

## Prevalence of goat's subclinical mastitis caused by coagulase negative *Staphylococci spp.* in Al-Diwanyia province.

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### Abstract

The study was carried out in Al-Diwanyia province .Two hundred and seventy four milk samples from both udder halves of 137 clinically health ,local breed does were collected and examined by california mastitis test , somatic cells counting , and bacteriological culture on Staphylococcal selective media.The prevalence of subclinical mastitis in does was 29.92% ,the percentage infection in one udder half was 21.29% and 38.54% in both udder halves. The percentage of affection of left half was 48.2% ,while the right halves give the percentage of 51.8% , with no significant difference.A total of 274 milk samples,73 samples were showed positive reaction to california mastitis test ,and according to CMT scoring, the highest percentage was score 2,which was reached 47.94% ,while the lowest one was score 4,which was reached 10.95%,while the mean somatic cells count was  $1.2 \times 10^6$  and  $2.15 \times 10^6$  cell/ml ,respectively.Out of 73 milk samples positive for chemical tests (CMT & SCC),the coagulase negative *Staphylococci spp.* was isolated from 53 samples (72.60%) ,while the coagulase positive *Staphylococci spp.* was isolated from 17 samples (23.28%).Only 3 samples were showed no bacterial growth on staphylococcal selective media.

### Introduction

Mastitis is one of the most important disease in farm animals ,it is characterized by physical ,chemical and bacteriological changes in milk and by pathological changes in udder (Radostitis *et al* ,1997). According to severity ,mastitis is classified into clinical and subclinical cases( Smith & Reguinsky,1977). The sub clinical mastitis has less obvious changes in udder ,and detected only by measures of cellular content of milk(Andrews *et al* ,1983). Many studies that revealed that the normal goat milk has a higher cell content than normal milk in cattle (East ,*et al* ,1986 ; Droke *et al* ,1992). The detection of somatic cells in milk samples from individual goat can be performed by using

several methods, but the california mastitis test CMT and somatic cell count SCC represent a valuable tool for prevalence assessment and screening (Poutrel & Lorandelle ,1983; Manser ,1986; Dominique *et al* , 2003).Many studies (Poutrel,1984; Manser ,1986 ; Deinhofer and Parnthaner,1995 ;Contreras *et al* , 1997 and Moroni *et al* , 2005) revealed that coagulase negative *Staphylococci spp.* were the main pathogens which responsible for goat subclinical mastitis ,this study was designed to determine the prevalence of subclinical mastitis caused by coagulase negative *Staphylococci spp.* in local breed goat in Al-Diwanyia province.

### Materials and methods

#### Animals

The study was carried out in Al-Diwanyia province (Nuf'fer,Dagara and City center of Al-Diwanyia),137 clinically health ,local breed does, aged between 2-5 years, at mid-lactation period ,were selected to milk sampling .

#### Milk-sampling

From 137 does,274 milk samples were

collected, the teat was cleaned and swabbed with cotton soaked in 70% ethyl alcohol .After the discarded of the first 3 streams,10 ml of milk were collected in sterile screw-capped plastic tubes ,which labeled previously and preserved at 4C<sup>o</sup>,until examination in laboratory with in 24 hours.

#### California Mastitis Test CMT

The CMT was carried out by using the method described by Hinckley and Leander, (1981), the reaction was visually scored as 1, 2, 3 and 4 depending upon the amount of gel that forms. The reaction was interrupted as follows: Score 1 = streak of gel which disappears with swirling; Score 2 = slight slime which disappears with continuous swirling; Score 3 = distinct slime gel formation and Score 4 = gel develops as convex surface and adheres to bottom of paddle.

### Somatic Cell Counting SCC

SCC were determined by spreading of 1  $\mu$  L of thoroughly mixed milk from each CMT positive sample over 1 cm<sup>2</sup> area on a glass slide, which left to air drying and were stained by Newman-Lampert stain as described by Hinckley and Leander

, (1981). SCC equal or above  $1 \times 10^6$  cells per milliliter milk was considered as positive (Sheldrake *et al*, 1981).

### Bacteriological culturing

The positive milk samples for CMT and SCC were inoculated on manitol salt agar (which contain 7.5% NaCl) plates, which divided into 2 sections. Plates were cultivated in 37°C under aerobic condition for 24-48 hours. The smears from the colony which developed on selective media, stained with Gram's stain to identify the Gram positive cocci. The isolates were identified according to their cultural, morphological and biochemical characteristics by using catalase test and coagulase test as suggested by (Poutrel, 1984).

## Results

Among 137 lactating does, subclinical mastitis were detected in 41 animals, in prevalence rate 29.92%, in other hand, a total of 274 udder halves which examined in this study, the mastitic milk was detected in 73 halves (26.64%). The number of does which have mastitic milk in one udder half was 9 in percentage of 21.95%, while the number of animal which have mastitic milk in both udder halves was 32, in percentage of 38.54%. There is no significant difference between the

percentage of affection of left half which was 48.2% and the percentage of affection of right half 51.8%. From 274 milk sample, 73 sample were positive to California mastitis test, and according to CMT scoring, the highest percentage was score 2, which was reached 47.94% with mean SCC  $1.2 \times 10^6$  cells per milliliter of milk, while the lowest one was score 4, which reached 10.95% with mean SCC  $2.15 \times 10^6$  cells per milliliter of milk. (table 1).

Table (1) CMT scoring and mean SCC of milk samples

CMT score	SCC (mean)	No. of samples	percentage
2	$1.2 \times 10^6$	35	47.9
3	$1.8 \times 10^6$	30	41.09
4	$2.15 \times 10^6$	8	10.95

Out of 73 positive milk samples for chemical test which cultured on Staphylococcus selective media, the coagulase negative *Staphylococci spp.* was isolated from 53 samples in percentage of

72.6%. In other hand, the coagulase positive *Staphylococci spp.* was isolated from 17 samples in percentage of 23.28%. Only 3 samples were showed no bacterial growth on Staphylococcal selective media.

## Discussion

Many studies were carried out to determine the prevalence of Staphylococcal subclinical mastitis in goat. Contreras *et al*, (1999) reported that the prevalence was 39% in commercial dairy goat farms in Southern Maryland. In

Connecticut and Rhode Island the prevalence of Staphylococcal subclinical mastitis was 36.4% (White and Hinckley, 1999). In New York, the prevalence of subclinical mastitis was 38.2% was found (Smith and Reguinsky, 1977), while

in Kenya it was 28.7% (Ndegwa *et al* ,2000).In Iraq the incidence rate of subclinical mastitis among goats at different places in Iraq was found 9.58% and on half bases 7.07%(Al-Graibawi,1983).The high prevalence in the other studies may be due to that these studies performed on commercial dairy goats herd ,which may use machine milking ,while our study was carried out on local breed in small herd, which not considers as dairy goat breed.The high percentage of affection of both udder halves revealed in this study are compatible with results of Contreras *et al* ,(1999) whose report prevalence of subclinical mastitis in both udder halves as 34.3% (the right half 36% vs. 33% left) and Manser ,(1986) whose report the prevalence of affection of both udder halves 36%.Although the normal goat milk has a higher cells content than normal milk of cattle, the somatic cells of goat's milk increases associated with breed, stage of lactation and parity(Wilson *et al* ,1995 ; Boscos *et al* ,1996 ; Zeng *et al* ,1997; and Dominique *et al*,2003) .In many reports, when classifying goat milk sample as either mastitic milk of non mastitic milk ,a threshold of  $1 \times 10^6$  cells per milliliter milk can be used, this threshold will detect the most infected halves with little false

positive errors (Sheldrake ,*et al*,1981; Poutrel & Lorandelle ,1983 and Kalidigrou-Vassiliadou *et al* ,1991).The high percentage of coagulase negative *Staphylococcus spp.* isolates 72.6% and relatively low percentage of coagulase positive *Staphylococcus spp.* isolates 23.28% in our study are compatible with Dominique *et al* ,(2003),whose report that the *Staphylococcus spp.* are the main causative agent of intra-mammary of small ruminants and the frequent isolate was *Staphylococcus aureus* in clinical cases and coagulase negative *Staphylococcus spp.* in subclinical cases, also these findings are in agreement with those reported by (Sheldrake ,*et al*,1981;Contreras ,*et al* ,1997 ; Ndegwa *et al* ,2000 and Moroni ,*et al* ,2005) they reported that most of subclinical intra mammary infection were caused by Coagulase negative *Staphylococci spp.* . Devries *et al* ,(1985) isolate 661 strains of Coagulase negative *Staphylococcus spp.* from the skin and nares of cattle, pigs ,poultry ,goat and sheep, and this fact may be interpreted the high prevalence of CNS in the study.The negative culture results of 3 samples in this study ,may be due to presence of other pathogens which can not grow on Staphylococcal selective media or may be false positive for chemical tests.

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## دراسة حدوث التهاب الضرع تحت السريري في الماعز المتسبب عن المكورات العنقودية السالبة لإنزيم التخثر في محافظة الديوانية

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### الخلاصة

أجريت الدراسة في محافظة الديوانية، حيث تم جمع ٢٧٤ عينة حليب من كلا شطري ضرع ١٣٧ أنثى ماعز محلي سليمة سريريا، فحصت العينات مختبرياً باستخدام اختبار كاليفورنيا لالتهاب الضرع، عدد الخلايا الجسمية والزرع الجرثومي على أوساط انتقائية للمكورات العنقودية. أظهرت النتائج إن نسبة حدوث التهاب الضرع تحت السريري في إناث الماعز كانت ٢٩.٩٢ %، وكانت نسبة إناث الماعز المصابة بالتهاب الضرع تحت السريري في احد شطري الضرع هي ٢١.٢٩ %، بينما كانت نسبة إصابة كلا شطري الضرع ٣٨.٥٤ %. لم يلاحظ أي فرق معنوي بين نسبة إصابة الشطر الأيمن ٥١.٨ % ونسبة إصابة الشطر الأيسر ٤٨.٢ %. من مجموع ٢٧٤ عينة حليب أظهرت ٧٣ منها تفاعل ايجابي لاختبار كاليفورنيا لالتهاب الضرع و اعتمدا على شدة التفاعل، كانت أعلى نسبة هي الدرجة الثانية حيث بلغت ٤٧.٩٤ %، بينما كانت أوطأ نسبة هي الدرجة الرابعة حيث بلغت ١٠.٩٥ %، أما معدل عدد الخلايا الجسمية لهذه الدرجتين فكانت  $1.2 \times 10^6$  و  $2.15 \times 10^6$  خلية /مل من الحليب وعلى التوالي. من مجموع ٧٣ عينة حليب موجبة لفحوصات التهاب الضرع، عزلت المكورات العنقودية السالبة لإنزيم التخثر من ٥٣ عينة بنسبة ٧٢.٦ % بينما عزلت المكورات العنقودية الموجبة لإنزيم التخثر من ١٧ عينة بنسبة ٢٣.٢٨ %، ولم تظهر ٣ عينات أي نمو على الوسط الانتقائي للمكورات العنقودية.