

## DETECTION OF SUBCLINICAL MASTITIS IN MILK OF DAIRY COWS IN SOHAG CITY, EGYPT

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### ABSTRACT

This study aimed to detect the incidence of subclinical mastitis (SCM) in the milk of dairy cows in Sohag city, Egypt. 100 quarter milk samples from all quarters of 25 dairy cows with apparently healthy udders were examined by strip cup test, California mastitis test (CMT), White side test (WST), chemical and bacteriological tests. From the obtained results, it was found that none of the strip cup test was positive, while CMT revealed 53% positive with various degrees, and the WST revealed 49% positive with various degrees. According to the estimation of chloride % and lactose %, the Koestler number was revealed 33% mastitic, 25% suspected and calculated 42% normal samples. According to the bacteriological examination, out of the 39 bacterial isolates from the examined milk samples, *Staph. aureus*, *Strept. agalactiae* and *E. coli* were estimated 64.10%, 28.21% and 7.69%, respectively.

**Key words:** Subclinical mastitis, CMT, WST, Koestler number

### INTRODUCTION

Milk is one of the most important foods for human beings; it is universally recognized as a complete diet due to its essential components. In recent years, the demand for liquid milk is increased tremendously worldwide due to increased population growth (Klaas, 2000). However, milk production has been affected by various factors like mastitis (Payne and Wilson, 1999). Mastitis occurs throughout the world wherever dairy animals are found. Mastitis may be classified as clinical and subclinical. In contrast to visible changes in the acute form of mastitis, there is absence of visible abnormalities in the milk or udder in case of SCM. Most of the mastitis are subclinical in nature and their prevention depends primarily on good management practices of dairy herd which include stress-free environment, proper maintenance and operation of milking equipment, good milking procedures (Konwar *et al.*, 2009).

SCM is of great economic importance to dairy farmers because it results in reduction in milk yield and undesirable changes in the milk's composition, as well as increased costs associated with control strategies (Halasa *et al.*, 2009). Over one hundred

different microorganisms have been isolated from bovine mastitis, but the most frequently isolated microorganisms are Staphylococci, Streptococci and Gram-negative bacteria (Oliver *et al.*, 2004; Hussain *et al.*, 2012; Hussain *et al.*, 2013). Staphylococci are considered as one of the main etiological agents of sub clinical mastitis in dairy cows (Unal and Yildirim, 2010).

There were several methods have been used for detection of SCM including physical and chemical changes of milk and isolation of associated organisms which is considered the most accurate although, it is expensive and time-consuming (Badiuzzaman *et al.*, 2015). Therefore, sensitive, simple, rapid and reliable tests are required to be applied on a large scale of animals, (Radostitis *et al.*, 2007); among these tests, CMT and WST (Greiner *et al.*, 2000).

The present study aimed to detect the SCM in the milk of dairy cows located in Sohag city, Egypt, and to detect some of the pathogens causing SCM.

### MATERIALS AND METHODS

#### The samples:

One hundred quarter milk samples were collected aseptically from all quarters of 25 dairy cows with apparently healthy udders in Sohag city, Egypt, according to the procedure recommended by Quinn *et al.* (2002). The collected samples were kept in an ice

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box and transported to the laboratory without delay where they were chemically and bacteriologically examined.

#### Preparation of the samples (APHA, 1992):

Each sample was divided aseptically into 2 portions; the 1<sup>st</sup> one was used for chemical examination, while the 2<sup>nd</sup> one was used for bacteriological examination.

#### Examination of the samples:

##### I- Field tests:

##### 1) Strip cup test (Thirapatsakun, 1999):

First few streams of milk were taken onto a strip plate. Mastitic milk will show discoloration, clots, or other abnormalities.

##### 2) California mastitis test (CMT) (Saloniemi, 1995):

A plastic vessel with four shallow wells was used for collecting approximately 2 ml of milk from each udder quarter; then equal amount of alkali reagent (Schalm reagent) was added. A gentle circular motion was applied to the mixtures in horizontal plane for 5 seconds and the different degrees of gel were recorded.

##### 3) White side test (WST) (Schalm *et al.*, 1971; Klastrup and Schmidt, 1974):

Five drops of milk were added to 2 drops of NaOH 4% on clean glass plate placed on dark black ground and mixed well and the reaction was graded according to the Scandinavian recommendations.

##### II- Chemical examination:

##### 1) Chloride% (Sanders, 1939):

In a beaker, 10 ml milk sample, 5 ml nitric acid 25% freshly prepared (act as catalyst), 5 ml silver nitrate N/10 (combined with all chloride) and 1 ml saturated iron alum solution (act as indicator) were added & thoroughly mixed by glass rod. Titration was done against ammonium thiocyanate N/10 until brownish color was obtained and remained for 1.5-2 minutes (end point).

##### 2) Lactose%:

All the milk samples were examined for lactose% using Lactoscan milk analyzer (Ultrasonic portable milk analyzer, LSSP001, Bulgaria), in the laboratory of Milk Hygiene, Department of Food Hygiene, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt.

##### 3) Koestler number (Koestler, 1920):

$$\text{Koestler number} = \frac{\text{chloride}}{\text{lactose}} \times 100$$

It is usually in normal milk less than 2, in suspected milk from 2 to 3, and in mastitic milk more than 3.

#### III- Bacteriological Examination:

All the milk samples that showed positive results with field and chemical tests were subjected to bacteriological examination.

##### 1) Isolation and identification of *Staph aureus*:

Enrichment procedure was done according to Bailey and Scott (1974) as the milk sample was inoculated into NaCl broth 10% and then incubated at 37<sup>o</sup> C for 24 h, then loopfuls from the incubated broth were streaked (AOAC, 2000) on mannitol salt agar plates and then incubated for 24 h at 37<sup>o</sup> C. Suspected colonies were picked up onto nutrient agar slants for further identification using anaerobic utilization of mannitol (Baird- Parker, 1962) and coagulase test (Cruickshank *et al.*, 1973).

##### 2) Detection of *Streptococcus agalactiae*:

By the using Hotis test (Hotis and Miller, 1936), 9.5 ml milk and 0.5 ml of sterile aqueous solution of bromocresol purple 0.5% were mixed thoroughly (purple color appeared, pH 6.5) and incubated at 37<sup>o</sup> C for 24 h; the positive result was indicated by appearance of yellow color and flakes of canary yellow color at the side of test tube and if negative further incubation for 24 h was applied. A loopful from the positive tubes was inoculated into a slope agar for further examination using hippurate hydrolysis (Mahon and Manuselis, 1995).

##### 3) Coliforms count (MPN/g) (AOAC, 1975):

1 ml of each 1:10, 1:100 and 1:1000 of the milk sample dilutions was inoculated into 3 replicate tubes of lauryl sulphate tryptose (LST) broth supplied with inverted Durham's tubes, and incubated at 35<sup>o</sup> C for 48 h. Tubes showed gas in Durham's tubes within 48 h were submitted for confirmatory test. A loopful was inoculated into brilliant green lactose bile (BGLB) broth tubes with inverted Durham's tube and incubated at 37<sup>o</sup> C for 48 h, and that showed gas in the Durham's tubes was recorded and considered positive for coliforms. The number of coliforms/g was calculated from the most probable number (MPN) table for 3 tubes dilution.

##### 4) Fecal coliforms count (MPN/g) (AOAC, 1975):

From all the positive BGLB broth tubes, a loopful was inoculated into *Escherichia coli* broth (EC broth) tubes with inverted Durham's tubes and incubated at 45.5±0.5<sup>o</sup> C for 48 h. Tubes showed gas production in Durham's tubes were recorded and considered positive for fecal coliforms. The number of fecal coliforms /ml was calculated using MPN table for 3 tubes dilutions.

##### 5) *E. coli* count (MPN/g) (AOAC, 1975):

The positive EC broth tubes were subcultured by streaking on Eosin Methylene Blue (EMB) agar plates and incubated at 37<sup>o</sup> C for 48 h. The typical nucleated dark center colonies with metallic sheen were considered to be *E. coli* positive.

## RESULTS

**Table 1:** The prevalence of SCM at udder-quarter level

No. of the infected animals		No. of the infected quarters		Strip cup test		CMT		WST	
				+ve		+ve		+ve	
No./25	%	No./100	%	No.	%	No.	%	No.	%
21	84	58	58	0	0	53	53	49	49

**Table 2:** Statistical analytical results of CMT in the examined samples

The examined quarter	The quarter No.	-ve CMT		+ve CMT		+ve CMT samples scores							
						Score ±		Score +		Score ++		Score +++	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
FL	25	15	60	10	40	1	10	6	60	1	10	2	20
FR	25	12	48	13	52	3	23.08	5	38.46	4	30.77	1	7.69
HL	25	10	40	15	60	2	13.33	10	66.67	3	20	0	0
HR	25	10	40	15	60	0	0	8	53.33	5	33.33	2	13.33
Total	100	47	47	53	53	6	11.32	29	54.72	13	24.53	5	9.43

FL=fore left quarter, FR= fore right quarter, HL= hind left quarter, HR= hind right quarter

**Table 3:** Statistical analytical results of WST in the examined samples

The examined quarter	The quarter No.	-ve WST		+ve WST		+ve WST samples scores					
						Score +		Score ++		Score +++	
		No.	%	No.	%	No.	%	No.	%	No.	%
FL	25	17	68	8	32	6	75	1	12.5	1	12.5
FR	25	12	48	13	52	7	53.85	5	38.46	1	7.69
HL	25	12	48	13	52	12	92.31	1	7.69	0	0
HR	25	10	40	15	60	11	73.33	4	26.67	0	0
Total	100	51	51	49	49	36	73.47	11	22.45	2	4.08

FL=fore left quarter, FR= fore right quarter, HL= hind left quarter, HR= hind right quarter

**Table 4:** Koestler number of the examined quarter milk samples

Parameter	Quarter%		Koestler number		
	Normal	SCM affected	Normal quarter% (< 2)	Suspected quarter% (2-3)	Abnormal quarter% (> 3)
Chloride%	42%	58%	42%	25%	33%
Lactose%	42%	58%			

**Table 5:** Frequency of the affected quarters in relation to the all affected quarters (58)

Quarter samples	Numbers affected	%
FL	11	18.96
FR	15	25.86
HL	16	27.59
HR	16	27.59
Total	58	100

FL=fore left quarter, FR= fore right quarter, HL= hind left quarter, HR= hind right quarter

**Table 6:** Frequency of causative microorganisms in affected quarters in relation to total isolates (39)

Type of the isolated microorganisms	Number of the isolates		Type of the quarter infection	
	No.	%	single	Mixed
<i>Staph. aureus</i>	25	64.10	20	(4) <i>Staph. aureus</i> + <i>Strept. agalactiae</i> (1) <i>Staph. aureus</i> + <i>E. coli</i>
<i>Strept. agalactiae</i>	11	28.21	6	(4) <i>Strept. agalactiae</i> + <i>Staph. aureus</i> (1) <i>Strept. agalactiae</i> + <i>E. coli</i>
<i>E. coli</i>	3	7.69	1	(1) <i>Staph. aureus</i> + <i>E. coli</i> (1) <i>Strept. agalactiae</i> + <i>E. coli</i>
Total	39	100	27	

**Table 7:** Number of the quarter milk samples in relation to coliforms counts

Count/ g.	No. of the quarter showed coliforms	No. of the quarter showed fecal coliforms	No. of the quarter showed <i>E. coli</i>
< 3	52	55	55
3 - < 10	2	1	1
10 - 100	2	1	1
> 100 - 1000	1	0	0
> 1000	1	1	1
Total	58	58	58

## DISCUSSION

As shown in Table 1, it was found that the rate of SCM among the examined dairy cows revealed an incidence rate of 84% at the cow level. Nearly similar result was obtained by Duguma *et al.* (2014) and lower results were obtained by Ayano *et al.* (2013); El- Kholy *et al.* (2018). The rate of positive SCM in the examined milk samples revealed an incidence of 53% depending on +ve CMT at the quarter level. Nearly similar results of SCM using CMT were reported by Wahba *et al.* (2005); Enany *et al.* (2007); Mureithi and Njuguna (2016). Lower results were

reported by Tanwar *et al.* (2001); Al-Hawary *et al.* (2003); Hussein (2012); Saidi *et al.* (2013); Barua *et al.* (2014); Sanotharan *et al.* (2016). Higher results were recorded by Kasikci *et al.* (2012); Kamal *et al.* (2014). The increased incidence of SCM among dairy animal may be attributed mainly to poor hygiene practices, inadequate housing & bedding, malfunctioning milking machines, improper milking procedures and inadequate treatment methods. Treatment failures of SCM are common and may be due to delayed treatment, poor selection of drugs & dose level, stopping treatment too soon, resistance of invasive organisms or deep seated infections that are protected by scar tissues (Philpot, 1984).

CMT principle is based upon the amount of cellular nuclear protein in the milk sample, thus correlated to SCC (Greiner *et al.*, 2000). CMT possesses many advantages, as the higher sensitivity, simplicity and accuracy; in addition, presence of foreign material, such as hair or other matter, does not interfere with the test.

The percentage of SCM among different quarters in the present study (Table 2) revealed that +ve CMT% samples were 40% in fore left, 52% in fore right, 60% in hind left and 60% in hind right quarters. The higher prevalence of infection in the hind quarters may be due, in part, to the morphological structure of the udder (Donagh and William, 2005). It may also be attributable to the greater milk yield produced by the hind quarters (Lancelot *et al.*, 1997).

As shown in Tables 1 & 3, it was found that the rate of SCM among milk samples by WST revealed an incidence rate of 49% at the quarter level. Nearly similar results of SCM by +ve WST were obtained by Shahid *et al.* (2011); Kabir *et al.* (2017); while lower incidences were obtained by Iqbal *et al.* (2004); Zahid (2004); Barua *et al.* (2014).

The obtained results in Table 3 showed +ve WST was in 2 quarters (4.08%) with degree +++, 11 ones (22.45%) with degree ++, 36 ones (73.47%) with degree +. The percentage of SCM among different quarters in the present study revealed that incidence of SCM in the hind quarters was higher than fore quarters.

Table 4 summarized the lactose% in relation to the percentages of normal and affected quarters; nearly similar result was reported by Swami *et al.* (2017). As the concentration of lactose decreased, compensation must be made to ensure that milk and blood maintain the same osmotic pressure. Most of this compensation is accomplished by increase of sodium and chlorides (Schalm, 1977). Ductal and secretory epithelium malfunction due to microbial infection leads to sharp increase of sodium and chlorine, in addition to break down of junctions between secretory cells, and the increased permeability of the blood capillaries. Thus, chlorine flowed into milk (Batavani *et al.*, 2007). This explains the possibility of adopting chlorine% in milk as an indicator for presence of SCM (Morsi *et al.*, 2000), however, chlorine% alone cannot judge the presence of mastitis as it usually give high results in colostrum or at late stage of lactation. Chloride% in the present study was different from that obtained by Bhoyar *et al.* (2010); and nearly similar to that reported by Kamal *et al.* (2014); Gupta *et al.* (2017).

Owing to the fact that chloride and lactose% in milk account for approximately 75% of its osmotic pressure, many attempts have been made to establish the correlation between them on a complementary basis; among these attempts was those of Koestler

(El-Sokkary and Hassan, 1950). The obtained results of Koestler number were listed in Table 4 as 42% of the quarter's milk samples as normal, 25% of the quarter's milk samples as suspected and 33% of the quarter's milk samples as mastitic. The listed results (Table 4) declared that all the positive milk samples to CMT and WST had Koestler number ranged from 2 to 3 as suspected or more than 3 as mastitic.

According to the mentioned results in Table 5, the incidence of SCM in the examined milk samples according to the affected quarters were 11 (18.96%) for FL, 15 (25.86%) for FR, 16 (27.59%) for HL and 16 (27.59%) for HR.

When focusing the light on the bacteriological examination listed in Table 6, it was found that a total of 39 bacterial isolates were identified as *Staph. aureus* (64.10%), *Strept. agalactiae* (28.21%) and *E. coli* (7.69%). The problem of these microorganisms not only economic or disturb animal health but also, produce a public health hazard to the human being.

It was concluded that there was a high incidence of SCM in the dairy cows located in Sohag city; and CMT & WST findings represented valuable diagnostic methods in detection of cows with secretion disorders that showed no clinical signs of mastitis.

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## الكشف عن إلتهاب الضرع الخفي في لبن الأبقار الحلاب في مدينة سوهاج ، مصر

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تم تجميع عدد ١٠٠ عينة لبن من كل ربع من أرباع ٢٥ بقرة حلابة بضرع سليم ظاهريا من مدينة سوهاج، مصر، وذلك لفحصهم للكشف عن إلتهاب الضرع الخفي بواسطة إختبارات strip cup test والكاليفورنيا CMT و White Side test (WST) وكذلك الإختبارات الكيميائية والبكتريولوجية. وقد أظهرت نتائج إختبار الكاليفورنيا أن ٥٣% من العينات كانت إيجابية لإلتهاب الضرع الخفي بدرجات مختلفة، وأن نتائج إختبار WST هي ٤٩% من العينات كانت إيجابية لإلتهاب الضرع الخفي بدرجات مختلفة كذلك. وقد تبين من خلال تقدير قيمة رقم كويسلر أن ٤٢% من العينات كانت أقل من ٢ أي أنها طبيعية و ٢٥% تراوحت من ٢ إلى ٣ أي أنها يحتمل إصابتها بإلتهاب الضرع الخفي وعدد ٣٣% من العينات كانت إيجابية لإلتهاب الضرع الخفي. وبالنسبة للإختبارات البكتريولوجية للعينات الإيجابية لإختبارات الكاليفورنيا و WST ورقم كويسلر والتي تم زرعها علي المستنبتات البكتيرية حيث تم عزل ٢٥ عترة من المكور العنقودي الذهبي و ١١ عترة من المكور السبحي أجالاكتيا و ٣ عترات من الإيشيريشيا كولاى بنسب ٦٤,١٠%، ٢٨,٢١%، ٧,٦٩% علي الترتيب وذلك من إجمالي ٣٩ عترة من المسببات البكتيرية.

**الكلمات الكاشفة:** إلتهاب الضرع الخفي، إختبار الكاليفورنيا، إختبار WST، رقم كويسلر.