3 (25%)**	9 (75%)	>10 <sup>5</sup> to <10 <sup>7</sup>	15 (9.9%)*	25 (16.4%)*
Farm iv	200	29 (14.5%)	12 (41.4%)	17 (58.6%)		50 (25%)	54 (27%)
Farm v	180	24 (13.3%)	9 (37.5%)	15 (62.5%)		7 (3.9%)	40 (22.2%)
<b>Total</b>	<b>532</b>	<b>65 (12.2%)</b>	<b>24 (36.9%)</b>	<b>41 (63.1%)</b>		<b>72 (13.5%)</b>	<b>119 (22.4%)</b>

\* From the total samples

\*\*from the CMT +ve samples

**Table(3):**Incidence of causative organisms in subclinically affected quarter milk samples collected from El-Gharbia farms.

Farms	No. of Examined samples	No. of +ve samples	Causative organisms				
			Coliforms	E-coli	Strept. agalactia	Staphylococci	Coagulase+ve Staph.aureus
Farm I	204	49 (24%)*	39 (79.6%)**	7 (14.3%)	0	12 (24.5%)	0
Farm ii	400	87 (21.7%)	81 (93.1%)	13 (14.9%)	0	24 (27.6%)	0
<b>Total</b>	<b>604</b>	<b>136 (22.5%)</b>	<b>120 (88.2%)</b>	<b>20 (14.7%)</b>	<b>0</b>	<b>36 (26.5%)</b>	<b>0</b>

\* % From examind samples

\*\* % Of the organisms calculated from the positive samples

**Table(4):**Incidence of causative organisms in subclinically affected quarter milk samples collected from Kafr El-Sheikh farms:

Farms	No. of Examined samples	No. of +ve samples	Causative organisms				
			Coliforms	E-coli	Strept.agalactia	Staphylococci	Coagulase +ve Staph.aureus
Farm iii	152	12 (7.9%)*	5 (41.7%)**	1 (8.3%)	0	7 (58.3%)	6 (50%)
Farm iv	200	29 (14.5%)	16 (55.2%)	2 (6.9%)	1 (3.4%)	13 (44.8%)	7 (24.1%)
Farm v	180	24 (13.3%)	13 (54.2%)	1 (4.2%)	0	9 (37.5%)	6 (25%)
<b>Total</b>	<b>532</b>	<b>65 (12.2%)</b>	<b>34 (52.3%)</b>	<b>4 (6.2%)</b>	<b>1 (1.5%)</b>	<b>29 (44.6%)</b>	<b>19 (29.2%)</b>

\* % From examind samples

\*\* % Of the organisms calculated from the positive samples

**Table(5):**Antibiotics sensitivity of the isolated strains from quarter milk samples

Antibiotic used	Amount	Staph.aureus (n=19)		E.coli (n=24)		St.agalactia (n=1)	
		No	%	No	%	No	%
A	20 Mg	0	0	2	8.3	0	0
C	5 Mg	15	78.9	0	0	0	0
F	30 Mg	19	100	24	100	1	100
G	10 Mg	19	100	24	100	0	0
N	10 Mg	8	42.1	3	12.5	0	0
O	30 Mg	16	84.2	4	16.7	0	0
P	I.U	13	68.4	0	0	0	0

A= Amoxicillin

N= Neomycin

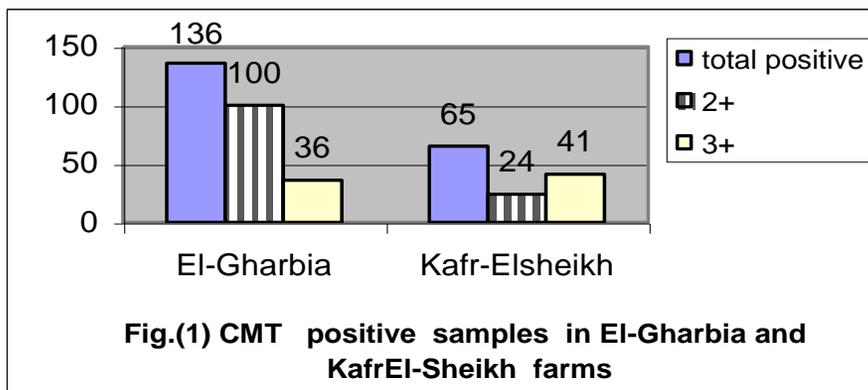
C= Cloxacillin

O= Oxytetracyclin

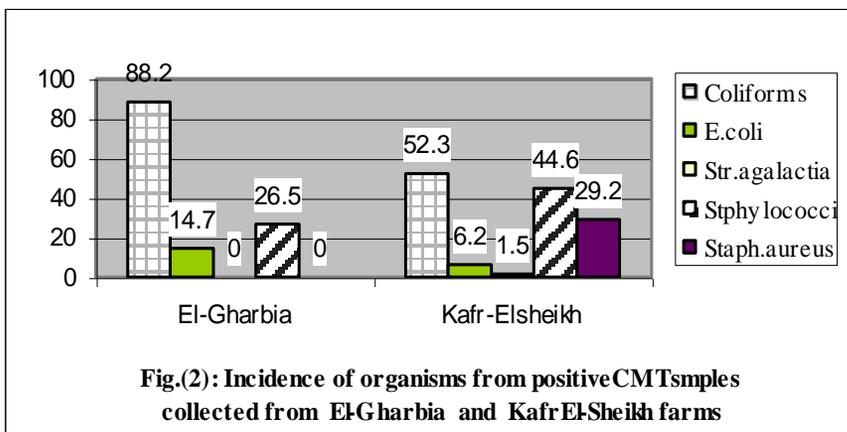
F= Flumequine

P=Penicillin

G= Gentamycin



**Fig.(1) CMT positive samples in El-Gharbia and KafrEl-Sheikh farms**



**Fig.(2): Incidence of organisms from positive CMTsmpls collected from El-Gharbia and KafrEl-Sheikh farms**

Assessment of high quality milk demonstrated by reductions of the levels of subclinical mastitis, as determined by the individual levels of somatic cell and chemical and bacteriological examinations.

### **Chemical examinations:-**

Table (1) and Fig. (1) show that out of 604 quarter milk samples collected from El-Gharbia farms (i,ii), 136 (22.5%) gave positive results with CMT of them 100 (73.5%) scored 2+ve and 36 (26.5%) scored 3+. In farm i, 33 samples scored 2+ and 16 samples scored 3+ with CMT, Vs 67 and 20 samples scored 2+ and 3+ in farm ii. On the other hand, 65 (12.2%) samples out of 532 quarter milk samples collected from three farms (iii,iv,v) in Kafr El-Sheikh gave positive with CMT of them 24 (36.9%) scored 2+ve and 41 (63.1%) scored 3+ve. In farm iii, three (25%) showed score 2+ve and 9(75%) had score 3+ve. Vs 12 (41.4%)& 9 (37.5%) scored 2+ve and 17 (58.6%)& 15 (62.5%) scored 3+ve with CMT in farm iv & v respectively (Table, 2 and Fig.1).

The obtained results were higher than that recorded by *El-Kholy & Hosein (1990)* and *Ahmed & Azza Deeb (2001)*, while the results were lower than that reported by *Patil et al., (1995)*. *Nazem and Azab (1998)* reported that the sensitivity of CMT was 88.02%. The variation between the incidence of subclinical mastitis in this study and other authors can be attributed to several factors including the rate of exposure of animals to infection and the hygienic conditions.

Herd consistently producing milk bulk milk somatic cell counts (BMSCC) less than  $2 \times 10^5$  cells/ml are common. In the major dairy producing countries of the world the standards for the upper legal threshold of BMSCC are currently at  $4 \times 10^5$  cells/ml within the European union  $5 \times 10^5$  cells/ml in Canada and  $7.5 \times 10^5$  cells/ml in the United States (*Larry Smith and Hogan, 1996*).

Our study declared that the CMT positive samples had SCC from  $>10^5$  to  $<10^7$ /ml in composite milk milk obtained from El- Gharbia and Kafr El-Sheikh farms (tables 1&2). Higher counts than the legal requirements of European union were recorded while within the legal of

Canadian and the United States standards. Nearly similar results were recorded by *Ahmed and Deeb (2001)*, while higher results were reported by *Abou- Zaid and El-Sawalhy (1999)*.

There is no clear threat to human health from SCC in excess to  $4 \times 10^5$  cell/ml and the cell count of milk has long been regarded as a measure of the quality of milk produced by herd (*Booth, 1996*). High BMSCC are caused by individual cows with high SCC as cows suffering from physiological disturbance or pathological udder damage also newly calved cow or drying off may lead to high SCC, subclinical mastitis caused by infectious germs is the most important cause of high BMSCC (*Logan and Gillespie, 1996*).

The examination of lactose content reveals that 120 (20%) samples collected from El- Gharbia farms recorded lactose % less than 4.7, 55 (27%) samples in farm i and 65 (16.3%) in farm ii. On the other hand, 72 (13.5%) samples collected from Kafr El-Sheikh farms had <4.7% lactose out of them 15 (9.9%) in farm iii, 50 (25%) in farm iv and 7 (3.9%) in farm v (Table 1,2). In mastitic cases, lactose sharply decreases and chloride percentage increases to maintain the osmotic pressure inside the inflamed quarters.

Results in Table 1 & 2 show that 140 (23.2%) milk samples collected from El-Gharbia farms, 60 (29.4%) in farm i and 80 (20 %) in farm ii, contained >0.14% while for milk samples collected from Kafr El-Sheikh farms 119 (22.4%) contained chloride greater than 0.14%, 25 (16.4%) of them in farm iii, 54 (27%) in farm iv and 40 (22.2%) in farm v. Lower results were obtained by *Ahmed and Azza Deeb (2001)* while higher results were recorded by *Azza Deeb (1996)*. The higher results may be attributed to the entrance of sodium chloride from blood to the udder suffering from mastitis leading to increase of chloride above normal (*Atherton and Newlander, 1977*).

### **Microbiological Examination:**

The most frequently organisms isolated from CMT positive samples collected from El- Gharbia Governorate were coliforms from

... (39.6%), ... 39(79.6%) in farm i and 81 (93.1%) in farm ii. While E.coli were isolated from 20 (14.7%) quarter milk samples 7 (14.3%) in farm i and 13 (14.9%) in farm ii (Table,3 and Fig.,2). On the other hand, coliforms were detected in 34 (52.3%) subclinically affected quarter milk samples collected from Kafr El-Sheikh farms, 5 (41.7%) in farm iii, 16 (55.2%) in farm iv and 13 (54.2%) in farm v, E-coli were isolated from 4 (6.2%) of examined quarter milk samples, 1 (8.3%) in farm iii, 2 (6.9%) in farm iv and 1 (4.2%) in farm v (Table,4 and Fig.2). Regarding E-coli higher results were obtained by *Schukken et al., (1989)*; *Nazem & Azab (1998)* and *Ahmed & Azza Deeb (2001)*, while nearly similar results were reported by *Tawfik et al., (1984)* and *Omya Saleh (1999)*.

Coliform infections are considered to be the major cause of environmental mastitis (*Anderson, 1989 and Larry Smith & Hogan ,1993*). *Larry Smith et.al., (1985)* estimated that 30 to 40% of all cases of clinical mastitis were caused by coliform bacteria and the most commonest of these organisms were E-coli.

Streptococcus agalactiae organisms failed to be isolated from all quarter milk samples collected from El-Gharbia farms while they were isolated from only one sample (1.5%) of the examined quarter milk samples collected from Kafr El-Sheikh farms (Table 3 ,4 Fig. ,2). *Abou-Zaid & El-Sawalhy (1999)* obtained nearly similar results, while higher results were obtained by *Nazem & Azab (1998)* and *Ahmed & Azza Deeb (2001)*.

Staphylococci were clearly observed in 36 (26.5%) quarter milk samples collected from El-Gharbia farms, 12 (24.5%) in farm i and 24 (27.6%) in farm ii (Table, 3 and Fig., 2 ). On the other hand , 29 (44.6%) samples collected from Kafr El-Sheikh Governorate had Staphylococci, 7 (58.3%) in farm iii, 13 (44.8%) in farm iv and 9 (37.5%) in farm v, (Table, 4 and Fig. , 2 ).

Coagulase +ve Staphylococcus aureus failed to be detected in all the examined samples collected from El-Gharbia farms, while it was detected in 19 (29.2%) quarter milk samples collected from Kafr El-

Sheih farms, 6 (50%) in farm iii, 7 (24.1%) in farm i. ...., ... farm v. Higher results were detected by *Hafez, et al., (1984)*; *Tawfik et al., (1984)*; *Nazem & Azab (1998)* and *Ahmed & Azza Deeb (2001)*.

*Staphylococcus aureus* is one of the major pathogenic causes of intramamary infection, field experience suggests that *Staphylococcus aureus* can persist for long periods in the herd with relatively low clinical or SCC problems but with high prevalence (*Nickerson, 1993 and Gaddo et al., 1996*).

*Wilson, et al., (1994)* reported that mastitis caused by *Staphylococcus aureus* was present in 78.7% of dairy herd in north eastern United States, while *Logan & Gillespie (1996)* found that the commonest bacteria isolated from subclinical mastitis were *Staphylococci* which were found in 98% of herds and *Streptococcus agalctiae* in 20% of herds.

Regarding at the sensitivity of the isolated microorganism to certain antibiotics, using seven different antibiotics to 19 strain of *Staphylococcus aureus*, 24 strain of *E-coli* and one strain of *Streptococcus agalactiae* (Table,5). All the isolated strains were sensitive to flumequine and gentamycin. Similarly the higher sensitivity of all isolates of *E-coli* to gentamycin had earlier been reported by *Ngeleka, et al., (1998)*. While 42.1 % of the *Staph. aureus* isolates and 12.5 % of the *E-coli* isolates appear to be sensitive to neomycin .The prevalence of resistance of *Staph. aureus* and *E-coli* isolates to amoxycillin were 100% and 8.3% respectively. On the other hand, 84.2% and 16.7% *Staph. aureus* and *E-coli* isolates were sensitive to oxytetracycline respectively .The *Staph. aureus* isolates were sensitive to cloxacillin and penicillin antimicrobials with a percentages of 78.9% and 68.4% respectively, while all (100%) *E-coli* isolates were resistant to them. The superior effect of Flumequin than other antibiotics might be due to the fact that Flumequin seldom used as a treatment for mastitis in contrast to other antibiotics which most frequently used and may leads to the development of resistant strains.

The resistance behaviour of 100% each *Staph. aureus* ,and 91.75 % *E.coli* and 100% *Strept. agalactiae* strains to Amoxycillin may developed

.....tment of mastitis , Penicillin resistance among E.coli and Staph.aureus isolates may be due to the development of B-lactomase from Penicillin- binding protein of the bacterial cell wall. In Germany, Belgium, England and Switzerland about 38% - 78% of the strains causing mastitis are estimated to form penicillinase. Therefore, insufficient dosage in intramammary administration of antibiotics or non-indicated application of dry cow therapy should be avoided.

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دراسات لاحقة عن مدى انتشار التهاب الضرع الغير ظاهري في الأبقار الحلوب بمحافظتى الغربية وكفر الشيخ مع ملاحظة حساسية الميكروبات للمضادات الحيوية

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تم تجميع 1136 عينة من أبقار حليب سليمة ظاهرياً من مزرعتين بمحافظة الغربية وثلاث مزارع بمحافظة كفر الشيخ جميع العينات تم فحصها كيميائياً باستخدام اختبار الشالم وتعيين نسبة الاكتوز واختبار الكلورايد النوعى وكذلك عدد الخلايا الجسمية **SCC**. تم فحص العينات الإيجابية لاختبار الشالم ميكروبيولوجيا للميكروبات المسببة لالتهاب الضرع .

وقد سجلت 136 عينة (بنسبة 22.5%) وكذلك 65 عينة (12.2%) من محافظتى الغربية وكفر الشيخ نتائج إيجابية لاختبار الشالم. كما أظهرت النتائج أن 120 (20%) و 140 (23.2%) من

عينات محافظة الغربية تحتوى على لاكتوز بنسبة أقل من 4.7% وكلو رايد بنسبة أعلى من 0.14%. بينما سجلت 24 عينة +2 و 41 عينة +3 لاختبار الشالم بمحافظة كفر الشيخ، 13.5% من العينات سجلت نسبة لاكتوز اقل من 4.7% و 22.4% من العينات سجلت نسبة كلورايد أعلى من 0.14%. بفحص العينات الإيجابية للشالم ميكروبيولوجيا، تم عزل الميكروبات القولونية بنسبة 88.2% والميكروب العنقودى بنسبة 26.5% وميكروب الايشريشيا كولاى بنسبة 14.7% وذلك من عينات محافظة الغربية بينما لم يتم عزل الميكروب العنقودى الذهبى coagulase+ve وكذلك الميكروب السبحى اجالكتيا. وكذلك تم عزل الميكروبات القولونية والعنقودية، والعنقودى الذهبى والايشريشا كولاى وكذلك الميكروب السبحى اجالكتيا من عينات محافظة كفر الشيخ بنسب 52.3%، 44.6%، 29.2% و 6.2% و 1.5% على التوالى.

بعد اجراء عد للخلايا الجسمية للعينات الإيجابية للشالم ، وجد أن العدد يتراوح من 5

إلى 10 7 .