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

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ABSTRACT
The Present study was designed to investigate the prevalence 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 × 10^3 ± 507.5 × 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.dysgalactiae, 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 Subclinical 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 neutrophils 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) seconds, consistancy and color of the mixture were visually determined.

**Measurement 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 (Milkscan 605, Foss, Electric, D.K-3400, Hillerod, Denmark).

**Chlorine Test (20)**

About (5ml) Silver Nitrate solution was added to (1ml) milk followed 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 loopfuls 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 mannitol salt agar and incubated at 37°C for 24 hr. After reading the colony morphology, the colonies were further streaked onto Baird-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%)CO₂ 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 sheen 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 to various biochemical tests as per standardized 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).**

<table>
<thead>
<tr>
<th>Sub clinical mastitis CMT</th>
<th>Negative(-)</th>
<th>Trace(±)</th>
<th>Score+</th>
<th>Score++</th>
<th>Score+++</th>
<th>Total positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>20</td>
<td>12.5</td>
<td>10</td>
<td>6.25</td>
<td>55</td>
<td>34.375</td>
<td>41</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Measured parameter</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCC</td>
<td>4×10⁶</td>
<td>1×10⁶</td>
<td>5×10⁵</td>
<td>5×10⁵</td>
</tr>
<tr>
<td>EC</td>
<td>4</td>
<td>9</td>
<td>6.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Fat%</td>
<td>1.5</td>
<td>3.8</td>
<td>2.65</td>
<td>1.15</td>
</tr>
<tr>
<td>protein%</td>
<td>2</td>
<td>4.1</td>
<td>3.1</td>
<td>1.1</td>
</tr>
<tr>
<td>lactose%</td>
<td>2.1</td>
<td>5</td>
<td>3.55</td>
<td>1.45</td>
</tr>
<tr>
<td>SNF%</td>
<td>6</td>
<td>9</td>
<td>7.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Chloride%</td>
<td>0.07</td>
<td>0.4</td>
<td>0.235</td>
<td>0.165</td>
</tr>
</tbody>
</table>
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)

<table>
<thead>
<tr>
<th>Types of pathogens</th>
<th>Bacteriological findings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>A) single infection</td>
<td></td>
</tr>
<tr>
<td>1- S. aureus</td>
<td>35</td>
</tr>
<tr>
<td>2- S. agalactiae</td>
<td>25</td>
</tr>
<tr>
<td>3- S. dysagalactiae</td>
<td>20</td>
</tr>
<tr>
<td>4- S. uberis</td>
<td>10</td>
</tr>
<tr>
<td>5- E. coli</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
<tr>
<td>B) Mixed infection</td>
<td></td>
</tr>
<tr>
<td>1- S. aureus + S. agalactiae</td>
<td>10</td>
</tr>
<tr>
<td>2- S. aureus + E. coli</td>
<td>6</td>
</tr>
<tr>
<td>3- S. agalactiae + E. coli + S. dysagalactiae</td>
<td>11</td>
</tr>
<tr>
<td>4- S. aureus + S. agalactiae + E. coli</td>
<td>8</td>
</tr>
<tr>
<td>5- S. uberis + S. epidermidis + E. coli</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
</tr>
<tr>
<td>C) No bacterial growth</td>
<td>20</td>
</tr>
<tr>
<td>Total no of samples</td>
<td>160</td>
</tr>
</tbody>
</table>

**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, Gethun, Varatanovic, Hshemi, Bhutto and Jinbo (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 Thromonuclease 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 subclinical mastitis. CMT positive and SCC 250,000 (cells / ml ) in individual quarter foremilk samples was found to be accompanied by several production effects and severe 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.

REFERENCES


الملخص العربي
دراسة عن البكتيريا المسببة لالتهاب الضرع الكامن في الأبقار الحليب وتأثيرها على الخلايا الجسدية ومكونات اللين الكيميائية الأساسية
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شيماء محمد إسماعيل علي شلبي
قسم مراقبة الأغذية – كلية الطب البيطرية – جامعة الزقازيق
قسم مراقبة الأغذية – معهد بحوث صحة الحيوان بالدقي

استهدفت هذه الدراسة الكشف عن معدل انتشار التهاب الضرع الكامن، حيث تم جمع (120) عينة حليب من الأبقار الحليب من المراعي المختلفة في محافظة الشرقية. لتشخيص مرض التهاب الضرع الكامن، أُجرت نتائج اختبار كاليفورنيا لالتهاب الضرع الكامن عند الدرجات المختلفة (++) (120) بنسبة حدوث (120% ) وتم قياس الناقلية الكهربية في العينات المفحوصة وكان متوسط مستوي الحدوث نحو (120% ) وتم متوسط مستوي عدد الخلايا الجسدية نحو (120% ) و(120%) . وتم قياس مكونات الحليب الكيميائية الأساسية في (120) عينة الحليب المفحوصة، حيث كان متوسط نسبة الدهن (120% ) وتم متوسط نسبة البروتين (120% ) وتم متوسط نسبة اللاكتوز (120% ) وتم إجراء الفحوصات البكترية في عينات الحليب المفحوصة حيث تم تحديد نوعية البكتيريا المسببة لالتهاب الضرع في العينات المفحوصة للآلات في (100) عينة حليب وتم تحديد نوعية البكتيريا المرضية في العينات المفحوصة والتي كان معظمها (استريتووكس أوريس) و (استريتووكس كولوني) بنسبة حدوث (120% ) ب约为 متوسط عينات الحليب السليمة والتي لم تنتج عنها أي نمو البكتيريا وذلك في (100) عينة ألبان بنسبة حدوث (120% ) وتم عزل بعض البكتيريا المسببة المرض، ولكن بصورة مختلفة بالأنواع المرضية الأخرى، وتم قياس بعض نسبه حدوث بحري بنسبة حديد (120% ) في عينات الحليب المفحوصة لاختبار كاليفورنيا وجدت نسبة الخلايا المسيلة لالتهاب الضرع الكامن بنسبة (120% ) وأقل ويكشف عن تأثير الخلايا الجسدية في الالتهاب المرضية الأساسي. ولهذا فإن الخلايا المسيلة لالتهاب الضرع الكامن وظيفة في ل richtig on the intention of the user and the content of the document. The translation may not be perfectly accurate and may require further refinement to ensure idiomatic and culturally appropriate language.