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 × 10^6 and 2.15× 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 diseases 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 Paranthaner, 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 4°C, 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 disappear with swirling; Score 2 = slight slime which disappear with continuous swirling; Score 3 = district slime gel formation and Score 4 = gel develop as convex surface and adhere to bottom of paddle.

**Somatic Cell Counting (SCC)**

SCC were determined by spreading of 1 μL of thoroughly mixed milk from each CMT positive samples over 1 cm² area on a glass slide, which lift to air drying and were stained by Newman-Lampert stain as described by Hinckley and Leander, (1981). SCC equal or above 1×10⁶ 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 at 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 1.2×10⁶ cells per milliliter of milk, while the lowest one was score 4, which reached 10.95% with mean SCC 2.15×10⁶ cells per milliliter of milk. (Table 1).

<table>
<thead>
<tr>
<th>CMT score</th>
<th>SCC (mean)</th>
<th>No. of samples</th>
<th>percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1.2×10⁶</td>
<td>35</td>
<td>47.9</td>
</tr>
<tr>
<td>3</td>
<td>1.8×10⁶</td>
<td>30</td>
<td>41.09</td>
</tr>
<tr>
<td>4</td>
<td>2.15×10³</td>
<td>8</td>
<td>10.95</td>
</tr>
</tbody>
</table>

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% (Ndewgwa 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 ; Bocos 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 1×10⁶ 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 ; Ndewgwa 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.

References


mas\textit{t}is related \textit{p}athogen in goat milk .Veterinary Microbiology .43 :161-166.


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 274 عينة حليب من كلا شطتي رطق 137 أنثى ماعز محلي سلالة سريريا. فحصت العينات مختبريا باستخدام اختبار كاليفورنيا لالتهاب الضرع، بعد الخلايا الجسمية والزرع الجرثومي على أوضاع انتقائية للمكورات العنقودية. أظهرت النتائج إن نسبة حدوث التهاب الضرع تحت السريري في إناث الماعز كانت 29.42%، وكانت نسبة إناث الماعز المصابة بالتهاب الضرع تحت السريري في أحد شطري الضرع هو 21.29%، بينما كانت نسبة إصابة كلا شطري الضرع كان 48.84%. لم يلاحظ أي فرق معنوي بين نسبة إصابة الشطر الأيمن 51.8% ونسبة إصابة الشطر الأيسر 88.2%. من مجموع 274 عينة حليب أظهرت 23 منها تفاعل إيجابي لاختبار كاليفورنيا لالتهاب الضرع واعتمادا على شدة التفاعل، كانت أعلى نسبة هي درجة الثانية حيث بلغت 47.94%، بينما كانت أدنى نسبة هي درجة الرابعة حيث بلغت 10.21%، أما معدل عدد الخلايا الجسمية لهذه الدرجتين فكانت 10^6 × 2.15 و 10^5 خلايا/مل من الحليب وعلى التوالي. من مجموع 73 عينة حليب موجبة لفحوصات التهاب الضرع، عزلت المكورات العنقودية السالبة لإنزيم التخثر من 53 عينة بنسبة 72.6% بينما عزلت المكورات العنقودية السالبة لإنزيم التخثر من 17 عينة بنسبة 23.28%، ولم تظهر 3 عينات أي نمو على الوسط الانتقائي للمكورات العنقودية.