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MICROBIOLOGICAL PROFILE OF SUBCLINICAL MASTITIC COW MILK AND ITS CORRELATION WITH FIELD TESTS AND THE SOMATIC CELL COUNT

(With 4 Tables)

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الصورة الميكروبيولوجية لألبان الأبقار المصابة بالتهاب الضرع الخفى وعلاقتها بالاختبارات الحقلية وكذلك العد الخلوى الجسمى

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بفحص عدد ٨٥ بقرة فريزيان هولشتين للالتهاب الضرع الخفى حقلياً بواسطة إختبار (MWT) واختبار كاليفورنيا ، تم أخذ ٢٦٠ عينة لبن من أرباع ضرع الأبقار وأظهرت النتائج ٢٤٤ (٩٣,٨%) ، ١٢٤ (٤٧,٧%) نتائج إيجابية على الترتيب. تم أخذ ٨٥ عينة لبن (مجمعة من الأرباع للبقرة الواحدة) وقد فحصت بنفس الاختبارين السابقين ومعهم اختبار عد الخلايا الجسمية فى اللبن فضلاً عن العزل البكتيرى للمسببات مع عدها. وقد أظهرت النتائج أن ٨٧ ، ٦٧ ، ٧٣% كانت إيجابية للاختبارات على الترتيب. تمت دراسة حساسية هذه الاختبارات وكانت ٨٦,١ ، ٤٦,٦ ، ٧٤,٧% للاختبارات الثلاثة على الترتيب بينما أظهرت دراسة التوافق لهذه الاختبارات الثلاثة مع العزل البكتيرى لميكروبات التهاب الضرع الكبرى أنها كانت ٨٠ ، ٦٠ ، ٧٢,٩% على الترتيب. نسب عزل ميكروبات التهاب الضرع الكبرى (السبحى ، الكوليفورم ، باسيلس سيريس ، سيدوموناس ايرجينوزا والعنقودى الذهبى) كانت ٦٤ ، ٤٧ ، ٣٣ ، ١٧ ، ١٠% على الترتيب ، أما الصغرى (العنقودى سلبى كوأجيولز، العفن والخمائر) فكانت ٦٦ ، ٥٩ ، ٥٧ على الترتيب. أثبت اختبار عد الخلايا الجسمية فى اللبن أنه هو الاختبار الاستكشافى الوحيد الذى يرتبط ارتباطاً ذو دلالة مع نسب العد الكلى البكتيرى فى حين أن اختبار الكاليفورنيا يرتبط ارتباطاً ذو دلالة عالية بكل من MWT واختبار عد الخلايا الجسمية فى اللبن. وخلصت الدراسة إلى أنه يجب استخدام أكثر من اختبار للكشف عن التهاب الضرع الخفى وخصوصا اختبار MWT والعد الخلوى الجسمى وكذا العد الكلى للميكروبات بصفة دورية وذلك حفاظا على صحة القطيع وصحة المستهلك.

SUMMARY

From a total of 85 Holstein Fresian cows which were subjected to subclinical mastitis using modified white test (MWT) and California mastitis test (CMT), 260 quarter milk samples showed 244 (93.8%) and 124 (47.7%) positive results respectively. 85 individual cow milk samples were subjected to MWT, CMT and somatic cell count (SCC) rather than bacteriological isolation and count. it is concluded that 87, 67 and 73% were positive results. test Sensitivity determination indicated that MWT, CMT and SCC showed 86.1%, 64.6% and 74.7% sensitivity. While their agreement with major pathogens isolation were 80, 60 and 72.9%, respectively. Major mastitis pathogens (Streptococcus spp., Coliforms, bacillus cereus, Pseudomonas aurogenosa and Staph aureus) showed prevalences of 64, 47, 33, 17 and 10%, respectively. Minor mastitis pathogens (Staph coagulase-ve, mold and yeast) showed 66, 59 and 57, respectively. SCC was the only screening test that correlated significantly (P>0.05) with the total bacterial count (TBC). CMT showed highly significant correlation coefficient (P>0.001) with each of MWT & SCC. The study recommended usage of more than an indirect screening tests to detect subclinical mastitis specially MWT and SCC. Moreover, periodical examination for TBC was essential either for herd health or milk hygiene and consumption.

Keywords: *Subclinical mastitis, field tests, somatic cell count, total bacterial count*

INTRODUCTION

Mastitis in both clinical and subclinical stages is a frustating, costly and complex disease that reduces the quality and quantity of milk. Annual losses in the dairy industry due to mastitis was approximately $ 2 billion in the United States, 526 million $ in India, in which subclinical cases are responsible for approximately 70% of these dollars losses (Crist and Harmon, 1991; Varshney and Naresh, 2004).

Mastitis affects the composition of milk and the degree of changes depends on the infecting agent and the inflammatory response (Pyorala, 2003). One of the early events of an infection is the movement of white blood cells or leukocytes into the udder to fight the infection, the end result is an increase in the number of somatic cells in milk

(Harmon, 1994). All pathogens resulted in a significant increase of somatic cell count (SCC) in individual milk samples (Janosi and Baltay, 2004). Identifying and eliminating intra mammary infection (IMI) may have significant benefits as preventing clinical mastitis, decreasing the amount of discarded milk and reducing the bulk milk somatic cell count (Wallace *et al.*, 2002). Additionally, knowledge of the clustering of an IMI, either within quarters of a cow or within a herd, may be of considerable interest and may lead to further understanding of the dynamics of the disease (Lam *et al.*, 1996).

Many recent investigations (National Mastitis Council, 1999; Dingwell *et al.*, 2003 and Milne *et al.*, 2003 had assured that bacteriological culture is the standard method for identifying the IMI but till nowadays the bacteriological sampling is not feasible as a routine test to identify subclinical mastitis. The indirect tests of mastitis seem to be more suitable for selecting cows with intramammary infections (Pyorala, 2003). Also, Sargeant *et al.* (2001) added that logestic and financial considerations involved in sampling all quarters for bacteriological culture have precluded the widespread adoption of this strategy in the dairy industry.

The prevalence of subclinical mastitis in dairy herds is often surprising to producers moreover, subclinically infected udder quarters can develop clinical mastitis and the rate of new infections can be high (Zdunczyk *et al.*, 2003). Cows with subclinical mastitis are those with no visible changes in the appearance of the milk or the udder, but milk production decreases. Bacteria are present in the milk and composition is altered (Rice, 1997). These unseen infections can be detected indirectly by several methods including The Modified White Side test (MWT), The California mastitis test (CMT) and SCC test. These tests are preferred to be screening tests for subclinical mastitis as they can be used easily, yielding rapid as well as satisfied results (El-Balkemy *et al.*, 1997 and Lesile *et al.*, 2002).

Rossetti (1993) and Alacam *et al.* (1994) found that MWT is more reliable and suitable for diagnosis of subclinical mastitis when compared with other indirect tests.

It is hypothesized that California Mastitis test (CMT) is an efficient, useful predictors of IMI in fresh cows than other inspection tests but it has some disadvantages as yielding some false positive reactions frequently when cows have been freshly less than 10 days calving or with cows that are nearly dry. Furthermore, scoring the test

may vary between individual testers and these scores represent a range of leucocyte content rather than the exact count (Rice, 1997).

On the basis of Ministry of Agriculture and Food, Ontario, Canada, quarter and cow SCC directly represent the inflammatory status of the mammary gland. So, SCC is considered the best over all indicators of subclinical mastitis and an effective tool in controlling mastitis (Nazem and Azab, 1998; Schukken *et al.*, 2003; Green *et al.*, 2004 and Janosi and Baltay, 2004). It is usually increased at least 10 folds in subclinical mastitis, thus it has been used as basis for designing rapid diagnostic tests for udder infection (Attia *et al.*, 2003). On the other hand, Middelton *et al.* (2004) referred to the necessity of bacteriological assessment procedures as neither CMT nor SCC is sensitive enough to be useful as a screening test for identifying infected mammary quarters among dairy cattle. In addition, the analysis technique of SCC is problematic for routine use in herds (Pyorala, 2003). So, new mastitis detection system which can be easily adopted are urgently needed and the standard of screening tests accuracy should be supported by bacterial assessments.

Otherwise, from public health view, the assessment of subclinical mastitis etiological pathogens aids to clasify the healthy sound milk samples from those of public health hazard as the limits recommended by European Countries Standards (International Dairy Federation, IDF) (1996) and Egyptian Standards (2001).

The present work may be of practical importance, it intended to establish whether which of the field tests-currently used as a mastitic indicator-could be contributed to misdiagnosis. It aimed also to study whether the bacteriological profile status of the udder correlated with field tests and the SCC.

MATERIALS and METHODS

I- Samples Collection:

Eighty five Holstein Fresian cows with clinically sound udder and secreting apparently normal milk were included in this study. These cows were milked twice daily in Alfa-laval milking parlor with automatic milk removal. Teats were prepared aseptically, prior to sample collection, according to the National Mastitis Council (1999).

A total of 340 quarter samples were examined, from which 80 quarters were blind non lactating (BQ). Milk samples (260) were examined at

once on each cow using the field tests (MWT and CMT) according to APHA (1992). Two reagents were used for CMT (Schalm reagent and Muv Feild Multitest reagent) according to the manufacturer's recommendations. Feild tests results were recorded and scored according to Rice (1997).

Individual cow milk samples (n=85) were collected in sterile screw capped bottles and subjected to the following examinations.

II- Laboratory tests:

A- Cytological examination: determination of the SCC was carried out and scored according to IDF (1984) and Rice (1997).

B- Microbiological examination and identification was adopted according to Quinn *et al.* (1994). Bacteriological causes of IMI were categorized as major and minor pathogens (Harmon, 1994 and Sargeant *et al.*, 2001).

* Isolation and bacterial count of major pathogens:-

1- Staph aureus count was applied by surface plate method on Baird Parker agar (Biolife, 20/28) as recommended by ICMSF (1978).

2- Streptococcal count was carried out using Sodium Blood Azide agar as described by Smeath *et al.* (1986).

3- Coliform count was adopted as recommended by Mercuri and Cox (1979) using Violet Red Bile agar (FAO, 1992).

4- Bacillus cereus count using KG media according to Kim and Geopfert (1971).

5- Pseudomonas aureginosa count was performed as Lowbury (1951) using Pseudomonas Selective Agar Base Cetrimide agar.

* Isolation and bacterial count of minor pathogens:-

1- Coagulase-negative staphylococci using Baird Parker agar (ICMSF, 1978).

2- Yeast and mold count according to Harrigan and Mc Cance (1976) using Sabaroud Dexrose agar (Difco lab.).

*Total bacterial count using standard plate count technique according to APHA (1992).

III- Sensitivity and agreement percentages were calculated according to Cochrane and Holland (1971) and Noon *et al.* (1980).

IV- Statistical analysis: Correlation coefficient was carried out among all parameter tested.

RESULTS

The results were manifested and tabulated in (1-4) tables.

Table 1: Mastitis field tests for quarter cow milk samples.

Score results	Quarter milk samples														
	Right fore BQ = 20			Left fore BQ = 20			Right hind BQ = 22			Left hind BQ = 18			Total udder quarters BQ = 80		
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
-	5	35	36	4	34	34	4	33	34	3	34	33	16	136	137
+	8	4	3	9	0	0	9	1	0	8	1	1	34	6	4
++	7	3	3	5	2	1	7	6	7	9	2	2	28	13	13
+++	45	23	23	47	29	30	43	23	22	47	30	31	182	105	106
Total reactors No.	60	30	29	61	31	31	59	30	29	64	33	34	244	124	123
%	92	46	45	93	47	47	93	47	46	96	43	50	93.8	47.7	47.3
Total	65			65			63			67			60		

BQ : Blind non lactating quarter.
a : Modified white side test
b : CMT using Schalm reagent
c : CMT using Muv field mastitest

Table 2: Statistical analytical results of Microbiological examination of individual cow milk samples (n= 85).

Microbial aspects	Positive samples		Min.	Max.	Mean
Cfu/mL	No.	%			
*** Major pathogens**					
Staph aureus	10	11.76	$1x10^2$	$4x10^3$	$1.3x10^2$
Strept organisms	64	75.29	$3x0^2$	$4x10^5$	$3.3x10^4$
Coliforms	47	55.29	$1x10^3$	$2x10^5$	$1.4x10^4$
Bacillus cereus	33	38.82	$1x10^2$	$8x10^3$	$4.8x10^2$
Pseudomonas auriginosa	17	20	$1x10^2$	$7.8x10^2$	$2.8x10^2$
*** Minor pathogens**					
Staph coagulase-ve	66	77.64	$2x10^2$	$3x10^4$	$5.5x10^3$
Mold	59	69.41	$1x10^3$	$1.5x10^4$	$9.6x10^3$
Yeast	57	67.1	$6x10^2$	$9.2x10^4$	$1.5x10^4$
Total bacterial count (TBC)			$8x10^3$	$5x10^8$	$1x10^7$

Table 3: Sensitivity of different tests and their agreement with the bacteriological profile.

Test	Positive		Negative		Sensitivity	Agreement
	No.	%	No.	%	%	%
MWT	74	87	11	13	86.1	80
CMT	57	67	28	33	64.6	60
SCC	62	73	23	27	74.7	72.9
Bacteriological results	79	93	6	7	-	-

Table 4: Pearson correlation coefficients among different tests and bacterial assessment.

	MWT	CMT	SCC	T.B.C.	Staph aureus	Strept	Coliforms	Pseu-domonas	yeast
MWT	-	0.001**	0.28	0.6	0.13	0.38	0.48	0.32	0.37
CMT		-	0.002**	0.38	0.02*	0.21	0.51	0.05*	0.2
SCC			-	0.04*	0.07	0.06	0.94	0.3	0.02*

* Significant correlation coefficient (P>0.05)
** Highly significant coefficients (P>0.001)
other species showing non significant correlation coefficient.

DISCUSSION

There is an increasing focus in milk quality in dairy industry. Interest in improving milk quality through mastitis control became intesified during the recent years. Current guidelines to evaluate subclinical infection status have been based on quarters within a cow (Hogan *et al.*, 1990). Additionally, transmission of intramammary infection occurs not only among cows but also among quarters within a cow (Lam *et al.*, 1997). So, measurements taken from individual animal, ignoring it among quarters could lead to a serious underestimation of treatment effects in such affected quarters (Barkema *et al.*, 1997).

Regarding the infection within quarters, the incidences of subclinical mastitis in the left hind (LH) quarters were 96, 49 and 50% using MWT, CMT with Schalm reagent and CMT with Muv field mastitest reagent respectively. While the incidences were nearly similar in other quarters (Table 1). So, LH appeared to be more susceptible to infection than the other ones. Most studies reported different prevalences between quarters; Adkinson *et al.* (1993), Lam *et al.* (1996) and Barkema *et al.* (1997) who proved that intrammary infections were found less often in front quarters than in near ones. However Adkinson *et al.* (1993) did not detect any incidence differences among quarters.

Bacteriological culture is considered as the standard method for identifying IMI (Dingwell *et al.*, 2003 and Milne *et al.*, 2003. Bacteria that most frequently cause mastitis can be classified into two main categories; major and minor pathogens (Harmon, 1994 and Sargeant *et al.*, 2001). Several studies have estimated the prevalence of IMI due to Staph aureus and have shown wide variations. The obtained finding

(11.76% with mean count of $1.3x10^2$) was coinsident with those recorded by Nazem and Azab, 1998 (17.6%), Abd El-Hafeez, 2002 (9.8%) and Dingwell *et al.*, 2003 (14.8%). While higher incidence obtained by Attia *et al.*, 2003 (80%); Janosi and Baltay, 2004 (32.5) and Shitandi and Kihumbu, 2004 (45.6%).

The strept organisms were the first bacteria to be incriminated as the cause of mastitis, the organism is not an active tissue invador but it multiplies in the milk within the udder elaborating an irritant causing an inflammatory reaction which is mostly sub clinical (Schalm *et al.*, 1971). In the present study, streptococci was the most predominant major pathogen, it was detected in 75.29% with mean count of $3.3x10^4$ (Table 2). These results were more higher than that obtained by Janosi and Baltay, 2004 (32.5%) and Shitandi and Kihumbu, 2004 (11.7%).

It is worth mentioning that coliforms, B. cereus and P. aureginosa were isolated from 55.29, 38.82 and 20% of the examined samples with mean count of $1.4x10^4$, $4.8x10^2$, $2.8x10^2$, respectively (Table 2). Regarding to minor pathogens, coagulase-ve staph organism were isolated in higher percentage (77.64%) while, yeast and mold were relatively parallel, their incidences were 67.1 and 69.41% with mean count of $1.5x10^4$ and $9.6x10^3$, respectively (Table 2). However, Nazem and Azab, 1998 failed to detect yeast and molds from their examined samples.

The importance of coagulase-ve staphylococci as a cause of subclinical mastitis was previously demonstrated by Hodges *et al.* (1984), Malinowski *et al.* (1992). Sargeant *et al.* (2001); Abdel Hafeez (2002) and Shitandi and Kihumbu (2004) added that the most common mastitis pathogen isolated were coagulase-ve staphylococci spp., it could be isolated from 40, 36, 90.1, 45.6 and 50% respectively.

The obtained results in tables 1 & 3 revealed that MWT showed 93.8 and 87% positive reactors for quarters and individual cow samples respectively. These results were more higher than El-Balkemy *et al.* (1997) findings which were 17.47 and 34.26% for quarters and cow samples, respectively. The test detected 45, 47, 43 and 47 (69, 72, 68 & 70%) as strong positive reactions for right front, left front, right hind and left hind quarters respectively (Table 1) which were more higher than those obtained by CMT using both reagents. The test was advisable to be used for diagnosis of subclinical mastitis by Rossetti (1993) and Alacam *et al.* (1994). The test was considered highly sensitive but with certain limitations that should be read within minutes since many milk samples-

both normal and mastitic-would gel upon prolonged contact with NaOH which is its reagent (Schalm *et al.*, 1971).

It is evident that CMT showed very close similar results for quarters 47.7 and 47.3% but the same identical for individual cow samples 67% (Table 1 & 3). They were more higher than those obtained by El-Balkemy *et al.* (1997) which were 15.97 & 13.98% for quarters and cow samples respectively. It is widespread used in dairy fields and recommended (Shitandi and Kihumbu, 2004).

SCC test in the present investigation resulted in 73% positive reactors (Table 3). SCC is considered as the best overall indicators of subclinical mastitis (Nazem and Azab, 1998; Green *et al.*, 2004 and Janosi and Balty, 2004). Otherwise, Harmon (1994) indicated that the use of SCC alone to classify cows as infected and uninfected will result in errors attributed to some factors that influence SCC, such as the normal fluctuation of SCC throughout the course of an infection and increasing SCC with advancing age, summer months, stage of lactation or stresses of various types. Moreover, Middelton *et al.* (2004) concluded that neither CMT nor SCC was sensitive enough to detect IMI because each of the forementioned tests has its limitations for its diagnostic value, thus the interdependance of one test may not be ideal for identifying the IMI.

Tests sensitivity were 86.1, 64.6 & 74.7% for MWT, CMT & SCC respectively. These results go parallel to those obtained by Sargeant *et al.* (2001) but somewhat lower than those detected by Nazem and Azab (1998) and Attia *et al.* (2003). On the other hand, Middelton *et al.* (2004) concluded that neither CMT nor SCC is sensitive enough to be useful as a screening test for identifying IMI.

Test results were in agreement with isolation of major pathogen showed 80, 60 & 72.9% for MWT, CMT & SCC respectively (Table 3). Similar results were obtained by El-Balkemy *et al.* (1997) and Nazem and Azab (1998).

Correlation of the screening tests and bacteriological pathogens-through the previous available literatures-conducted just with the existance of major pathogen. While, in the present investigation, correlation coefficient analysis among all parameters studied was applied into directions. Firstly among and inbetween the indirect screening tests, secondly between these tests and different pathogen count rather than total bacterial count.

The present findings showed that CMT had highly significant correlation coefficient with each of MWT and SCC, while significant correlation between MWT and SCC (Table 4). SCC showed significant correlation with TBC and yeast count (Table 4). The high positive correlation of SCC with bacteriological isolation was obtained by Nazem and Azab (1998) and Attia *et al.* (2003).

From the public health view point, for the sanitation of milk yielded, Egyptian standards required milk to be sold suitable for human consumption to have SCC of 750,000/mL or less. In the present study 62 (72.9%) of the examined samples lied with the range of 300,000-900,000 SCC. High SCC scores affect milk quality as off flavour, poor shelflife and other undesirable characteristics (E.S., 2001). According to European Counteries Standards, milk TBC must not be exceeded than $1x10^5$ cfu/mL. In the present study, 69 (81%) milk samples showed count above that limit. MWT detected 47 (68%), SCC detected 37 (53.6%) and CMT detected only 30 (43%) of these unfit milk sample while all these tests failed to detect two samples. The findings coincided with those of Abdel Hamid, 2002.

From the present findings, it is concluded that a battery of screening tests must be required to judge and decide subclinical mastitis which must include more than an indirect test especially MWT & SCC either for herd health or milk hygiene and consumption. As the satisfied correlation between SCC and TBC, it recommended to examine bulk milk tank daily for SCC and periodically for TBC especially in automatic milking systems as they cause statistically significant increase in TBC as there is no direct control for the appearance of milk and udder, furthermore the great probability of transmission of mastitis pathogen from cow to another (Rotz *et al.*, 2003).

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