Chemical, Microbiological and Enzymatic Evaluation of Mastitic Milk

Abd El-Rahman.S.A, Ahmed. H.S.and Jehan I.I.
Department of Food Hygiene and Control, Faculty of Veterinary Medicine Suez Canal University, Ismailia, Egypt

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Abstract
A total of 400 quarter milk samples were collected from 100 dairy animals breaded in three dairy farms in Suez Canal area examined by California Mastitis Test (CMT)and found that 102 samples were positive for subclinical mastitis(SCM). Positive samples microbiologically revealed thatthe most common bacterial isolates from subclinical mastitis cases were *Staphylococcus aureus* 92(90.20%), *Staphylococcu sepidermidis*18 (17.65%), streptococcus spp.79(77.45) *and E.coli* 35(34.31%). On the other hand, total yeast and mould count were 74(72.54). Chemical and enzymatic examinations inpositive CMT milk samples revealed that mean level of milk lactose %was 3.16±0.79, while the mean level of milk chloride %was 0.13±0.04 significantly higherin positive CMT milk samples, mean value of LDH andALP enzyme was503.52±14.21IU/ML and 723.77±21.30 IU/ML which significantly higher in positive CMT samples than negative CMT samples. Therefore, our study concluded that milk lactose, chloride and enzymes are consideredto be suitable diagnostic methods for diagnosis of SCM in dairy animals.

Key words: Subclinical mastitis, etiology, milk LDH, ALP, lactose, chloride; *Staphylococcus aureus,streptococcus spp.,E.coli.*

Introduction

Milk considered as good supplement of nutrients for human diet.It contains all the food constituents required. Its composition is affected by the breed of animals, species, health of udder, stage of lactation and diseases affected udder **(Sharif *et al.*, 2007).** Mastitis is an inflammatory change of the mammary gland characterized by an increase in somatic cells in the milk and pathological changes in mammary tissues. **(Souto *et al.*, 2010).** It is a dangerous disease of dairy animals which is found in clinical and subclinical forms causing great economic losses and of public health concern. **(Sharif *et al.*, 2007).** Mastitis is the first cause of elevated somatic cell count, so affects both quality and quantity of milk. Elevated milk SCC is associated with changes in protein

quality, change in fatty acid composition, lactose, and mineral concentration, increased enzymatic activity and PH of rawmilk **(Auldist*et al.*, 1996 and Coulon *et al.*, 2002).** Bacteria that causing mastitis in cattle are transmitted through raw milk from infected udder and cause disease problems in human., such type of bacteria includes Mycobacterium, Brucella and Staphylococcus and Streptococcus species. **(Dagnaw, 2015).** Many studies were revealed that some changes detected in enzyme activities due to mastitis **(Andrei *et al.*, 2011).** Detection of enzyme activity in milk considered as reliable markers for early diagnosis of subclinical mastitis **(Babaei*et al.*, 2007; Guha*et al.*, 2012).** Milk enzymes ALP and LDH were markedly increased in mastitis and they considered both as the early indicators of acute mastitis **(Larsen *et al.*, 2010).**

There for the aim of this study was based on: 1- Detection of subclinical mastitis in dairy animals by using screening test (CMT),2- Determination of lactose and chloride percent,3- Determination of lactate dehydrogenase enzyme and alkaline phosphates enzyme,4- Microbiological analysis of positive CMT samples.

Materials And Methods

1. Animals:

A total of 400quarter milk samples from100 dairy animals breaded in three dairy farms at Suez Canal area, Egypt were subjected to this investigation. Animals selection based on the age and stage of lactation.

2.Sampling:

Before milking each udder was washed with water and soap then washed with potassium permanganate 1/1000 solution, dried with clean towel, The teat was disinfected with 70% ethyl alcohol. The first three streams were rejected then 150 ml of milk for each sample were sent aseptically in a sterile screw capped bottlesto be examined. Each sample was thoroughly mixed before divided into three subsamples used for screening tests, microbiological and chemical examination.

3. California mastitis test (CMT) APHA, (2004)

Equal amount (2ml) of milk and frieso-test reagent were mixed thoroughly in a cup of black plastic paddle and swirl gently of the paddle for 10seconds. Results were recorded according to the tendency of gel formation and expressed as strong positive (+++), positive (++), weak positive (+)or negative (-).

4. Microbiological examination:

2.1-Preparation of samples for microbiological examination according to *APHA (2004):* Milk samples were microbiologically examined for**Enumeration,** Isolation and identification of Staphylococcus spp. according to ***Deibel and Herrttman (1984)*** on Baird-Parker agar plates andincubated at 37ºC for 48

hoursand isolation of *E.coli*was carried out according to ***APHA (2004)*** on eosin methylene blue agar(EMB)platesand incubated at 37 ºC for 24 hours. Isolation and count ofstreptococcus spp. according to ***APHA (2004)*** onasculinazid agar medium platesand incubated at an inverted postion at 36± 1º Cfor 48 hours andIdentification of streptococcus species done according to ***Koneman et al. (1988)*** and ***Quinn et al (1994).*** Total Yeast and mould count was carried usingsabourauddextrose agar medium according to ***APHA (2004).***

5. Chemical examination:

Estimation of milk lactose and chloride levels according to ***Analysis of milk and its product (2005).***

Measurement of alkaline phosphatase and lactate dehydrogenase enzyme according to ***Bergmeyer (1974) and Goldberg and Spooner (1983)*** respectively**:**Milk samples were skimmed by centrifugation at 10,000 gm for 20 min at 4°C. Defatted milk samples were used for enzyme activity estimations of lactate dehydrogenase enzyme (LDH) and alkaline phosphatase (ALP) activity were assayed by spectrophotometer.

Results

Table 1: *Incidence of subclinical mastitis in examined quarter milk samples according to California mastitis test.*

NO. of animals	No. of quarters milk Samples	Positive samples NO.	Positive samples %
100	400	102	25.5

Table 2: *Correlation between positive CMT score, % of mastitic milk samples and microbiological results.*

Positive CMT scores	No. of samples	% of mastitic milk samples	NO. of Microbiological positive samples	Accuracy %
+	38	37.25	38	100%
++	39	38.24	39	100%
+++	25	24.51	25	100%
Total	102	100	102	100%

Table 3: *Incidence and count of some microorganisms in examined positive CMT quarter milk samples (n=102).*

Isolated organism	Incidence		Total count(cfu/ml)		
	NO	%	Min	Max	Mean± SE.
S. aureus	92	90.20	6×10^2	5×10^6	$2.8\times10^5 \pm 8.7\times10^2$
S. epidermidis	18	17.65	2×10^2	1×10^5	$1.2\times10^4 \pm 1.7\times10^2$
Strept.spp.	79	77.45	1×10^2	2×10^9	$2.6\times10^7 \pm 1.5\times 10^2$
E.coli	35	34.31	1×10^2	1.3×10^6	$8.4\times10^4 \pm 0.53\times10^2$
Total yeast and mold count	74	72.54	1×10^2	1×10^7	$1.7\times10^5 \pm 1\times10^2$

Table4: *Statistical analytical results of Milk Chemical and Biochemical parameters values based on CMT in examined quarters' milk samples (n=400).*

Biochemical Parameters	CMT positive milk samples(n=102)			CMT negative milk samples(n=298)		
	Min	Max	Mean ± SE	Min	Max	Mean ± SE
Lactose%	2.23	5.7	*3.16 ± 0.79	3.15	6.17	4.12±0.20
Chloride%	0.07	0.24	*0.13 ± 0.04	0.03	0.10	0.06±0.001
ALK.Ph. Enzyme	276.18 IU/ML	2561 IU/ML	***723.77 ± 21.30	81.70 IU/ML	256.40 IU/ML	186.4±15.6
LDH. Enzyme	165 IU/ML	844 IU/ML	***503.52±14.21	68.40 IU/ML	190.17 IU/ML	158.7±7.04

*Significance at P< 0.05. *** Significance at P < 0.001.

Discussion

1- Incidence of subclinical mastitis by California Mastitis Test (CMT).

Results summarized in table (1) revealed that 102 out of 400 examined quarter milk samples (25.5%) were positive for CMT. The obtained result was nearly similar to those obtained by ***Islam (2011) and Saidiet al. (2013),*** while relatively higher incidence were obtained by ***Ayanoet al. (2013), Murugaiyahet al. (2014) and Rahmanet al.(2014).***Whereas comparatively lower incidence were recorded by ***Hashemiet al. (2011) and Hussein*** **(2012).**California mastitis test generally used as rapid test for detection of sub-clinical mastitis which detects somatic cell nuclear material, depending on a threshold of 300,000 SCC per milliliter ***(Radostitset al., 2000).*** Results given in table (2) proved that 100% positive CMT samples in all scores were microbiologically positive. The obtained results clarified a good correlation between CMT in all scores and microbiological results. Lower correlation was reported by ***Hussein (2012).*** Nearly similar results were recorded by ***Saidiet al. (2013)*** and ***Sanotharanet al. (2016)***, they found that 97%, 95% and 93.9% of CMT yielded

bacterial growth, respectively. Inspection of table (2) showed that 38(37.25%), 39(38.24%) and 25(24.51%) of examined samples were positive for CMT scores (+), (++) and (+++) respectively. These results were being disagreed with those obtained by ***Sabuncuet al. (2013)*** who reported that 82.58%, 14.83% and 2.58% were positive of CMT score (+), (++) and(+++), respectively. Nearly similar result was reported by ***Nabih and Abd-El. Rahman (2015)***, they reported that 16%, 32% and 52% of examined samples were positive for CMT score (+), (++) and (+++) respectively.

2- Microbiological evaluation of positive samples.

It was evident that *S. aureus* could be isolated from 92 out of 102 (90.2%) of microbiologically positive samples in single and/or mixed infection. Results in table (3) revealed that the total *S. aureus count in microbiologically positive mastitis* milk samples ranged from 6×10^2 to 5×10^6 with a mean count value of $2.8\times10^5\pm 8.7\times 10^2$cfu / g. The results indicated that *S. aureus* was the first major pathogenic organism incriminated in subclinical mastitis and this was potenciated by what had been reported by several authors; ***(Nagwaet al., 2015; Abdel Tawabet al.,2016 and Sanotharan et al.,2016).*** Data tabulated in table (3) revealed that *S. epidermidis* count in microbiologically positive mastitis milk samples was ranged from 2×10^2 to 1×10^5 with a mean count of $1.2\times10^4\pm1.7\times 10^2$cfu / g. *S. epidermidis*, is often regarded as a culture contaminantbut its importance as a pathogen has been recognized in recent years. *S. epidermidis* is a common cause of infection indwelling foreign devices, surgical wound and bacteremia in immunocomprised patients ***(Blum and Rodvold, 1987).*** Results given in table (3) revealed that the totalStreptococcus speciescount in microbiologically positive mastitis milk samples ranged from 1×10^2 to 2×10^9 with a mean count of $2.6\times 10^7 \pm 1.5 \times 10^2$ cfu / g. streptococcus spp. classified to Strept.pyogens was isolated from 44(43.14) where organism was isolated from subclinical mastitis milk samples by many authors with different incidence ***(Saidiet al., 2013, Murugaiyahet al. 2014 and Mureithi and Njuguna 2016).*** While*Strept.agalactiae*was isolated from 30 (29.41%) Nearly similar result was recorded by ***Ahmed et al. (2008).*** Higher results were recorded by ***Plozzaet al. (2011) and Ramirez et al. (2014).*** Lower records were obtained by ***Ayanoet al. (2013), El Sayedet al. (2015) and Sztachanskaet al. (2016).****E.coli* represent the third important causative bacterial agents isolated from examined mastitis milk samples in this work, Inspection of table (3) revealed that the total *E.coli* count in microbiologically positive mastitis milk samples

ranged between 1×10^2 and 1.3×10^6 with a mean count of $8.4\times10^4 \pm 0.53\times10^2$ cfu/g., Nearly similar results were reported by ***Plozzaet al. (2011), Hussien (2012) andAbd-ELrahman (2013).*** Lower findings were recorded by ***Ali et al. (2015) and Sanotharanet al., (2016).*** Higher results were recorded by ***Ahmed et al. (2008)*** and ***Nagwaet al. (2015).*** Result recorded in table (3) revealed that the total yeast and mould counts of examined samples ranged from 1×10^2 to 1×10^7 with a mean count value of $1.7\times10^5 \pm1\times10^2$cfu / g., Lower results of yeast and mould counts were recorded by ***Rajeev et al. (2011)*** and ***Murugaiyahet al. (2014).*** On contrary, sporadic incidence of subclinical mastitis due to yeast had been reported by ***Dudkoet al. (2003) and EbrahimandNikookhah. (2005).***

3- Chemical and enzymatic evaluation of positive CMT quarter milk samples:

Table (4) revealed that lactose content in examined positive CMT quarter milk samples ranged from 2.23 to 5.7% with a mean value of 3.16 ± 0.79, while in negative CMT quarter milk samples was ranged from 3.15 to 6.17 % with mean value of 4.12±0.20. lactose content showed significant ($p<0.05$) decreased in positive CMT milk samples($P<0.05$), The obtained result, similar to those obtained by ***Sharif et al. (2007), Hamid et al. (2012) and Nagwaet al. (2015).*** chloride content in examined positive CMT quarter milk samples was ranged from 0.07 to 0.24 with a mean value of 0.13 ± 0.04, while in negative CMT milk samples ranged from 0.03 to 0.10 with a mean value of 0.06±0.001., Results obtained in this work showed that chloride content increased significantly ($P\leq 0.05$) in positive CMT quarter milk sample. Inspection of table (4) revealed that Alkaline phosphatase enzyme in examined positive CMT quarter milk samples ranged from 276.18 IU/ML to 2561 IU/MLwith a mean value of 723.76 ± 21.30. While in negative CMT samples ranged from 81.70 IU/ML to 256.40 IU/ML with a mean value of 186.40±15.60. Also lactate dehydrogenase enzyme content in examined positive CMT quarter milk samples ranged from 165IU/ML to 844 IU/ML with a mean value of 503.52±14.21, while in negative CMT quarter milk samples ranged from 68.4 to 190.17 with a mean value of 158.7±7.04.The obtained results indicate that Alkaline phosphatase enzyme and lactate dehydrogenase enzyme increased significantly ($P\leq 0.001$) in positive CMT quarter milk samples. The obtained results were nearly similar to those obtained by ***Aliaaet al. (2013) and Nagwaet al. (2015).*** Lower results were reported by ***Zeinhomet al. (2013)andNabih&abd. El Rahman (2015).***

Conclusion and Recommendations

The obtained results revealed that CMT and lactose and chloride as well as enzyme evaluation of mastitis milk can be considered as efficient tests for detection of subclinical mastitis in cows and buffaloes.Therefore, in order to minimize the risk of infection of milk and to safeguard consumers,the following suggestions should be applied:

1- The herd should bePeriodicallyexamined for subclinical mastitis using screening tests and confirmed by detection of enzymes level in milk.

2- Separation of the infected animals from healthy one and milked last or by special precautions and the milk from infected quarter should not be mixed with the bulk milk and discarded.

3- Efficient treatment of infected animals using effective drugs and retest them after suitable time to prove their complete cure.

4- Application of good herd management and strict hygienic measures including:

a- Functionally adequate milking machine should beused in a correct manner.

b- Proper hand –milking procedures with efficient washing and drying of milker's hand and udder.

c- Good cleaned and sanitized milking equipmentshould be used.

d- Application of teat dip after milking using a suitable and effective antiseptic solution.

5- Using a suitable scheme for prophylactic treatment of udder during drying period.

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التقييم الكيميائي والميكروبي والانزيمي للبن الناتج عن التهاب الضرع

سارة احمد السيد عبد الرحمن* واحمد حسن سعد وجيهان اسماعيل ابراهيم**

قسم الرقابه الصحيه علي الأغذية, كليه الطب البيطري, جامعه قناة السويس,الاسماعيليه,مصر

اجريت الدراسه علي 400 عينه لبن من ارباع الضرع للحيوانات التي تم تجميعها من100 حيوان من ثلاث مزارع مختلفه في منطقه قناه السويس وفحصها باختبار كاليفورنيا ووجد ان102 عينه موجبه للالتهاب الضرع تحت السريري وكشف الفحص الميكروبيولوجي للعينات الموجبه ان المكورات العنقوديهالذهبية كانت92(90.20%), المكورات العنقوديه كانت 18 (17.65%),المكورات السبحيه 79(77.45%),ميكروب الايشرشيا كولاي(القولونيه)35(34.31%)و للفطريات والخمائركانت74 (72.54%).وقد كشف الفحص الكيميائى والانزيمى للعينات الموجبه ان متوسط نسبه اللاكتوزللبن كانت 3.16±0.79اقل من النسبه الطبيعيه بينما كان متوسط نسبه الكلورايد للبن 0.13±0.04 اكثر من النسبه الطبيعيه,وكان متوسط نسبه انزيم اللاكتات ديهيروجيناز503.52±14.21وحده دوليه /مللى و كان نسبه انزيم الفوسفاتيز القلوي723.76±21.35 وحده دوليه /مللى, حيث كانت الانزيمات نسبتها مرتفعه في العينات الموجبه لاختبار الكاليفورنيا عن نسبتها في العينات السالبه لاختبار كاليفورنيا,من النتائج السابقه نستنتج ان قياس نسبه اللاكتوز والكلوريد والانزيمات للبن يعتبر طرق تشخيص مناسبه للكشف عن التهاب الضرع في قطيع الحيوانات الحلابه .