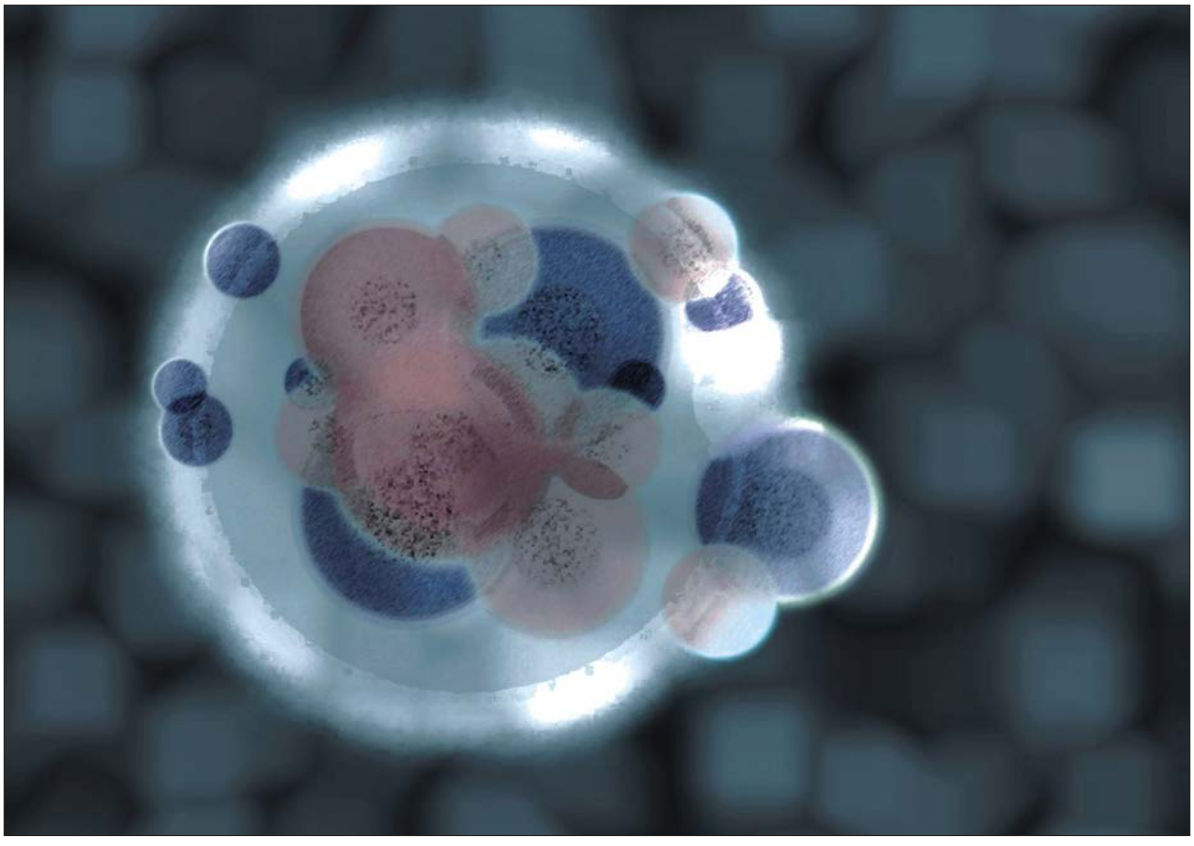

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# Mastitis in One Humped She-Camels (*Camelus dromedarius*) in Jordan

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**Abstract:** This study was conducted to establish data on mastitis in she-camels in Jordan. Milk samples were collected from 90 dromedary camels in south province of Jordan. California Mastitis Test (CMT) gave results with 70 milk samples; 42 samples (60%) showed positive CMT. Infection with some bacterial species was associated with positive CMT. About 21% of the camels revealed clinical signs of mastitis. The highest percentage of bacterial count, which range from $3.0\times10^2$ to $<3.0\times10^3$ cfu $mL^{-1}$, was founded in the milk samples. The most predominant bacterial isolates were *Micrococcus* spp., *Staphylococcus aureus*, *Streptococcus* spp. and *Corynebacterium* spp. Gentamycin, Ampicillin and Tetracycline were the most effective antimicrobial agents against the bacterial isolates.

**Key words:** *Camelus dromedarius*, mastitis in camels, California mastitis test

## INTRODUCTION

Mastitis has both an extreme zoonotic and economic importance. It is the cause of multiple hazardous effects on human health and animal production (Makovec and Ruegg, 2003; Hegazi *et al*., 2004; Al-Majali *et al*., 2008). Camel milk is one of the main components of diet of the nomads in semiarid and arid zones and is an essential food for livelihood of people and it maybe the only milk available in places where other milking animals cannot be maintained (Kazmi, 2002; Abdurahman, 2006). Little work has been done on mastitis in camels comparing to studies on sheep and cows. Three decades ago, there was no mention of mastitis problem at herd level; today it is reported from almost all camel rearing countries (Al-Ani and Al-Shareefi, 1998; Guliye *et al*., 2002; Khedid *et al*., 2003; Mohammed *et al*., 2005).

Many different bacteria has been isolated from mastitic mammary glands in camels either in the form of pure or mixed infection (Barbour *et al*., 1985; Abdurahman, 1996; Bekele and Molla, 2001; Younan *et al*., 2001; Woubit *et al*., 2001; Hegazy *et al*., 2004; Abdurahman, 2006; Abdel Gadir *et al*., 2006). In Jordan, information on mastitis in one humped she-camel (*Camelus dromedarius*) is almost non-existed and has recently received more veterinary attention as a disease of camels.

This study was undertaken to establish data on mastitis in single-humped subtropical camels (*Camelus dromedarius*) in Jordan, to determine if a relationship exists between California Mastitis Test (CMT) results and bacterial counts and to study the susceptibility of these bacteria to different antimicrobial agent.

## MATERIALS AND METHODS

This study was conducted during the years 2006 and 2007. Milk samples were collected from 90 lactating she-camels that selected randomly from five herds in the south province of Jordan. All udders were subjected to clinical examinations such as swelling and presence of lesions or anatomical malformations. Prior to sampling, the udder was washed, dried and the teat was disinfected. After discarding the first 15 mL of foremilk, about 15 mL were collected in sterile universal bottles and transported immediately to the laboratory in ice boxes for analysis.

The milk samples were examined for their consistency, color and other visible abnormalities. Clinical mastitis was recognized by abnormal milk, signs of udder infection and detection of mastitis pathogens by bacteriological culture; whereas subclinical mastitis was recognized by apparently normal milk and an increase in somatic cells as evidence by CMT and positive culture results. CMT was used to give an indication of the number of somatic cells present in each of the milk samples.

To determine the total bacterial count, a volume of 0.1 mL of each milk sample was spread using a sterile

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L-shaped glass rod on Plate Count Agar (Oxoid); plates were incubated at 37°C for a period of 18-24 h and then counted. Direct streaking was done on duplicate 7% sheep blood agar and MacConkey agar plates; plates were incubated aerobically and anaerobically using Gas Pak System at 37°C and examine after 24 and 48 h. Bacteriological examinations were carried out. MacConkey agar plates were used to detect *Enterococcus* spp. and any Gram-negative bacteria. The Gram-negative bacteria were identified by biochemical reactions. Bacterial isolates present at $3.0\times10^2$ cfu $mL^{-1}$ or greater were identified to the genus level and in certain cases to the species level using standard procedures (Finegold and Scott, 1978).

Muller-Hinton Agar (oxoid) was used for disk diffusion method to test the susceptibility of the isolates to some antibiotics: 10 μg Gentamycin, 10 μg Ampicillin, 10 μg Streptomycin, 30 μg Tetracycline and 10 IU Penicillin. For fastidious organisms the Muller-Hinton Agar was supplemented with 7% sheep blood. The recorded data for the Sensitivity test to antibiotics were subjected to the analysis of variance (ANOVA); the Least Significant Differences (LSD) at probability <0.05 was used to asses the differences among means.

## RESULTS

Seventy milk samples out of 90 collected from individual camels were scored by the CMT technique. Camels with obvious signs of inflamed udders had a mean lactation of about four months. Some (21%) of the camels had clinical signs of mastitis. The visible signs of inflammation included acute and edematous swelling of the udder and formation of pus in the mammary exudates resulting in a visible alteration of the milk.

Table 1 shows differences between percentages of CMT negative and positive samples. The negative samples distributed in three different bacterial count ranges namely $<3.0\times10^2$, $3.0\times10^2$ to $<3.0\times10^3$ and $>3.0\times10^3$ cfu $mL^{-1}$; the highest percentage of CMT negative milk samples (69.45) was found in the total bacterial count range of $<3.0\times10^2$ cfu $mL^{-1}$. For CMT positive samples, the highest percentage (54.08) was found in the range of $3.0\times10^2$ to $<3.0\times10^3$ cfu $mL^{-1}$.

Table 2 shows the relationship between specific organisms, which mostly are the causative agent of mastitis and the respective percentage of samples with negative and positive CMT. The bacteria (*Micrococcus* spp., *Staphylococcus aureus* and *Streptococcus* spp.) show highest percentages for positive CMT; while the bacteria (*Corynebacterium pyogenes*, *Corynebacterium pseudotuberculosis* and *Pseudomonas aeruginosa*) show only positive CMT; whereas the bacteria (*E. coli*, *Mannheimia haemolytica* and *Pasteurella multocida*) show only negative CMT.

Table 3 shows that the total bacterial count range for different bacteria infecting the camel's udder was most commonly $3.0\times10^2$ to $3.0\times10^3$ rather than $>3.0\times10^3$ cfu $mL^{-1}$. This same table indicates that the most frequent bacterial flora from different camels was: *Micrococcus* spp., *Staphylococcus* spp., *Streptococcus* spp. and *Corynebacterium* spp. Six other aerobic bacteria were isolated. *Bacteroid* spp. was the only anaerobic bacterium isolated. *Candida albicans* was isolated from one sample.

Table 4 shows the result of sensitivity tests of the orgamisms isolated to antibiotics. The *in vitro* susceptibility testing of 61 bacterial isolates showed that the most effective drugs were Gentamycin and Ampicillin. The less effective drugs were Streptomycin and Penicillin.

Table 1: The relationship between positive and negative CMT scores and the percentages of camel milk samples of different bacterial counts

| | | Percentage of samples within the total bacterial count range[1] | | |
|---|---|---|---|---|
| CMT score | No. of samples | $<3.0\times10^2$ | $3.0\times10^2$ to $<3.0\times10^3$ | $>3.0\times10^3$ |
| Positive | 42 | 22.50 | 54.08 | 23.42 |
| Negative | 28 | 69.45 | 26.20 | 4.35 |

Table 2: Bacterial identified and percentage of camel milk samples with different CMT scores

| | | Percentage of samples within CMT score range | |
|---|---|---|---|
| Bacterial species | No. of samples | Positive | Negative |
| *Micrococcus* spp. | 15 | 67.22 | 32.78 |
| *Staphylococcus aureus* | 12 | 75.00 | 25.00 |
| *Streptococcus* spp. | 7 | 89.80 | 10.20 |
| *Corynebacterium pyogenes* | 2 | 100.00 | 0.00 |
| *Corynebacterium pseudotuberculosis* | 2 | 100.00 | 0.00 |
| *Escherichia coli* | 3 | 0.00 | 100.00 |
| *Mannheimia haemolytica* | 2 | 0.00 | 100.00 |
| *Pasteurella multocida* | 2 | 0.00 | 100.00 |
| *Pseudomonas aeruginosa* | 2 | 100.00 | 0.00 |

Table 3: The percentages of camel milk samples that included in two different bacterial counts of various organisms

| Bacterial species | No. of samples | Percentage of samples within total bacterial count range* | |
|---|---|---|---|
| | | >3.0×10² to <3.0×10³ | >3.0×10³ |
| *Micrococcus* spp. | 18 | 72.20 | 27.80 |
| *Staphylococcus aureus* | 14 | 78.60 | 21.40 |
| *Streptococcus* spp. | 8 | 75.00 | 25.00 |
| *Corynebacterium pyogenes* | 3 | 66.67 | 33.33 |
| *Corynebacterium pseudotuberculosis* | 4 | 75.00 | 25.00 |
| *E. coli* | 3 | 66.67 | 33.33 |
| *Mannheimia haemolytica* | 2 | 50.00 | 50.00 |
| *Pasteurella multocida* | 2 | 50.00 | 50.00 |
| *Pseudomonas aeruginosa* | 2 | 50.00 | 50.00 |
| *Klebsiella pneumoniae* | 2 | 100.00 | 0.00 |
| *Enterococcus* spp. | 1 | 100.00 | 0.00 |
| *Bacteroides* spp. | 2 | 100.00 | 0.00 |
| *Candida albicans* | 1 | 100.00 | 0.00 |

*Counts of <3.0×10² were not considered as such bacteria were not identified

Table 4: Sensitivity test for bacterial isolates against different antibiotics using disc diffusion method

| Bacterial species | No. of isolates | Percentage of sensitivity to antibiotics | | | | |
|---|---|---|---|---|---|---|
| | | GM | AM | TE | S | P |
| *Micrococcus* spp. | 18 | 100.00 | 88.90 | 88.30 | 83.30 | 33.30 |
| *S. aureus* | 14 | 92.80 | 92.80 | 100.00 | 85.70 | 57.10 |
| *Streptococcus* spp. | 8 | 100.00 | 100.00 | 100.00 | 25.00 | 25.00 |
| *C. pseudotuberculosis* | 4 | 100.00 | 100.00 | 75.00 | 0.00 | 0.00 |
| *C. pyogenes* | 3 | 66.70 | 100.00 | 66.70 | 0.00 | 66.70 |
| *E. coli* | 3 | 100.00 | 33.30 | 33.30 | 0.00 | 0.00 |
| *Mannheimia haemolytica* | 2 | 100.00 | 100.00 | 66.70 | 66.70 | 33.30 |
| *P. multocida* | 2 | 66.70 | 100.00 | 66.70 | 66.70 | 0.00 |
| *Ps. aeruginosa* | 2 | 100.00 | 0.00 | 50.00 | 0.00 | 0.00 |
| *Enterococcus* spp. | 1 | 100.00 | 100.00 | 100.00 | 100.00 | 0.00 |
| *Bacteroides* | 2 | 0.00 | 100.00 | 100.00 | 0.00 | 0.00 |
| *Kl. pneumoniae* | 2 | 100.00 | 100.00 | 100.00 | 100.00 | 50.00 |
| Mean* | - | 85.51a | 84.58a | 78.89a | 43.95b | 22.11b |

GM = Gentamicin (10 μg), AM = Ampicillin (10 μg), TE = Tetracycline (30 μg), S = Streptomycin (10 μg), P = Penicillin (10 IU). *Percentages means among columns which are followed by different letter(s) differ significantly ($p<0.05$)

## DISCUSSION

The relation among CMT, the presence of inflamed udders and the bacteriological findings indicated that camel milk is like that of cows (Djabri *et al*., 2002); it also indicated that camels have phagocytic cells, which constitute one of the essential defenses against microbial infection of the mammary glands.

CMT can be used to detect subclinically infected udders of camels (Barbour *et al*., 1985; Sargeant *et al*., 2001). Table 1 and 2 indicated that bacterial infection was involved in mastitis of *Camelus dromedarius*. Table 1 shows that higher bacterial counts were present in positive CMT samples than in the negative ones. The relation of CMT with the presence of mastitis pathogens in camel milk showed that CMT is a useful screening test in the detection of mastitis in camels and may serve to segregate udders infected with major pathogens in a subclinical form. An increase in the number of somatic cells in camel milk is a good indication of inflammation.

Table 2 indicated that the majority of camels react to infecting bacteria by raising the somatic cells in milk. Woubit *et al.* (2001) and Abdurahman *et al.* (1995) found that the results of the somatic cell counts of the milk samples were between $3\times10^5$ to $1.5\times10^7$ of cells $mL^{-1}$.

Table 3 shows that in many cases of infection with a variety of bacteria, the organisms are present at less than $3.0\times10^3$ $mL^{-1}$ and a minority exceed this level which is similar to that reported by Barbour *et al.* (1985). This may indicate that there is a limit to bacterial multiplication in the camel's udder probably due to the complex immune system. The relative number of the various pathogens (Table 3) especially *S. aureus* and *Streptococcus* spp. is very similar to that reported by Woubit *et al.* (2001) and Abdurahman (2006). *S. aureus* and *Streptococcus* spp. along with *Micrococcus* spp. seem to be the major causative agents of mastitis in camels and this is in agreements with that of Barbour *et al.* (1985) and Woubit *et al.* (2001).

The *in vitro* susceptibility test of the bacterial isolates indicated that Gentamycin, Ampicillin and

Tetracycline were the most effective drugs. The bacterial flora showed greatest resistance to penicillin and streptomycin; these two drugs are the most commonly used for domestic animals in Jordan and this may lead to an accumulation of resistant bacteria to these drugs. The percentage average of resistance of Gram-positive cocci to penicillin was 63.587% as shown in Table 4.

In conclusion, the results of this study indicated that mastitis was prevalent in dromedary camels in Jordan and the Gram-positive cocci were the dominant mastitis pathogens. More efforts are needed to improve the general udder health. It is possible to recommend a control program for camel mastitis in Jordan taking in consideration using effective antibiotics therapy during lactation and at drying off; this would be essential part of such a program.

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