PATHOGENESIS AND BACTERIOLOGICAL ETIOLOGY PROFILE OF SUBCLINICAL MASTITIS OF SHE-CAMELS INFLUENCED IN ECONOMIC LOSSES IN AL-TAIF GOVERNORATE (SHORT TITLE: SUBCLINICAL MASTITIS IN SHE-CAMELS AT TAIF)

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ABSTRACT

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Subclinical mastitis is a disease for lactating animals which leads to loss of milk production, and reduces its quality. Sometimes progress to mastitis as well as the affection of udder my lead to dryness. A total of 160 quarters milk samples were collected from (n=40) she-camels (*Camelus dromedarius*) in herds in Al-Taif area. All she-camels apparently clinically healthy, the milk samples were examined for subclinical mastitis by using California Mastitis Test (CMT), Somatic Cell Count (SCC) and bacteriological tests. The results showed that the prevalence rate of subclinical mastitis was 23.8%. The main positive of CMT was scores (1+ - 3+), SCC ($2X10^4 - 4.2X10^5$) cell/ml. The predominant isolates were *CNS* (19.4%); *Staph.aureus* (14.4%); *E.coli* (5.6%); *Strept.dysagalactiae* (3.8%); *Strept.agalactiae* (2.5%) and *Klebsiella pneumonia* (1.9%). The accuracy of tests (CMT and SCC) used had got objective goals with the results of microbial isolation and identification. The results of present study showed that the percentage of subclinical mastitis was about of one quarter of tested milk samples and indicated that, there are losses in milk by one quarter. In conclusion, subclinical mastitis must be controlled through upgrading the environmental pasture, regular test of milk, early diagnosis, and all precautions for decreasing and prevention of infection must be taken, in consideration.

Keywords: *CMT, SCC, CNS, E.coli.*

الهيئة المرضية والأسباب البكتيريولوجية لالتهاب الضرع الخفي للنوق مؤدية لخسائر اقتصادية بمنطقة الطائف

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التهاب الضرع الخفي هو مرض للمرضعات من الحيوانات يؤدي إلى فقدان إنتاج الحليب، والجودة، وإحراز تقدم في بعض الأحيان إلى التهاب الضرع، فضلا عن قد يؤدي إلي جفاف الضرع. تم جمع مجموعه ١٦٠ عينة من الحليب من أرباع النوق لعدد ٤٠ ناقة، من قطعان في منطقة الطائف يظهروا بصحة جيدة بدون أي علامات مرضية في مرحلة الرضاعة. تم إجراء اختبار كاليفورنيا وعد الخلايا الجسدية بالإضافة إلي العزل والتصنيف البكتيريولوجي للعينات بوجود خمسة من النوق كعينات ضابطة. أظهرت النتائج أن معدل ايجابية التهاب الضرع الخفي ٢٣،٨%، أكثر ايجابية لاختبار كاليفورنيا انحصرت بين معدلي (+١ – +٣)، عدد الخلايا الجسدية بين ($X٤،٢ ^{٥١٠} - ^{٤}١٠X٢$) خلية/ملل. العزلات الأكثر في النسبة المكورات العنقودية السالبة للتجلط ١٩،٤%، تليها المكورات العنقودية الذهبية ١٤،٤%، ثم

العصويات القولونية ٥،٦%، *Strept.dysagalactiae* ٣،٨%، *Strept.agalactiae* ٢،٥%، وأخيرا كلابسيلا الرئوية ١،٩%. قد حصلت دقة الاختبارات المستخدمة على أهداف موضوعية مع نتائج العزلات الميكروبية وتحديد الهوية. كشف الهدف من التهاب الضرع الخفي في النوق في الطائف كمصدر للحليب ومنتجاته خصيصا للعائلة. نتائج الدراسة الحالية تبين النسبة المئوية حوالي ربع عينات الحليب المختبرة، التي تشير إلى أن هناك خسائر في الحليب بمقدار الربع. ويجب أن تسيطر علي التهاب الضرع الخفي من خلال رفع مستوى البيئة المراعي، واختبار للحليب وكذلك لتشخيص المرض في وقت مبكر مع اتخاذ جميع الاحتياطات لتقليل والوقاية من الإصابة.

INTRODUCTION

Subclinical mastitis is a form of mastitis characterized by change in milk composition with no signs of gross inflammation or milk abnormalities. Changes in milk composition can be detected. Chief pathogens causing subclinical mastitis include *Strept.agalactiae Staph.aureus. Strept.dysagalactiae.* The organisms live inside the udder and survives only for a short time outside the mammary gland. It spreads primarily during milking, contaminated hands and materials. The *Staph.aureus* lives inside and outside the udder on the teat skin. The microorganism can cause subclinical mastitis and spreads by the same way as *Strept.agalactiae*. Yeast can also cause mastitis, overuse of antibiotics and poor sanitation contribute to yeast mastitis was recorded by Macdonald Campus of McGill University, 2012.

The most predominant bacterial isolates were *Micrococcus spp.*, *Staph.aureus, Strept.spp.* and *Coryne. spp.* followed by eight other flora (Barbour *et al.*, 1985). She-camels are able to produce milk in valuable quantity (Faye, 2005). However, (*Camelus dromedarius*) could be affected by udder infection as mastitis like diary animals, a complex disease occurring worldwide among dairy animals, which lowering milk quality, with heavy economic losses largely due to subclinical mastitis which needs special fast diagnosis (Matofari *et al.*, 2003). Evidence indicates that subclinical mastitis causes suffering of she-camels, reduce milk yield, alters milk properties, impairs preservation and processing and is a public health concern for consumers of camel milk (Tibary and Anouassi, 2000). Very little medical information is known about subclinical mastitis concerning their etiology and occurrence in she-camels *(Camelidae)* (Kalla *et al.,* 2008). Cases of subclinical mastitis in she-camels have recently been reported in Saudi Arabia, Egypt, and Somalia (Barbour *et al.*, 1985; Mostafa *et al.*, 1987; Abdurahman *et al.*, 1991). The early clinical and laboratory diagnosis for subclinical mastitis and treatment greatly reduces the incidence of clinical mastitis and improve health of she-camels. For monitoring mastitis, a number of tests to detect changes in milk can be routinely used for screening purposes in milking herds. One of the screening procedures, for subclinical mastitis, involves the measurement SCC in milk. SCC is a count of the number of neutrophils and normal udder cells present in milk. An increase in the SCC to more than $5x10^5$ cells/ml is considered to be an indication of udder infection in she-camel (Eberlein, 2007).

SCC, CMT, Adenosine tri-phosphate (ATP), N-acetyl-D-gluco-samimidase (NAGase) and Serum albumin has been used as indirect diagnostic tools for infected and non-infected quarters of the she-camel mammary gland (Abdurahmann, 1996). The reading of test is the leading to the diagnosis, because the basal levels of cells and their physiological variations in the she-camels were still not yet established (Abdurahmann *et al.*, 1992).

Previously it was found that milk of she-camels type *(Camelus bactrianus)* contains not only leukocytes but also large number of a nuclear cell-like particle, so-called 'cell fragments' (Abdurahmann *et al.*, 1992). During the past decade there have been several reports on subclinical mastitis in she-camels (*Camelus dromedarius*) and a few on *(Camelus bactrianus)* (Barbour *et al.*, 1985; Obeid, 1983; Arush *et al.*, 1984; Kospakov, 1976a), but a work has been done to study subclinical mastitis and the udder's response to bacterial invasion by applied CMT to composite milk samples from the she-camels *(Camelus dromedarius)* and resulted in the

test was useful for screening subclinical infected udders (Barbour *et al.*, 1985; Saber *et al.*, 2010). Quarter milk samples that contained bacteria had significantly higher mean values for SCC, but the mean NAGase levels were not significantly different for the bacteriological negative and positive samples.

There was a low correlation coefficient between the SCC and NAGase in the quarter milk samples from which bacteria were not isolated. The type of bacteria had a significant effect on the SCC but not on the NAGase activity. Quarter samples from which *Staph.aureus* (coagulase positive) was isolated showed the highest mean SCC and this organism is therefore suspected to be the underlying cause of the subclinical mastitis.

The SCC gave a better indication of the presence of pathogenic microorganisms in milk samples than did NAGase (Guljye *et al.*, 2002). Subclinical mastitis was detected in she-camels milk samples, the causative agents was isolated from 66.7% of the samples. The most prevalent groups were *Strept.* group D 30%, CNS *Staph.* 20.1%, *Staph.aureus* 16%, *Strept.dysagalactiae* 3.6%, and *Strept. agalactiae* 1.5%. Other isolatates were *Coliforms* and *Micrococci. Strept. dysagalactiae* and *Strept.agalactiae* had a greater association with subclinical mastitis than the other pathogens. *Strept.agalactiae* and *Staph-aureus* were ranked as infectious pathogens while *Strept.* group D, *Strept.dysagalactiae, CNS, Coliforms* and *Micrococci* were ranked as environmental pathogens (Atofari *et al.*, 2005). Examined milk samples of she-camels, the microbiology identification showed the most predominant pathogens were *Micrococcus spp. Staph.aureus, Strept.spp.* and *Coryne.spp.* CMT test gave results with which 60% positive, 21% of she-camels revealed subclinical mastitis (Azmi *et al.*, 2008).

Subclinical mastitis is prevalent in Saudi camels, and its incidence is influenced by breed, parity, and stage of lactation (Aljumaah *et al.*, 2011). Samples gathered from clinically healthy dromedary camel from Al-Jouf, Saudi Arabia were cultured to detect subclinical udder infection. The milk samples were screened by SCC and CMT. Gram-positive *Cocci* were the dominant recovered udder pathogen. The mean value of SCC was 125,000 cells/ mm3. Infected quarter had generally higher mean values for SCC and CMT scores. Both SCC and CMT were of value in predicting the infection status of the udder (Saleh and Faye, 2011).

The objectives of this study were to diagnose the subclinical mastitis of she-camels with references to diagnosis tests SCC, CMT and microbial isolation and identification of milk, which related to reproduction and economic losses in Al-Taif area.

MATERIALS and METHODS

Animals: Forty lactating she-camels (*Camelus dromedarius*) from the farms present at Taif area, KSA, was screened for detection of subclinical mastitis. She-camels were of (5-15) years ages, within lactation stages (l-15 months) and suckling their calves. They were housed together and fed with ideal food, all the she-camels were free from clinical mastitis during the sampling period.

Sampling procedure: The quarters milk samples (160) were collected from lactating she-camels (n=40). Before collections of samples the udder and the teats were washed and cleaned with 70% alcohol. Discarded the for-milk from each teats. Tem ml of milk was taken from each quarter under sterile condition in sterile containers. Recorded the animal number, the quarter (Right or left front, Right or left back), date, time, the samples were kept in ice box till reach laboratory. The quarter milk samples were subjected to microbiological isolation, identification, SCC and CMT tests.

Bacteriological examination: Inoculated 0.01 ml of each milk sample separately and were streaked on blood and MacConkey agar plates; were incubated for 24- 48 h at 37°C. The plates were then examined for growth colony morphology. Individual colonies were picked for identification according to the

Scandinavian recommendations on examination of quarter milk samples (Klastrup, 1975). For fastidious organism the Muller-Hinton agar was supplemented with 5-7% sheep blood. Confirmed identification by API and Micro-scan.

California mastitis test (CMT): CMT was carried out using the method (Schalm and Noorlander, 1974). An equal volume of CMT reagent and milk was mixed and the reaction was graded 1,2,3,4 or 5, according to the Scandinavian recommendations,corresponding to 0, trace, 1, 2 and 3 (Klastrup and Schmidt, 1974). The test was performed by a trained technician. The reactions were interpreted as follows: score 1 = no reaction; score 2 = slight slime which tends to disappear with continued swirling; score 3= distinct slime but without gel formation; score 4 = immediate formation of gel which moves as a mass during swirling; score 5 = gel develops a convex surface and adheres to the bottom of the paddle.

Somatic cell count (SCC): The somatic cell counts (cells/ml) for the quarter milk samples were determined using Nucleo Counter SCC-100 (coulter electronic–ChemometecA/s, Denmark)

Statistical analysis: SCC values were transformed into log in order to get homogeneous variance. Mean and standard deviation were calculated for quantitative data. The relationships between SCC and CMT were estimated by the correlation of Spearman. The parity effect, the udder localization effect, the type of bacterial, contamination effect and the level of CMT reaction were estimated by variance analysis on logSCC. For measuring the parity effect, the variable parity was divided into 3 modalities: primiparous (n=1), second parity (n=7), more than second parity (n=11). The types of bacterial contamination were identified by Hierarchical Classification Analysis (HCA) after Multiple Correspondence Analysis (MCA). These analyses were applied to a table of presence/absence of different pathogens agents. The software SPSS, 18 version and XLSTAT (Addinsoft ©) used for data management and statistical analysis.

RESULTS

The results in Table 1 show the majority of she-camels milk samples were identified for subclinical mastitis which total samples were identified 160 resulted in 38 samples (23.8%) positive, were higher than the normal range, identified as subclinical mastitis according to CMT and SCC. Table 1 shows also the explanation of positive for CMT and SCC as flow first score 1+ = $2X10^4$ -which identified in 20 milk samples referred to 12.5%, on the other hand second score 2+ = $3.1X10^4$ showed in 11 milk samples less than first score equal to 6.9%, lastly third score 3+ = $4.2X10^5$ lower than second score and equal to 4.4%. The isolated pathogens types in Table 2 and Figure 1 show the 76 isolates (47.5%) were identified from the milk sample, as well as founding of isolation for more pathogens from once milk sample. Isolated pathogenes divided into Gram-positive included (*Staph.aureus*, *CNS*, *Strept.agalactiae* and *Strept.dysagalactiae*) and Gram-negative included (*E.coli*, *Klebsiella pneumonia*). The predominant isolates were *CNS* as 19.4%, then *Staph.aureus* 14.4%, *E.coli* 5.6%, *Strept.dysagalactiae* 3.8%, *Strept.agalactiae* 2.5%, the last *Klebsiella pneumonia* 1.9%.

Table 1: CMT and SCC from subclinical milk sample

Positive	1+	m* 2 X 10^4	20	12.5%
	2+	m* 3.1 X 10^4	11	6.9%
	3+	m* 4.2 X 10^5	7	4.4%
Samples n*=160			38	23.8%

n*= numbers
m* = main

Table 2: Isolated pathogens from milk samples

Gram-positive	*Staph.aureus*	23	14.4%
	CNS	31	19.4%
	Strept.agalactiae	4	2.5%
	Strept.dysagalactiae	6	3.8%
Gram-negative	*E.coli*	9	5.6%
	Klebsiella pneumoniae	3	1.9%
Samples n*=160		76	47.5%

n*= numbers

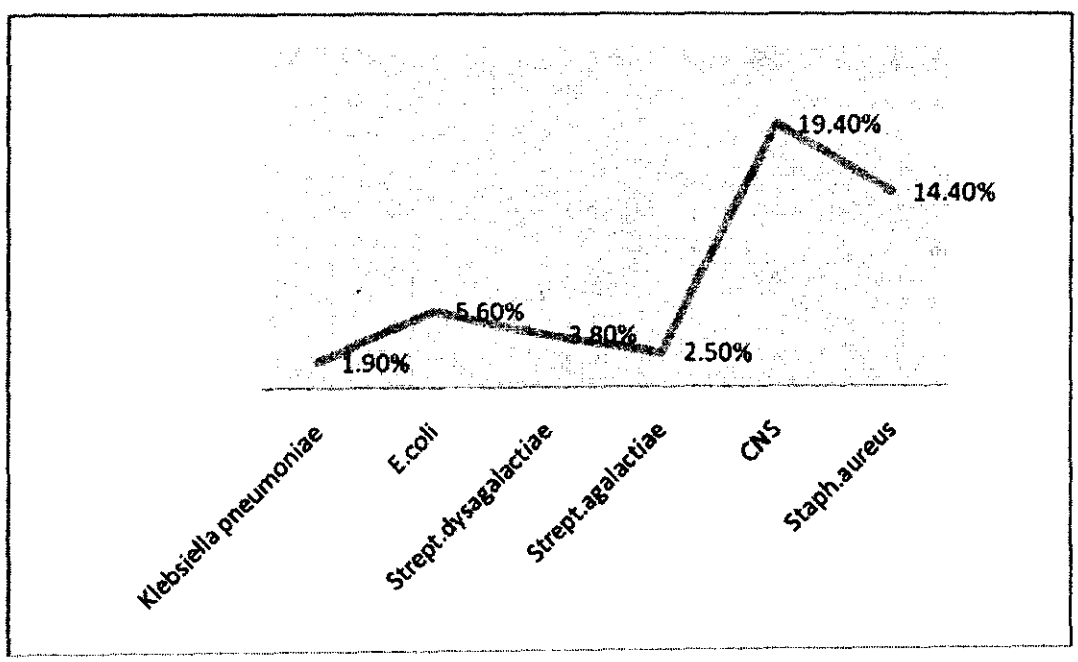

Figure 1. Isolated pathogens from milk samples

DISCUSSION

Subclinical mastitis is a form of mastitis, changes in milk composition, chief pathogens *Strept.agalactiae Staph.aureus. Strept. agalactiae,* lives inside the udder and survives only for a short time outside the mammary gland. It spreads primarily during milking, contaminated hands and materials.

Microorganism can cause subclinical mastitis and spreads the same way as *Strept.agalactiae*. Factors help in subclinical mastitis type of bacteria, physiological status, age, level of milk production, inherited featured, milking and environment Diagnosis of subclinical mastitis by SCC, CMT, WMT and ESCC tests plus microbiological isolation and identification (Macdonald Campus of McGill University, 2012). Tests to detect changes in milk can be routinely used for screening purposes in milking herds. One of the screening procedures, for subclinical mastitis, involves the measurement SCC in milk. SCC is a count of the number of neutrophils and normal udder cells present in milk. An increase in the SCC to more than $5x10^5$ cells/ml is considered to be an indication of udder infection in she-camel (Eberlein, 2007).

The present study gave incidence of subclinical mastitis in milk of she-camels (*Camelus dromedarius*) as 38 positive she-camels milk samples equal 23.8% from Al-Taif area, different subclinical mastitis prevalence rates were obtained from studies performed in many she-camels rearing countries, such as 81% in Palestine (Guljye *et al.*,2002), also cases of subclinical mastitis in she-camels have recently been reported in Saudi Arabia, Egypt, and Somalia (Barbour *et al.*, 1985; Mostafa *et al.*, 1987; Abdurahmann *et al.*, 1991). These variation may be due to weather and expose of udder to trauma due to ticks or desert plant and anti-suckling devices which used by camel's owner to allow the young calves older than one year are herded together with their harms. All these factors are predispose the udders to bacterial infections.

CMT test gave 12.5% first score, 6.9% second score and 4.4% third score and SCC gave $2X10^4$ for 12.5%, 6.9 as $3.1X10^4$ and 4.4 as $4.4X10^5$ showed in this work, the results of the somatic cell counts of the milk samples (between $2X10^4 - 4.2X10^5$ cells/ml) (Kospakov, 1976a; Abdurahman *et al.*, 1991), an increase in the number of somatic cells in camel milk with infected quarters has also been reported by (Mostafa *et al.*, 1987).

In present study the data of CMT scores, threshold of somatic cells count and its significant correlation to SCC are in agreement. The strong positive correlation of CMT and SCC with the bacteriological findings indicates that she-camels milk (Guljye *et al.*, 2002). During the past decade there have been several reports on subclinical mastitis in she-camels (*Camelus dromedarius*) and a few on *(Camelus bactrianus)* (Barbour *et al.*, 1985; Obeid, 1983; Arush *et al.*, 1984; Kospakov, 1976a), but a work has been done to study subclinical mastitis and the udder's response to bacterial invasion by applied CMT to composite milk samples from the she-camels (*Camelus dromedarius)* and resulted in the test was useful for screening subclinical infected udders (Barbour *et al.*, 1985; Saber *et al.*, 2010). The CMT scores were vary according to severity of inflammation, type of isolates and lactation stage (Mostafa *et al.*, 1987). The main pathogens isolated grouped in Gram-positive and Gram-negative, the main pathogens was *CNS* 19.4% which is normal flora, *Staph.aureus* 14.4% present always on skin can cause masitis, *E.coli* 5.6% as contamination from stools, environmental articles, *Strept.dysagalactiae* 3.8% main pathogens for mastitis, *Strept.agalactiae* 2.5% second pathogens for mastitis after *Strept.dysagalactiae* (Guljye *et al.*, 2002). The most prevalent groups were *Strept.* group D 30%, CNS *Staph.* 20.1%, *Staph.aureus* 16%, *Strept.dysagalactiae* 3.6%, and *Strept.agalactiae* 1.5% (Atofari *et al.*, 2005; Azmi *et al.*, 2008). In the present study, the accuracy of various tests for

subclinical obtained taking SCC and SCC as a standard tests. The accuracy was noted in the order of microbiological isolation and identification as in results.

CONCLUSION

Results of the present study showed that subclinical mastitis was prevalent in she-camels type (*Camelus dromedarius*) of Al-Taif area, Gram-positive were the dominant subclinical mastitis pathogens isolated. The positive correlation of CMT and SCC with the presence of subclinical mastitis pathogens were isolated in she-camels milk showed that CMT and SCC are a useful screening test in the detection of subclinical mastitis in she-camels and may serve to segregate udders infected with major pathogens in a subclinical form. Increase in the somatic cell counts of infected quarters indicated that camels reacted to inflammation induced by agents of mammary tissue by raising the number of somatic cells in the milk. However, further investigation is needed to determine the infection. The subclinical tests are pre-diagnosis detection tests have roles as well as used in this study are of great important in diagnosis of subclinical mastitis in she-camels, so fare are good to diagnosis as a first, stop the progress of pathogens to cause mastitis, treatment of she-camels, isolation of infected she-camels milks to prevent contamination of milk tank, improving economic income of she-camels milk and raising the she-camels milk quality. Beside that all reducing losses of she-camels milk and lowering treatment coast for infected she-camels on the owners of herd.

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