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DETECTION OF SUBCLINICAL MASTITIS IN A DAIRY FARM IN BENI-SUEF CITY, EGYPT

ADEL MUSTAFA EL-KHOLY; GAMAL MOHAMED HASSAN; MOHAMED MAAROUF ALI ZEINHOM and MOHAMED ADEL AHMED MOHAMED EL-KLAWY
Food Hygiene Department, Faculty of Veterinary Medicine, Beni-Suef University, Egypt.

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ABSTRACT

A total of 116 quarter milk samples were collected aseptically from apparently healthy udders of 29 cows in a dairy farm in Beni-Suef city, Egypt; for detection of subclinical mastitis using California mastitis test (CMT), somatic cell count (SCC), chemical and microbiological examination. Thirteen cows (44.83%) were subclinically mastitic with 27 mastitic quarters (23.28%).The scores of CMT showed 11 quarters (40.74%) as +1 and 16 quarters (59.26%) as +2. The SCC of fore left (FL) quarter milk samples was $4.3\times10^5\pm1.2\times10^5$, while of fore right (FR) quarter milk samples was $3.8\times10^5 \pm1.1\times10^5$, but for SCC of hind left (HL) quarter milk samples was $2.4\times10^5\pm9.5\times10^4$ and SCC of hind right (HR) quarter milk samples was $2.2\times10^5\pm7.9\times10^4$. The isolated micro-organisms from the examined milk samples were *Staphylococcus aureus*, *Coagulase negative Staphylococci* (CNS), *Streptococcus spp*, *E.coli* and *Aspergillus fumigatus*. The present study assured that the indirect tests of subclinical mastitis are more suitable for selecting cows with intramammary infections for subsequent bacteriological sampling.

Key words: Subclinical mastitis, CMT, SCC.

INTRODUCTION

Among the animal diseases which affect the profitability of rearing animals, mastitis is considered to be one of the most expensive diseases in terms of production losses (Bardhan, 2013). Mastitis is a very devastating disease of dairy animals which influences the quality and quantity of milk (Akhtar *et al*., 2012). In case of mastitis, dairy industry suffers economic losses because of low quality milk that is not fit for human consumption, decrease in milk yield, premature culling of animals and replacements (Batavani *et al*., 2007).

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 gross abnormalities in the milk or udder in case of subclinical mastitis. Most of the mastitis is subclinical in nature and its prevention depends primarily on good management practices in dairy herd which includes stress-free environment, proper maintenance and operation of milking equipment, good milking procedures (Konwar *et al*., 2009).

Corresponding author: Dr. MOHAMED ADEL
E-mail address: m.adel39@yahoo.com
Present address: Food Hygiene Department, Faculty of Veterinary Medicine, Beni-Suef University, Egypt.

Subclinical mastitis is an inflammation of the mammary gland without noticeable signs, although it is accompanied by 15-45% reduction in daily milk yield and altered milk composition (Swinkels *et al*., 2005; Halasa *et al*., 2007). Subclinical mastitis 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). Subclinical mastitis can be recognized indirectly by several diagnostic methods including the California mastitis test (CMT) and somatic cell count (SCC); These tests are preferred to be screening tests for subclinical mastitis as they can be used easily, yielding rapid as well as satisfied results (Joshi and Gokhale, 2006).

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 the main etiological agents of mastitis in dairy cows (Unal and Yildirim, 2010). Although *Staphylococcus aureus* has been described as one of the most important mastitis pathogens in cattle, coagulase-negative Staphylococciare increasingly becoming recognized as etiologic agents associated with intramammary infections (IMI) in most countries (Unal *et al*., 2012).

Seriousness of mycotic infection of mammary glands depends upon the species of the fungus involved as well as the percentage of infectivity (Tarfarosh and Purohit, 2008). Bovine mycotic mastitis is usually caused by yeasts, but mastitis due to filamentous fungi mostly *Aspergillus fumigatus* has been reported; it occurs as sporadic cases affecting a small percentage of cows or as outbreaks affecting the majority of animals (Abd El Razik *et al*., 2011). Fungal infections account for 2%–13% of all cases of mastitis in cows in Poland (Krukowski *et al*., 2000; Krukowski *et al*., 2006).

Otherwise, from public health view, the assessment of subclinical mastitis etiological pathogens aids to classify the healthy sound milk samples from those of public health hazard as the limits recommended by European countries standards (IDF, 1996) and Egyptian standards (Egyptian Standards, 2001). Therefore, the aim of this work is to detect the subclinical mastitis in a dairy farm in Beni-Suef city by using field tests, chemical examination, isolation and identification of different pathogens causing subclinical mastitis.

MATERIALS AND METHODS

Collection of the samples:
A total of 116 quarter milk samples were collected aseptically from apparently healthy udders of 29 cows in a dairy farm in Beni-Suef city according to the procedure recommended by Quinn *et al*. (2002). The samples were properly packed, stored in an ice box (at 4^{o} C) and transferred to the laboratory with a minimum of delay to be examined chemically and microbiologically.

Preparation of the samples (APHA, 1992):
Each sample was divided aseptically into 2 parts. The 1st was transferred to the lab for chemical examination and numbering of the somatic cell count, while the 2nd one was used for microbiological examination.

Examination of the samples:

1- California mastitis test (CMT), (Saloniemi, 1995):
A plastic vessel with 4 shallow wells was used for collecting approximately 2 ml of milk from each udder quarter, then equal amount of alkali reagent (kerbl® 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, according to the system used in the Nordic countries as the scoring is made from 1-5.

2- Somatic cell count (SCC):
All the milk samples were examined automatically for somatic cell count by using The Nucleo Counter® SCC-100™. The sample was warmed in water bath at 35°C for 5 minutes, and then mixed automatically before reading (Radostitis *et al.,* 2000).

3- Chemical examination:
All the milk samples were examined using Lactoscan milk analyzers (Ultrasonic portable milk analyzer, LSSP001, Bulgaria) for lactose, protein and fat%.

4- Microbiological examination:
4-a) Cultivation of the samples (Sayed *et al*., 2011).
All the milk samples were examined microbiologically by collecting 10 ml of a well-mixed milk sample and added in a sterile plastic centrifugated tube, then centrifugated at 3000 r.p.m. for 20 minutes and the cream and supernatant fluids were discarded. A loopful from the sediment was taken and streaked onto the surface of Azide maltose agar for *Streptococcus spp*, Baired parker agar for *Staphylococcus spp*, MacConkey agar for Enterobacteriacae and Sabouraud dextrose agar with chloramfenicol (CMF) 500 mg for yeasts and molds. The suspected organisms were cultured on nutrient and Sabouraud slope agars which incubated at 37^{o}C for 24-48 hours as well as 25^{o}C for 5-7days, respectively.

4-b) Identification of the isolated organisms was done according to APHA (1992); Koneman *et al*. (1992); Collee *et al*. (1996); Quinn *et al.* (2002) based on their Gram-reaction, colony growth and further confirmation.

RESULTS

Table 1: The prevalence of subclinical mastitis at udder–quarter level.

No. of the infected animals		No. of the examined quarters	CMT				SCC				Bacteriological result				Yeasts & molds Result			
			Positive		Negative		Positive		Negative		Positive		Negative		positive		Negative	
No.	%		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
13	44.83	116	27	23.28	89	76.72	27	23.28	89	76.72	27	23.28	89	76.72	11	9.48	105	90.52

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

The examined quarter	The quarters No.	Negative CMT samples		Positive CMT samples		Positive CMT Samples							
						Score -		Score +		Score ++		Score +++	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
FL	**29**	**20**	**68.97**	**9**	**31.03**	**0**	**0.00**	**2**	**22.22**	**7**	**77.78**	**0**	**0.00**
FR	**29**	**20**	**68.97**	**9**	**31.03**	**0**	**0.00**	**4**	**44.44**	**5**	**55.56**	**0**	**0.00**
HL	**29**	**24**	**82.76**	**5**	**17.24**	**0**	**0.00**	**3**	**60**	**2**	**40**	**0**	**0.00**
HR	**29**	**25**	**86.21**	**4**	**13.79**	**0**	**0.00**	**2**	**50**	**2**	**50**	**0**	**0.00**
Total	**116**	**89**	**76.72**	**27**	**23.28**	**0**	**0.00**	**11**	**40.74**	**16**	**59.26**	**0**	**0.00**

FL=foreleft, FR=foreright, HL=hindleft, HR=hindright.

Table 3: Frequency percentages of single and mixed infection in the quarter milk cow's samples.

The examined quarter	Single infection		Mixed infection					
			Double infection		Triple infection		Tetra infection	
	No.	%	No.	%	No.	%	No.	%
FL	**2**	**7.41**	**4**	**14.81**	**3**	**11.11**	**0**	**0.00**
FR	**2**	**7.41**	**4**	**14.81**	**2**	**7.41**	**1**	**3.70**
HL	**5**	**18.52**	**0**	**0.00**	**0**	**0.00**	**0**	**0.00**
HR	**3**	**11.11**	**1**	**3.70**	**0**	**0.00**	**0**	**0.00**
Total	**12(44.44%)**		**9**	**33.33**	**5**	**18.52**	**1**	**3.70**
			15(55.56%)					

FL=foreleft, FR=foreright, HL=hindleft, HR=hindright.

Table 4: Incidence of the identified microorganisms in relation to the total isolates (49).

Type of the isolated microorganisms	Number of isolates		Type of infection
	No.	%	
Staphylococcus aureus	**7**	**14.28**	**Single and mixed**
Coagulase negative Staphylococci (CNS)	**6**	**12.24**	**Single and mixed**
Streptococcus Spp.	**5**	**10.2**	**Mixed**
E. coli	**20**	**40.82**	**Single and mixed**
Aspergillus fumigatus	**11**	**22.45**	**Mixed**
Total	**49**	**100.00**	

Table 5: Statistical analytical results of SCC/ml of the examined samples.

The examined quarter	Normal				Subclinical mastitis				Mean± S.E.M.
	No.	%	Min.	Max.	No.	%	Min.	Max.	
FL	**20**	**68.97**	**1×10^4**	**3.2×10^5**	**9**	**31.03**	**3.8×10^5**	**2×10^6**	**$4.3\times10^5\pm1.2\times10^5$**
FR	**20**	**68.97**	**1×10^4**	**2.9×10^5**	**9**	**31.03**	**4×10^5**	**2×10^6**	**$3.8\times10^5\pm1.1\times10^5$**
HL	**24**	**82.76**	**1×10^4**	**2.5×10^5**	**5**	**17.24**	**3.8×10^5**	**2×10^6**	**$2.4\times10^5\pm9.5\times10^4$**
HR	**25**	**86.21**	**1×10^4**	**3.5×10^5**	**4**	**13.79**	**4×10^5**	**2×10^6**	**$2.2\times10^5\pm7.9\times10^4$**

FL=fore left, FR=fore right, HL=hind left, HR=hind right, Min. = minimum, Max. =maximum

Table 6: Frequency distribution of SCC/ml of the examined samples.

The quarter state	Intervals	The examined quarter							
		FL		FR		HL		HR	
		No.	%	No.	%	No.	%	No.	%
Normal	1×10^4 - < 3.5×10^5	20	68.96	20	68.96	24	82.76	25	86.20
Subclinical mastitis	3.5×10^5 - < 6.9×10^5	2	6.90	4	13.79	3	10.34	2	6.90
	6.9×10^5 - < 1×10^6	1	3.45	0	0.00	0	0.00	0	0.00
	1×10^6 - < 1.3×10^6	1	3.45	1	3.45	0	0.00	1	3.45
	1.3×10^6 - < 1.7×10^6	2	6.90	2	6.90	0	0.00	0	0.00
	1.7×10^6 - ≤ 2×10^6	3	10.34	2	6.90	2	6.90	1	3.45
	Total	29	100	29	100	29	100	29	100

FL=foreleft, FR=foreright, HL=hindleft, HR=hindright.

Table 7: Correlation between the positive CMT and the microbiological results of the examined samples.

CMT score	No. of the samples	Bacteriological result		Agreement %	Yeasts & molds result		Agreement %
		+ve	-ve		+ve	-ve	
-	89	0	89	-	0	89	-
+	11	11	0	100	4	7	36.36
++	16	16	0	100	7	9	43.75
+++	0	0	0	-	0	0	-

Table 8: Compositional changes in the milk constituents associated with elevated SCC.

Constituent %	Average of normal milk according to NMC (1987)	Average of milk constituents with high SCC (%)			
		FL	FR	HL	HR
SNF	8.9	8.5	8	9	8.9
Fat	3.5	2.8	2.8	3.2	4.3
Lactose	4.9	4.7	4.8	5.1	4.9
Total protein	3.61	3.4	2.9	3.4	3.4

DISCUSSION

CMT principle is based upon the amount of cellular nuclear protein present in the milk sample, thus correlated to SCC (Greiner *et al*., 2000).

The results listed in Table (1) showed that 13(44.83%) cows of the total examined 29 dairy cows had subclinical mastitis and consequently, the 116 examined cows' quarter milk samples classified into 89(76.72%) CMT negative and 27(23.28%) CMT positive samples.

The summarized data of SCC as compared with bacteriological examination showed a positive correlation between SCC and bacteriological status in the examined quarter milk samples (Table 1). These results were similar to Fox *et al*. (1985). Some observations in this study were different in results than other studies which may be attributed to the prevention and control programs, sampling, methods of isolation, type of management employed and other factors. The right management leads to a reduction of mastitis and vice versa. Also, the most infectious diseases, mastitis risk factors depend on three components: exposure to udder pathogens, cow defense mechanisms, environmental and management factors (Suriyasathaporn *et al*., 2000).

The obtained results in Table (2) showed that among the CMT positive samples, the highest incidence was recorded in CMT (+2) as 59.26%, while none of the positive CMT showed score +3.

It was clear from the obtained results that CMT used as indicator and screening of bovine mastitis and microbiological status of milk. The CMT has the advantages of being animal – side, inexpensive and rapid to be performed (Contreras *et al*., 1996). From the other side, this test may give positive result as in case of very early (colostrum), late lactation, teat end injury, fluctuating and irregular milking vacuum and abnormal health of cow such as foot rot and uterine infection, one to two weeks following treatment and with non-infected quarters (Robert and Edmondson, 1993; Abdurahman, 2006). So, it should carry out with other tests as SCC and microbiological examination to detect the cause or products of mastitis.

As shown in Table (3) the incidences of single and mixed infection were 44.44% and 55.56% in the positive cows' milk samples for subclinical mastitis, respectively. These findings reflect an idea about the level of environmental microbial contamination (Sayed and Abdel-Hafeez, 2009). In addition, *Staphaureus* may predispose the animals to infection by *coliforms* or other pathogens (Ibtisam *et al*., 1993). On the other hand, Srinivasan *et al*. (2013) reported that single quarter infection was more common compared to mixed quarter infection, but the incidences of mixed and single infection were 80.77% and 19.23% in positive cows' milk samples for subclinical mastitis as obtained by Sayed *et al*. (2011).

Inspection of Table (4) revealed that the main isolated organisms from the examined milk samples were *Staphylococcus aureus*, *CNS*, *Streptococcus spp*, *E.coli* and *Aspergillus fumigatus* in a percentage of 14.28, 12.24, 10.2, 40.82 and 22.45%, respectively. The obtained data were in agreement with that reported by Kassa *et al.* (2014); Alekish, (2015); El-Bagory and Zayda (2015). The problem of these microorganisms not only economic or disturb animal health but also, produce a public health hazard to human being.

Although results of some screening tests often show good correlation with the bacteriological findings, yet no single test was completely satisfactory for detection of subclinical mastitis (EL-Kholy *et al*., 1994). The most important factor affecting the SCC of the milk from an individual quarter depends upon the infection status of the quarter (Dohoo and Meek, 1982).

The listed results in Table (5) declared that all the cows positive CMT had SCC≥ 200.000 cells/ml so, all these cows defined as having subclinical mastitis (Haltia *et al*., 2006; Moroni *et al*., 2006).

The results of CMT as compared with bacteriological, yeasts and molds examination were recorded in Table (7) from which it was evident that 89 out of the 116 quarters cow's milk samples showed (-) CMT score, negative bacteriological and negative yeasts and molds examination; and in compatable, all the isolated microorganisms were from the positive CMT samples.

CONCLUSION

SCC, CMT and intramammary infection are associated significantly; therefore, these parameters provide good information to evaluate udder health status in cows.

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الكشف عن إلتهاب الضرع الخفي في مزرعة حلاب ببني سويف، مصر

عادل مصطفي الخولي ، جمال محمد حسن ، محمد معروف علي زينهم ، محمد عادل القلاوي

E-mail: m.adel39@yahoo.com Assiut University web-site: www.aun.edu.eg

فى هذه الدراسة تم جمع إجمالي ١١٦ عينة لبن من أرباع ٢٩ بقرة حلاب بضرع ظاهري سليم من مزرعة حلاب ببني سويف، مصر، وتم فحص العينات للكشف عن إلتهاب الضرع الخفي وذلك بواسطه إختبار الكاليفورنيا وعد الخلايا الجسدية والإختبارات الكيميائية والميكروبيولوجية وأوضحت النتائج أن إلتهاب الضرع الخفي وجد في ١٣ بقرة (٤٤,٨٣%) تحتوى على ٢٧ ربع مصاب (٢٣,٢٨%) مقسمة إلى ١١ ربع (٤٠,٧٤%) معطياَ المستوى رقم ١ وعدد ١٦ ربع (٥٩,٢٦%) معطًياَ المستوى رقم ٢ طبقاَ لنتائج إختبار الكاليفورنيا وكان عدد الخلايا الجسدية فى الأرباع الأمامية شمال $٤,٣ \times ١٠^{٥} \pm ١,٢ \times ١٠^{٥}$ والأمامية يمين $٣,٨ \times ١٠^{٥} \pm ١,١ \times ١٠^{٥}$ والخلفية شمال $٢,٤ \times ١٠^{٥} \pm ٩,٥ \times ١٠^{٤}$ والخلفية يمين $٢,٢ \times ١٠^{٥} \pm ٧,٩ \times ١٠^{٤}$. وقد تم عزل المكور العنقودى الذهبي والمكور العنقودي سالب التجلط والمكورات السبحية والإيشرشيا كولاي والأسبرجيلس فيومجاتس من عينات اللبن المفحوصة. وأكدت الدراسة الحالية على إن الإختبارات الغير مباشرة لفحص إلتهاب الضرع الخفي أكثر ملائمه لإختيار الأبقار الذين لديهم عدوى داخلية بالضرع وذلك لأخذ عينات للفحص البكتريولوجى لاحقاَ.

الكلمات المفتاحية: إلتهاب الضرع الخفي ، إختبار الكاليفورنيا، عدد الخلايا الجسدية.