



Relationship between Somatic Cell Count and Bovine Subclinical Mastitis in Raw Milk in Egypt

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Abstract

A total of 150 raw milk samples (75 each from Holstein Friesian cows and 75 buffaloes) from Egyptian farms were collected and transferred to the Laboratory to estimate Somatic Cell Count and bacteriological examination for diagnosis of subclinical mastitis. Prevalence of *S.aureus*, *Staphylococcus* spp., *Streptococcus agalactiae* and *Streptococcus* spp. in cow milk samples were 60.0%, 34.7%, 17.3% and 54.7% respectively; the prevalence of the corresponding bacterial species in buffaloes were 48.0%, 32.0%, 10.7% and 50.7%, respectively. On the other hand, prevalence of *E.coli*, *Klebsiella*, *Proteus*, *Morganella*, *Providencia* and *Citrobacter* in cows milk samples, and 14.7%, 70.7%, 76.0%, 49.5%, 56.0% and 2.6% in buffaloes milk, respectively. Milk samples contained SCC lower than 200 000 SCC/ml were mostly culture negative. Samples having 200 000- 500 000 of SCC/ml were mainly infected with Enterobacteriaceae spp. Samples with high SCC (500 000 to 1000 000/ml) was associated with infections caused by most studied bacteria especially *S.aureus* (54.3%), whereas samples with very high SCC ($\geq 1000\ 000$ /ml) was associated with infections caused by *Staphylococcus* spp (62.0%), *Streptococcus agalactiae* (73.0%), *S.aureus* (43.2%), *Streptococcus* spp (53.1%), *E.coli* (43.0%), *Klebsiella* (45.9%), *Proteus* (40.9%), *Morganella* (56.4%), *Providencia* (47.7%) and *Citrobacter* (66.7%). The present study concluded that most of milk samples analyzed contained high bacterial and SCC, therefore attention should be directed towards the health status of the bovine udder and the appropriate measures to minimize the incidence of mastitis.

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Introduction

Mastitis remains to be the most important costly disease of dairy cows (19) which is originated by an extensive spectrum of pathogenic agents that invade the teat canal and proliferate in the udder cistern (13), pathogens include *Staphylococcus aureus*, *Streptococcus agalactiae*, *Corynebacterium bovis* and *Mycoplasma* spp. or environmental pathogens include *Escherichia coli*, *Enterococcus faecalis*, *Streptococcus dysgalactiae* and *Streptococcus uberis* and Coagulase-Negative Staphylococci (CNS) (22). Mastitis can harmfully change the quality of milk and profitable efficiency of farms (41) as the bacterial infection of milk from the diseased cows makes it unfit for human consumption and has zoonotic significance (33). In the dairy industry, both clinical and subclinical mastitis cause great economic losses (1). About 70 to 80% of the estimated \$140 to \$300 loss per cow per year from mastitis belong to low milk production caused by in apparent signs subclinical mastitis (24). Subclinical mastitis is not easy to be diagnosed due to the nonappearance of any clinical symptoms also requires the facilities of a rapid screening test for early onset disease detection (39). Different methods of diagnosis of SCM have been set up including evaluation of SCC which is an evidence of inflammation. Food safety regulations in European countries, Australia, and New Zealand mentioned that SCC more than 400 000 cells/ml milk is unfit for human consumption, the USA above 750 000 cells/ml milk and Canada and

South Africa 500 000 cells/ml milk. As a result, dairy producers have responsibility make sure that milk SCC formed by own herd is constantly at lowest permitted ratio and hence meets the qualification levels in force (20). In Kosovo, Bytyqi et al. (12) showed that the bacterial infection can cause Somatic Cell Count (SCC) as high as above 1×10^6 cells/ml, and they mentioned that the contagious bacteria (*Staphylococcus aureus*, *Streptococcus agalactiae*) generally cause the highest SCC elevation while considerably less SCC in case of the environmental bacteria *Streptococcus dysgalactiae*, *Streptococcus Uberis*, *Corynebacterium* Spp., as well as Coagulase Negative Staphylococcus (CNS). Therefore Somatic Cell Count (SCC) is still an important means to distinguish between healthy and infected animal (5). In addition to SCC estimation, the bacteriological examination of milk samples served as a gold standard method for estimation of different tests used for diagnosis of SCM and evaluation of intramammary infection (26).

The purpose of the present study is to evaluate the correlation between SCC and investigating the occurrence of some subclinical and to discuss the public health importance of subclinical mastitis and its control in dairy farm.

Materials and Methods

2. 1. Animals:

A number of 75 clinically healthy milk-producing cows of Holstein Friesian breed in special dairy farm and 75 buffaloes from private owners were used for milk sampling in Dakahlia governorate, Egypt.

2.2. Collection of milk samples :

Sampling of milk was carried out as previously described (31). Milk samples were obtained from the dairy cows and buffaloes before morning milking. All animals had no symptoms of clinical mastitis at the time of collection. Teat orifices were cleaned and swabbed by a piece of cotton immersed in 70% ethyl alcohol. The first amount of foremilk was discarded; then 50 ml of milk was obtained aseptically from each animal into sterile test tubes. Milk samples were reserved cold during transportation at 4°C and reached to the laboratory to be examined within 2 hours after collection. Each sample was agitated thoroughly before being divided into two parts. The first part was used for cytological examination, while the second was served for bacteriological examinations

2.3. Detection of subclinical mastitis:

2.3.1. Somatic cell count (SCC) :

Collected milk samples were tested for SCC automatically by a Bentley Soma count, 150 (Bentley, U.S.A) as previously described (42). The sample was warmed in a water bath at 40°C for 5 minutes then mixed automatically before automatic evaluation of SCC by Bentley Soma count 150 for dispersion of fat globules. Somatic Cell Count (SCC) indicates the number of white blood cells (which is consisting of macrophages, neutrophils, eosinophils, lymphocytes), and numerous epithelial cell types of the mammary gland in milk that were existing in a large number in case of subclinical mastitis.

2.3.2. Bacteriological examination of milk samples:

From milk samples, 10 µl were inoculated onto Mannitol salt agar, blood agar, Edward's media and MacConkey agar plates according to (26). Plates were incubated aerobically at 37°C for 24-48 h. After that, the plates were examined for colonial morphology, hemolytic characteristics, and pigmentation at 24-48h. Presumptive identification of bacterial isolates was determined according to their colonial characteristics, Gram's reaction and morphology. Identification was confirmed by additional laboratory tests (3, 29, 30).

Results and Discussion

Milk remains to be one of the most essential foods of human beings. Because of its necessary components, it is internationally known as a complete diet. However, mastitis decreases the value of milk and is one of the most frequent and expensive disease of dairy industry (8). In addition to that, it is multi-factorial and a complex disease, the occurrence of which depends on variables related to the animal, environment and pathogen (28). The inflammatory response increases Somatic Cell Count (SCC) in milk. Somatic Cells are very specific, and are only elevated in the mammary once infection occurred (38).

3.1. prevalence rate of microorganisms isolated from subclinical mastitis milk samples of dairy cows and buffaloes

There was an increase in bacterial isolation frequency of *S. aureus*, *Staphylococcus* spp., *Streptococcus agalactiae*, *Streptococcus* spp., and *E.coli*

from cows milk in comparison with that from buffaloes milk. Conversely, there was an increase in bacterial isolation frequency of *Proteus*, *Citrobacter* and others microorganism from buffaloes milk in comparison with that from cows milk.

Results summarized in Table (1) mentioned that causative agents implicated in subclinical mastitis and their frequency of isolation in examined milk samples. *Proteus*, *S. aureus*, Streptococcus spp. and *Klebsiella* were the most prevalent microorganisms in cows milk, where they were detected at high percentages of 73.3%, 60%, 54.7% and 45.3% respectively. In this respect, Sudhan et al. (37) investigated the microbial isolates of subclinical mastitis in cows milk and showed that *S. aureus* was the major bacteria (56.8%) followed by Micrococcus spp. (15.5 %), *Klebsiella* (3.4 %), and *E. coli* (1.7 %). In addition, Abdel-Rady et al. in Egypt (2), Ayano et al. in Ethiopia (9), Elango et al. in India (15), Hameed et al. in Poland (18) and Shrestha et al. in Nepal (35) reported nearly similar prevalence rates for *S. aureus*, Staphylococcus spp. and Streptococcus spp. in subclinical mastitic cows milk samples.

On the other hand, *proteus*, *klebsiella*, *providencia*, Streptococcus spp., *Morganella* and *S. aureus* were the most prevalent microorganisms in buffaloes milk; Where they were detected at high percentages of 76%, 70.7%, 56%, 50.7%, 49.5% and 48% respectively. The prevalence rates of *S. aureus* and Streptococcus spp., in the present study are

in agreement with those reported previously (4,6,36).

Our results (table 1) also revealed significant differences ($p < 0.05$) in the numbers of samples positive for *klebsiella*, *Morganella* and *providencia* between cows and buffaloes milk.

3.2. Correlation between bacterial species and SCC x (10^5 /ml) in raw milk samples

Somatic Cell Count was assessed in correlation with the type of bacterial isolates from the examined subclinical milk samples of cows and buffaloes. Generally, there are a positive correlation between the microbial populations and Somatic Cell Count. As the microbial population increased, the somatic cell counts increased. The results presented in Table (2) showed that milk samples had level of SCC lower than 200 000 were mainly associated with low microbial population.

Nonetheless, Samples that contained 200 000- 500 000 of SCC/ml were mainly infected with enterobacteriaceae spp. High SCC of 500 000- 1000 000 /ml was associated with high contamination with the most bacterial species isolated especially *S. aureus* (54.3% amongst all isolates).

Much higher SCC (≥ 1000 000) /ml) was associated with higher microbial populations especially *Streptococcus agalactiae* (73.0%), followed by *Citrobacter* (66.7%) and Staphylococcus spp. (62.0%). These results are in agreement with (12, 17, 40), who concluded that the contagious pathogenic

agents (*Streptococcus agalactiae* and *S.aureus*) mainly produce the greatest SCC rise, while considerably less SCC in case of the environmental pathogenic agents (*Streptococcus dysgalactiae*, *Coagulase Negative Staphylococcus* (CNS), *Streptococcus Uberis* also *Corynebacterium spp.*).

3.3. The influence of different microbial species on Somatic cell count (SCC x 10⁵ /ml) in subclinical mastitic milk of cows and buffaloes

Somatic Cell Count is an important method for estimation of subclinical mastitis and milk value. The normal count of SCC in milk should not be more than 200 000 cells/ml. Higher SCC indicates udder infections; moreover high SCC causes a rise in whey protein and a decrease in casein, leading to a considerable lower cheese yields. In addition, shorter shelf life and adverse milk flavor are other consequences of high SCC (10).

Results summarized in Table (3) revealed that when *Streptococcus agalactiae*, *S.aureus*, *Staphylococcus spp.*, *Streptococcus spp.*, *E. coli*, *Morganella* and *providencia* were detected as predominant species, the SCC (x 10⁵ /ml) showed significant (p < 0.05) increases in buffaloes milk in comparison with cows milk.

On the other hand, when *Klebsiella*, *Proteus*, *Morganella*, and *Citrobacter* were the predominant microorganisms, higher significant increases (p < 0.001) in SCC (x 10⁵ /ml) were detected in buffaloes milk versus cows milk.

It has been indicated that buffaloes had higher absolute and relative resistance to subclinical mastitis (25), and hence buffaloes showed high levels of MSCC/ml in raw milk samples in case of subclinical mastitis (14, 27, 32). Conversely, other researches revealed that there were higher values of SCC/ml for SCM milk samples of cows (7, 11, 21).

Table 1: prevalence rate of microorganisms isolated from subclinical mastitis milk samples of dairy cows and buffaloes

Microorganism	Cows (n = 75)		Buffalos(n = 75)		P value; X ²
	Present	%	Present	%	
<i>S. aureus</i>	45	60.0%	36	48.0%	2.17; 0.14 (NS)
<i>Staphylococcus spp.</i>	26	34.7%	24	32.0%	0.12; 0.73 (NS)
<i>Streptococcus agalactiae</i>	13	17.3%	8	10.7%	1.38; 0.24 (NS)
<i>Streptococcus spp.</i>	41	54.7%	38	50.7%	0.24 ; 0.62 (NS)
<i>E. coli</i>	14	18.7%	11	14.7%	0.43 ; 0.51 (NS)
<i>Klebsiella</i>	34	45.3%	53	70.7%	9.88; 0.002* (S)
<i>Proteus</i>	55	73.3%	57	76.0%	0.14; 0.71 (NS)
<i>Morganella</i>	18	24.0%	37	49.5%	10.36; 0.001* (S)
<i>Providencia</i>	23	30.7%	42	56.0%	9.80; 0.002* (S)
<i>Citrobacter</i>	1	1.4%	2	2.6%	0.32; 0.57 (NS)
Others Microorganism	69	92.0%	70	93.3%	0.09 ; 0.75 (NS)

(NS) : Non Significant

(S): Significant

Table (2): Correlation between bacterial species and SCC x (10⁵/ml) in raw milk samples

microorganism species	SCC x (10 ⁵ /ml) range				total
	≤ 2	2 - 5	5– 10	≥ 10	
<i>S. aureus</i>	0.2%	2.2%	54.3%	43.2%	100.0%
Staphylococcus spp.	1.0%	3.0%	34.0%	62.0%	100.0%
<i>Streptococcus agalactiae</i>	1.2%	2.0%	23.8%	73.0%	100.0%
Streptococcus spp.	1.3%	1.3%	44.3%	53.1%	100.0%
<i>E.coli</i>	1.0%	4.0%	52.0%	43.0%	100.0%
<i>Klebsiella</i>	2.3%	4.6%	47.2%	45.9%	100.0%
<i>Proteus</i>	5.4%	9.8%	43.9%	40.9%	100.0%
<i>Morganella</i>	1.8%	3.6%	38.2%	56.4%	100.0%
<i>Providencia</i>	3.1%	9.2%	40.0%	47.7%	100.0%
<i>Citrobacter</i>	0.0%	0.0%	33.3%	66.7%	100.0%
Others Microorganism	2.9%	3.6%	41.0%	52.5%	100.0%

% : indicates the percentages of certain bacterial species amongst all isolates detected

Table (3):The influence of different microbial species on Somatic cell count (SCC x 10⁵ /ml) in subclinical mastitic milk of cows and buffaloes

Microorganism	SCC x (10 ⁵ /ml) in Cows (n = 75)			SCC x (10 ⁵ /ml) in Buffaloes (n = 75)			P value
	Min	Max	Mean ± SD	Min	Max	Mean ± SD	
<i>S. aureus</i>	6.7	17.8	9.88±2.07	7.1	19.0	11.38±3.96	< 0.05
Staphylococcus spp.	5.2	16.0	10.10±2.46	5.9	19.0	12.55±3.73	< 0.05
<i>Streptococcus agalactiae</i>	6.50	18.2	10.45±2.78	7.1	18.7	12.81±2.82	< 0.05
Streptococcus spp.	4.90	15.8	9.70±2.60	3.8	16.2	11.43±3.92	<0.05
<i>E. coli</i>	5.0	13.6	8.39±3.74	6.1	14.3	11.15±4.38	< 0.05
<i>Klebsiella</i>	3.3	12.8	8.54±3.78	4.2	17.0	10.57±3.69	<0.001
<i>Proteus</i>	4.1	14.9	9.11±2.65	4.6	17.2	9.95±4.23	<0.0001
<i>Morganella</i>	5.0	12.0	8.57±2.09	3.7	18.0	10.04±3.36	< 0.05
<i>Providencia</i>	4.4	16.0	9.21±5.01	3.8	16.7	11.05±3.62	< 0.05
<i>Citrobacter</i>	5.3	10.0	8.00±2.04	7.20	12.00	11.10±2.04	<0.0001
Others Microorganism	2.2	19.0	8.81±2.97	2.8	19.0	10.30±3.88	<0.01

Conclusion

The present study concluded that estimation of Somatic Cell Count in addition to identification of the causative microorganisms are very important tools that can be used for evaluation of subclinical mastitis (SCM); in addition, there is a strong correlation between SCM and elevation of SCC in raw milk. So, attention should be directed towards the status of health of the bovine udder and the appropriate measures applying to minimize the incidence of mastitis and eliminate the reservoir of the disease.

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العلاقة بين الخلايا الجسدية و التهاب الضرع الكامن للماشية فى الحليب الخام بمصر

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الملخص العربي

10.7% , 50.7% , 14.7% , 70.7% , 76% , 49.5% , 56% , 2.6%) بالتتابع . ومن ناحية أخرى قد أقرت النتائج ان العينات التى تحتوى على اقل من (200,000 خلية/مل) تعتبر عينات طبيعيه لا تحتوى على البكتريا المسببة لالتهاب الضرع الكامن . فى حين انه مع زيادة نسبة الاصابة بالعدوى البكتيرية تزداد نسبة الخلايا الجسدية عن (200,000 خلية/مل) . أوضحت الدراسة أيضا ان العينات المحتوية على بكتريا الايشيريشيا كولاى تحتوى كل منها على اكثر من (500,000 خلية/مل) فى حين انه مع تواجد المكورات العنقودية الذهبية (ستافيلوكوكس أوريس) ترتفع النسبة ل (5000,000 خلية/مل) على أقل تقدير . كما أوضحت النتائج ان بكتريا العقديية (استربت كوكس اجالاكتيا) تزيد نسبة الخلايا لأكثر من (10,000,000 خلية/مل) . ومن هنا نجد انه كلما زادت أعداد و انواع البكتريا كلما ارتفعت نسبة الخلايا الجسدية فى الحليب الخام . لذا يعتبر قياس الخلايا الجسدية وسيلة لمعرفة مدى تواجد التهاب الضرع الكامن فى الحليب الخام . وخلصت الدراسة الى أن تحسين جودة اللبن و حماية الحليب المجمع بالمزارع من التلوث الميكروبي ويتم ذلك من خلال الكشف المبكر عن التهاب الضرع الكامن فى الحليب الخام , وعزل الحيوانات المصابة، وتنظيف وتطهير حلمات الحيوانات، واستخدام معدات حلب نظيفة وتهيئة بيئة صحية.

يعتبر فحص الحليب الخام للتأكد من سلامته وجودته من الضروريات الملحة فى عصرنا الحالى . لذا أجريت هذه الدراسة لقياس نسبة الخلايا الجسدية (كريات الدم البيضاء) الموجودة فى الحليب الخام بالاضافة للفحص البكتريولوجى لهذه العينات وذلك للكشف عن التهاب الضرع الكامن للماشية وارتباط وجوده بزيادة نسبة الخلايا الجسدية عن المعدل الطبيعى فى عينات اللبن الخام. و قد أجريت الدراسة على 150 عينة لبن خام (75 بقرة حلاب و75 جاموس حلاب). قد تم الفحص البكتريولوجى لجميع العينات بالاضافة لقياس نسبة الخلايا الجسدية. وقد خلصت الدراسة ان نسب البكتريا فى عينات الالبان الناتجة من الابقار كالاتى: المكورات العنقودية الذهبية (ستافيلوكوكس أوريس), استافيلو كوكس اسبيشز , بكتريا العقديية (استربت كوكس اجالاكتيا) , استربت كوكس اسبيشز, الايشيريشيا كولاى , كليسيلا , بروتيس , مورجانيللا , بروفيديشيا بالاضافة الى ستروبيكتر (60% , 34.7% , 17.3% , 54.7% , 18.7% , 45.3% , 73.3% , 24% , 30.7% , 1.4%) بالتتابع . أما بالنسبة لنسب البكتريا فى عينات الالبان الناتجة من الجاموس كالاتى: المكورات العنقودية الذهبية (ستافيلوكوكس أوريس), استافيلو كوكس اسبيشز, بكتريا العقديية (استربت كوكس اجالاكتيا) , استربت كوكس اجالاكتيا , استربت كوكس اسبيشز, الايشيريشيا كولاى , كليسيلا , بروتيس , مورجانيللا , بروفيديشيا بالاضافة الى ستروبيكتر (48% , 32% ,