A Study On The Bacteria Causing Subclinical Mastitis In Dairy Cows and Its Effect On Somatic Cell Count and Milk Chemical Composition parameters

El-Sayed MS*, Awad EI*, Mira EKI and Shalapy SMIA***

*Food Control Department, Faculty of vet. Med., Zagazig University, Egypt.

**Food Hygiene Department, Animal Health Research Institute, Dokki-Cairo.

ABSTRACT

The Present study was designed to investigate the prevelance of Subclinical mastitis. A total number of One hundred and sixity (160) cow's quarter milk samples were collected from different dairy farms at Sharkia – Governorate for detection the causative agents of Subclinical mastitis , the results revealed that California mastitis test (CMT) was graded as (- , ± , + , ++ , +++) with incidence of (12.5 , 6.25 , 34.375 , 25.526 , and 21.25 %) respectively , the mean ± SEM of milk electrical conductivity (EC) (ms / cm) of 6.5 ± 2.5 , and the mean ± SEM of milk Somatic cell count (SCC) (cells / ml) was $547.5 \times 10^3 \pm 507.5 \times 10^3$, the mean ± SEM of milk Chloride % was 0.235 % ± 0.165 % , the mean ± SEM of measured Fat % was 2.65 % ± 1.15 % , the mean ± SEM of measured Protein % was 3.1 % ± 1.1 % , the mean ± SEM of measured Lactose % was 3.55 % ± 1.45 % , the mean ± SEM of measured SNF % was 7.5 % ± 1.5 % , The most predominant single pathogens in 100 Out of 160 milk samples was *(S . aureus, S.agalactiae, S.dysagalactiae, S.uberis and E.coli)* with incidence of (21.875, 15.625, 12.5 , 6.25 and 6.25 %) respectively, and normal healthy control 20 (12.5 %) milk samples (didn't yield any pathogens), and 40 (25 %) milk samples yield mixed bacterial pathogens . It can be concluded that CMT was used to determine the severity of Suclinical mastitis. CMT positive and SCC>250.000 (cells / ml) in individual quarter foremilk samples was found to be accompanied by several production effects and sever depression in milk chemical parameters, Bacterial contamination of milk from affected cows render it unfit for human consumption, and there is correlation between SCC and decrease chemical milk parameters .

INTRODUCTION

Subclinical mastitis is the most serious form as both infected udder and milk show no obvious clinical abnormalities, whereas several causative organisms are discharged with the milk for long time . This may cause sever harm from the epizootiological and epidemiological as well as economic points of view *(1)*. The term "sub –clinical mastitis "means that, although there are no visible udder external changes, the infection is present and the inflammation is occurred. It leads to undesirable effect on milk constituents and its nutritional value *(2)*. Many infectious agents have been implicated . The cause of subclinical mastitis mainly as *Staph aureus, Streptococcus species and E. coli (3)*. Other responses to subclinical mastitis are reduced milk yield and changes in the chemical composition of the milk caused by cellular damage and increased permeability in the membranes of the mammary tissue *(4)*. Mastitis disease negatively affects the physical-chemical characteristics composition and yield of milk *(5)*.

Mastitis affects the milk quality in terms of decrease in milk protein , fat , sugar (lactose) contents and increase in somatic cell count *(6)*.The extent of various changes in composition depends on the inflammatory response *(7)*. Fernandes investigated the relationship between SCC and composition (Total solides , Fat , Protein and Lactose content) of milk reduced lactose content of milk

in inverse proportion to the number of leukocytes *(8)*.

The concentration of sodium and chloride must be considered in content with lactose, because the combination of these parameters are responsible for the osmolar equilibrium. The contents of sodium and chloride showed minor changes between SCC groups to permit elevated movement of ions from blood into milk ***(9,10)***. Typical Electrical conductivity (EC) of normal milk appears to be between (4 and 5.5) (ms/cm) at (25°C) .If the EC is higher than (5.6) (ms/cm), it means the cows suffers from mastitis or the milk is suspected of mastitis. The EC of milk has also been expressed as a concentration of NaCl with the same conductivity as the examined milk ***(11)***. Electrical conductivity (EC) measured by a hand-held meter and chloride concentration of milk were studied as auxiliary methods for the diagnosis of bovine subclinical mastitis in the identification of affected mammary quarters ***(12)***.

Somatic cell count (SCC) are accepted as the international standard measurement of milk quality. Milk somatic cells are primarily leukocytes or white blood cells, which include phagocytes and lymphocytes during mastitis the major increase in SCC is due to the influx of neutophils to the milk to fight infection ***(13,14)***. The aim of detecting the rate of subclinical mastitis in cows was conducted to perform the following : California mastitis test (CMT), Electrical conductivity test (EC), Somatic cell count (SCC), Effect on milk composition parameters (Fat, Protein, Lactose and Solide Not Fat), Chlorine test, and isolation of some pathogens *(Staph.aureus, Strept. agalactiae, E.Coli)*.

MATERIALS AND METHODS

Milk Sampling *(15)*

The udder was properly washed by water, dried with clean towel, then disinfected by 70% ethyl alcohol just before milk sampling. the 1st two strips of milk (foremilk) were discarded from each quarter. (15-20ml)of milk was drawn in a clean sterile screw capped bottle then labeled for the quarter, animal number, animal age and date of sampling. the milk samples were kept in an ice container till delivered to laboratory.

California Mastitis Test (CMT) ***(16)***

The CMT reagent (Alkyl-Aryl-sulphate) was used Special white plates were filled with (2ml) of test solution and mixed with (2ml) of examined milk samples after turning the plate for (5-10) secondes, consistancy and color of the mixure were visually determined.

Measurment Of Electrical Conductivity (EC) ***(17)***

Milk samples were subjected to conductivity test Using MAS –D-TEC (wescor, logan , Utah , USA) .

Somatic CellCounting (SCC) ***(18)***

SCC was measured by fossmatic 360 and fossmatic 5000 (A / S N foss Electric, Hillerod , D . K .) according to IDF standared 148 A : 1995 , methods.

Measuring Milk chemical Parameters ***(19)***

Infrared milk analyzer (Milkoscan 605, Foss, Electric ,D.K-3400 , Hillerod , Denmark).

Chlorine Test ***(20)***

About (5ml) Silver Nitrate solution was added to (1ml) milk followd by two drops of Potassium Chromate solution. Development of yellow color indicates positive and the chloride level 0.14%.

Isolation and identification of bacteria causing subclinical mastitis

Preparation and cultivation of milk samples) ***(21,22)***

All milk samples were incubated at 37°C for 24 hrs., then loopfulls of incubated milk were streaked onto plates of blood agar (for detection of hemolysis), Mannitol salt agar (selective media for *Staphylococci*), MacConkey's agar (selective media of *Enterobacteriaceae*), and Edward's media (selective media for *Streptococci*).

Isolation and identification of *Staphylococcus aureus (23)*

For isolation of *Staphylococci* , 0.1ml of milk samples was initially enriched in nutrient broth for 6 hrs at 37°c and then streaked onto manitol salt agar and incubated at 37°c for 24 hr. After reading the colony morphology, the colonies were further streaked onto Baired-parker agar media, the black with narrow white margin and surrounded by clear halo zone extended into the opaque medium were picked up and inoculated for further identification procedures .

Isolation and identification of *Streptococcus species (23)*

For isolation of *Streptococci*, 0.1 ml of milk sample was initially enriched in Trypticase soya broth, with (5-10%)CO_2 tension for 6 hr at 37°C and then streaked the colonies onto blood agar plates and incubated further at 37°C for 48 hr after reading the hemolysis pattern and colony morphology , these pure culture were streaked onto Edwards media ,the small round and translucent colonies were picked up and inoculated for further identification procedures.

Isolation and Identification of *Escherichia coli (23)*

For isolation of *E. Coli* , 0.1ml of milk samples was initially enriched in nutrient broth for 18 hr at 37°c and then streaked onto MacConkey agar and incubated at 37°c for 24 hr. The lactose fermenting colonies were further streaked onto Eosine Methylene blue (EMB) agar and incubated at 37°c for 24 hr . The metallic shen colonies were streaked for further identification procedure.

Biochemical identification

Pure cultures of isolates of *(Staphylococcus aureus, Strept. agalactiae and E.coli)* were streaked onto nutrient agar slants and preserved at 4°c. From these slants , the pure cultures were subjected for various biochemical tests as per standared procedures.

RESULTS

Table 1. The Severity of Subclinical mastitis in examined cow's quarter milk samples according to the results of California mastitis test (CMT)(N=160).

Sub clinical mastitis CMT											
Negative(-)		Trace(±)		Score+		Score++		Score+++		Total positive	
No	%	No	%	No	%	No	%	No	%	No	%
20	12.5	10	6.25	55	34.375	41	25.625	34	21.25	140	87.5

Table 2. Statistical analytical results of the measured chemical parameters of the examined (N=160)cow's quarters milk samples

Measured parameter	(N=160)			
	Minimum	Maximum	Mean	±SEM
SCC	40×10^3	1055×10^3	547.5×10^3	507.5×10^3
EC	4	9	6.5	2.5
Fat%	1.5	3.8	2.65	1.15
protein%	2	4.1	3.1	1.1
lactose%	2.1	5	3.55	1.45
SNF%	6	9	7.5	1.5
Chloride%	0.07	0.4	0.235	0.165

Table 3. Incidence of single and mixed bacterial pathogens causing subclinical mastitis in the examined cow's quarter milk samples for subclinical mastitis examination(N=160)

Types of pathogens	Bacteriological findings	
A)single infection	**No.**	**%**
1- *S.aureus*	35	21.875
2- *S.agalactiae*	25	15.625
3- *S.dysagalactiae*	20	12.5
4- *S.uberis*	10	6.25
5- *E-coli*	10	6.25
total	100	62.5
b)Mixed infection		
1-*S.aureus*+ *S. agalactiae*	10	6.25
2-*S.aureus* + *E-coli*	6	3.75
3- *S. agalactiae* + *E-coli*+ *S.dysagalactiae*	11	6.875
4-*S.aureus* + *S.agalactiae* +*E-coli*	8	5
5- *S.uberis*+ *S.epidermidis*+ *E-coli*	5	3.125
Total	40	25
C)No bacterial growth	20	12.5
Total no of samples	160	100

DISCUSSION

Subclinical mastitis is considered to have vital importance to public health due to its association with many zoonotic diseases in which the milk may act as a vehicle for transmission of infectious agents ***(24)***.

The results listed in table (1) Revealed that out of 160 examined cow's milk samples according to CMT, 20 (12.5%) were negative while 140 (87.5%) were positive for subclinical mastitis of positive samples 10 (6.25%), 55 (34.375%), 41 (25.625%) and 34 (21.25%) were listed as Trace ± , Score + , Score ++, and Score +++, respectively. Nearly similar finding were detected by Al-Hawary and Karimuribo ***(25,26)*** Lower results were reported by Iqbal, Getahun, Varatanovic, Hshemi, Bhutto and Jin-bo ***(27-32)***. CMT is the most widely used test for routine screening of subclinical infected quarters on the farm as a simple cowside, inexpensive and rapid test for subjective evaluation of quarter SCC at cowside. The CMT was developed to test milk from individual quarters but has also been on composite milk samples and bulk milk samples ***(33)***. The use of CMT identify infected quarters has been extensively validated ***(14,34,35)***.

The results listed in table (2) Declared that, the minimum SCC was (40×10^3) cells / ml, the maximum was (1055×10^3) cells / ml, and the mean value was (547.5×10^3 ± 507.5×10^3) cells / ml . These findings were in agreement with those reported by Egyptian Standards ***(36)***. Total SCC in cow's raw milk must not more than 750,000 cells/ml. Higher values of SCC / ml for mastitis milk samples were recorded by Sharif and Elango ***(37,38)*** while lower figures were recorded by Spakauskas and Bhutto ***(16,31)***. The mammary gland infection is the most important factor affecting SCC during subclinical mastitis ***(39)***. Somatic cells acts as natural defence mechanism and first line of defence against invading pathogens in the mammary gland and include eosinophils, monocytes, lymphocytes, macrophages, neutrophils and few epithelial cells ***(40-42)***.

While Electrical conductivity (EC) was ranged from (4 to 9) (ms/cm) with a mean value of (6.5 ± 2.5) (ms/cm) . These findings were in agreement with those reported by

Cavero, Spakauskas and El-Barawy and Ali *(16,43,44)*. Higher findings were reported by Janzekovic *(45)*. While lower values were recorded by Mansell and Seguya *(46)*. Electrical conductivity (EC) is a measure of the resistance of a particular material to an electric current *(11)*. Normally, milk has a resistance of between 4.0 and 5.5 mS/cm at 22°C *(47)*. The concentration of sodium chloride (NaCl) is often expressed as milk Electrical conductivity (EC) *(48-51)*.

While the results of chlorine % was ranged from (0.07-0.4%) and the mean value was (0.235 ± 0.165 %) These findings were in agreement with those reported by Elango *(38)* Who reported that the normal range of chlorine content of healthy animal was 0.08 to 0.14 %. While Higher values of chlorine content (0.12%) for normal milk samples were recorded by Elango *(38)* lower values were reported by Sharma *(52)* Who found that the chlorine content of normal milk samples was 0.91%. While Batavani reported that Milk from quarters with subclinical mastitis showed elevated chlorine (>0.14 vs <0.14 g/dl) which is significantly higher in the milk of inflamed quarter than those in normal ones (P<0.01) *(53)*.

The results found in table (2) Summarized that, Fat%, Protein%, Lactose% and S.N.F.% in the examined samples were (1.5, 2, 2.1 and 6 %) of minimum value, respectively, and (3.8, 4.1, 5 and 9 %) of maximum value respectively, with mean value of (2.65 ± 1.15 , 3.1 ± 1.1 , 3.55 ± 1.45 and 7.5 ± 1.5 %) respectively. Mastitis reduces milk yield and alters milk composition . The magnitude of reduced milk yield and alterations in milk composition is influenced by the severity of the inflammatory response, which in turn is influenced by the mastitis pathogen causing the infection *(54)*. Subclinical mastitis reduced Lactose , Non Fat Solides and Total Solides content, but no difference was found in the Protein and Fat content between infected and uninfected quarters . Mastitis causing pathogens affected Protein , Lactose, Non Fat Solids and Total Solids content but not milk Fat content *(55)*.

Table (3) mentioned that, the most predominant single isolates in examined quarter milk samples were (21.875, 15.625, 12.5, 6.25 and 6.25%) for *(S.aureus, S.agalactiae, S.dysagalactiae, S.uberis and E-coli)*, respectively. While 40 (25%) show mixed infection and the predominant mixed infection were *(S. agalactiae + E-coli+ S.dysagalactiae)* in percentage of (6.875%) . The distribution of pathogens causing intramammary (IMI) varies widely among dairy herds . However, Some reports for the evaluation of new tests to categorize the causative agents of mastitis as either Gram – negative or Gram – positive have been published knowledge of the microbiological status of milk and the different structures in the mammary gland has a great importance in elucidating the pathogenesis of mammary gland infection *(56)*. Rapid and accurate identification of mastitis pathogens is important for disease control . Bacterial culture and identification are considered the gold standard in mastitis diagnosis but are time consuming and results in many culture-negative samples *(57)*. The economic importance of the *Staph aureus* causing clinical and subclinical bovine mastitis is largely recognized *Staph aureus* is a contagious pathogen commonly transmitted among the cows by contact with infected milk and the infection reach up to 32% of the herd *(58)*. In the present study, the isolates were *Staph. aureus* based on Mannitol Fermentation, Catalase, Coagulase and Thermonuclease tests. Several workers also found that *Staphylococcus* species were the predominant isolates in subclinical mastitis cases *(59-61)*.

Conclusion

It can be concluded that California mastitis test was used to determine the severity of suclinical mastitis. CMT positive and SCC □ 250.000 (cells / ml) in individual quarter foremilk samples was found to be accompanied by several production effects and sever depression in milk chemical parameters Bacterial contamination of milk from affected cows render it unfit for human consumption , and there is correlation between increase

number of somatic cell count and decrease chemical milk parameters, presence of pathogens in milk samples increase chlorine % and milk electric conductivity.

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الملخص العربي

دراسة عن البكتريا المسببة لالتهاب الضرع الكامن في الأبقار الحلوب وتأثيرها علي الخلايا الجسدية ومكونات اللبن الكيميائية الأساسية

مجدي شرف السيد*، عصمت إبراهيم عوض* ،انشراح خليل ابراهيم ميره**

،شيماء محمد إسماعيل علي شلبي

***قسم مراقبة الأغذية – كلية الطب البيطرى – جامعة الزقازيق**

****قسم مراقبة الأغذية – معهد بحوث صحة الحيوان بالدقى**

استهدفت هذه الدراسة الكشف عن معدل انتشار التهاب الضرع الكامن ، حيث تم جمع (١٦٠) عينة حليب من الأبقار الحلوب من المزارع المختلفة بمحافظة الشرقية لتشخيص مرض التهاب الضرع الكامن ، أسفرت نتائج اختبار الكاليفورنيا لالتهاب الضرع الكامن عند الدرجات المختلفة (- ، ± ، + ، ++ ، +++) بنسبة حدوث (١٢،٥ ، ٦،٢٥ ، ٣٤،٣٧٥، ٢٥،٥٢٦ ، ٢١،٢٥ ٪) وتم قياس الناقلية الكهربية في العينات المفحوصة وكان متوسط مستوي الحدوث نحو (٦،٥± ٢،٥) (مليسيمنز/سم) ومتوسط مستوي عدد الخلايا الجسدية نحو (٥٤٧،٥ × ١٠^{٣} ±٥٠٧،٥ ×١٠^{٣}) (خلية / ملي لبن) ، وصل متوسط نسبة الكلور في عينات الحليب المفحوصة (٠،٢٣٥ ± ٠،١٦٥ ٪) . وتم قياس مكونات الحليب الكيميائية الأساسية في (١٦٠) عينة الحليب المجمعة ، حيث كان متوسط نسبة الدهن (٢،٦٥ ±١،١٥٪) ومتوسط نسبة البروتين (٣،١ ±١،١٪) ومتوسط نسبة اللاكتوز (٣،٥٥±١،٤٥٪) و متوسط نسبة المواد الجافة اللادهنية (٥،٧ ±٥،١٪) وتم إجراء الفحوصات البكترية في عينات الحليب المجمعة حيث تم تحديد نوعية البكتريا المسببة لالتهاب الضرع في العينات الموجبة للبكتريا في (١٠٠) عينة حليب وتم تحديد نوعية البكتريا الممرضة في العينات الموجبة والتي كان معظمها (*استافيلوكوكس اوريس و استربتوكوكس اجالاكتياو استربتوكوكس ديساجالاكتيا و استربتوكوكس يوبيريس و الايشيريشيا كولاي*) بنسبة حدوث (٢١،٨٧٥ ،١٥،٦٢٥ ،١٢،٥ ،٦،٢٥ ،٦،٢٥٪) ،و تم تحديد عينات الحليب السلبية السليمة والتي لم ينتج عنها أي نمو للبكتريا وذلك في (٢٠) عينة ألبان بنسبة حدوث (١٢،٥%) وتم عزل بعض البكتريا المسببة للمرض ولكن بصورة مختلطة بالأنواع الممرضة الاخري وبنسب اقل وذلك في (٤٠) عينة حليب بنسبة حدوث (٢٥%) . الخلاصة أن اختبار الكاليفورنيا لالتهاب الضرع يستخدم لتحديد شدة الإصابة بمرض التهاب الضرع الكامن في عينات الحليب الموجبة لاختبار الكاليفورنيا وذات عدد الخلايا الجسدية الأكثر من (٢٥٠،٠٠٠) خلية /ملي من الحليب لربع الضرع الواحد في البقرة الحلوب الذي يكون مصحوبا بتأثيرات مختلفة علي الإنتاج وعلي مكونات الحليب الكيميائية الأساسية ، و تلوث اللبن بالبكتريا الممرضة يجعله غير صالح للاستخدام الادامي، حيث يوجد ارتباط بين عدد الخلايا الجسدية وقلة مكونات الحليب الكيميائية الأساسية.