

Bacteria, Mycoplasma and Fungi Associated with Sub-clinical Mastitis in Camel.

Suheir, I. Abdalla¹; Salim, M.O¹. and Yasin, T. E².

(1) Central Veterinary Research Laboratories, P.O. Pox 8067 (Al-Amarat) Khartoum- Sudan. (2) Department of Preventive Medicine and Veterinary Public Health, Faculty of Veterinary Medicine, University of Khartoum. P.O. Box 32- Khartoum north.

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Summary

One hundred and sixty milk samples were collected from lactating she-camels from different localities in the Sudan. They were examined for sub-clinical mastitis by California mastitis test (CMT). Isolation of bacteria, mycoplasmas and fungi was carried out.

Sub-clinical mastitis based on CMT and bacteriological examination was detected; as 68(42.5%) and 59(36.87%) cases out of total samples. More mastitis cases were observed in the last stages of the lactation period and during the fourth and fifth calving. *Staphylococcus aureus* was dominant (20.2%). *Streptococcus* spp, *Corynebacterium*, *Enterobacteria* and *micrococcus* spp were isolated in variable degree. *Mycoplasma arginini*, *Acholephasma laidlawii*, mould and yeast were also encountered.

Introduction

Mastitis is a complex disease occurring worldwide among dairy animals, with heavy economical losses. More work was carried out in bovine, caprine and ovine mastitis, but little is known about mastitis in camel (Abdalgadir, 2001).

Compared to cattle, the disease in camel is infrequent, but it may shoot up due to the increased use of camel milk (Almaw and Molla, 2000). In the Sudan, acute and chronic camel mastitis were clinically diagnosed (Obeid, 1996), while the sub-clinical mastitis can be detected by indirect tests such as California Mastitis Test (CMT) and Somatic cell count (SCC) as well as microbial examination. In this study the aerobic bacteria, mycoplasma and fungi associated with camel sub-clinical mastitis in different localities in the Sudan were identified.

Materials and Methods

A total of 160 milk samples were collected from she-camels (*Camelus dromedarius*), 85 samples from Kordofan, 60 from Khartoum and 15 from port Sudan. Sub-clinical mastitis was detected by the California mastitis test. Isolation and identification of the microbial isolates were carried out according to Barrow and Feltham (1993). The relationships between the stage of lactation and number of calving and the prevalence of mastitic she-camel were also explained depending on the questionnaire carried out in the areas where milk samples were collected.

The lactation period was classified according to Radostits *et al.* (2000) into three stages: First stage (1-120 day), second stage (121-240 days) and a third or last stage (>240 days).

Results

California mastitis test (CMT):

When the 160 she-camel milk samples were tested by CMT, 59(36.87%) proved positive (Table 1).

According to the CMT, only 59 (36.87%) out of 160 milk samples showed positive sub-clinical mastitis; 99 (61.88%) isolates were obtained from them. Most isolates (n=54; 63.5%) were obtained from 35 camel milk samples collected from Kordofan State. In Khartoum State, 35 (58.34%) were isolated from the 60 samples tested and 10 isolates (66.7%) from 15 samples brought from Port Sudan (Table 1).

On the basis of cultural, morphological and biochemical characters the main bacterial isolates encountered were belonged to the genera *Staphylococcus*, *Streptococcus*, *Micrococcus*, *Corynebacterium*, *Bacillus*, *Bordetella parapertussis* and *Enterobacteria*. *Mycoplasma arginini* and *Acholeplasma laidlawii*, yeasts and moulds were also isolated. Number and prevalence of the isolated microorganisms are shown in Table 2.

The frequency of isolation of different microorganisms from camel milk obtained from the three states is shown in Table 3, 4 and 5.

Relationship between the stage of lactation and the prevalence of mastitis:

Cases of mastitis increased with progress of lactation. According to the questionnaire, few cases of mastitis were observed (25%) at the first stage, (30%) at the second stage and higher number of cases at the last stage of lactation (45%).

Relationship between calving number and incidence of mastitis:

There was a direct relationship between the frequency of mastitis and the calving number. During the first, second and third calving the incidence prevalence of mastitis was 25% while at the fourth and fifth calving the incidence increased to 43.8%. However, mastatic cases decreased to 16.7% in the sixth, seventh and eighth calving.

Table 1: Results of CMT, Number and Percentage of total organisms isolated from different areas

| Study area | No. of samples tested | CMT No. +ve (%)* | No. of isolates (%)* |
|-------------------|-----------------------|--------------------|----------------------|
| Kordofan | 085 | 36 (42.35%) | 54 (63.5%) |
| Khartoum | 060 | 18 (30.0%) | 35 (58.3%) |
| Port Sudan | 015 | 05 (33.33%) | 10 (66.7%) |
| Total | 160 | 59 (36.87%) | 99 (100%) |

+ ve= positive *Percentages are calculated from the number of samples tested in each area.

Discussion

The present work was performed to identify the causative agents of camel sub-clinical mastitis in three areas of the Sudan, and to disclose the relationship between mastitis, number of calving and stage of lactation.

The last stage of lactation was found to be associated with high incidence of mastitis; a finding that agrees with that of Salwa (1995). The gradual increase in incidence of mastitis corresponded with the increase in calving number until the 5th calving before it reversed at the 6th calving and on word, is in agreeent with those of Omer (1991) and Salwa (1995).

Increase and decrease in cases of mastitis in both lactation stage and number of calving may be attributed to the immune response of the host against the pathogen.

Sub-clinical mastitis was detected by CMT in 59 (36.87%) of the tested samples. This finding is in line with the results obtained by Amel (2003), Abdelgadir (2001), Mostafa *et al.* (1987) who reported a

Table 3: Frequency of microorganisms isolated from camel milk in Kordofan State during the period

| Species | No. isolates obtained | isolates % | Prevalence of each isolate to total isolates% |
|-----------------------------------|-----------------------|-------------|---|
| <i>Staphylococcus aureus</i> | 12 | 30 | 15.792 |
| <i>Staphylococcus epidermidis</i> | 02 | 05 | 02.63 |
| <i>Staphylococcus hyicus</i> | 02 | 05 | 02.63 |
| <i>Staphylococcus simulans</i> | 01 | 02.5 | 01.32 |
| <i>Staphylococcus sciuri</i> | 01 | 02.5 | 01.32 |
| <i>Staphylococcus warneri</i> | 01 | 02.5 | 01.32 |
| <i>Streptococcus agalactiae</i> | 05 | 12.05 | 06.58 |
| <i>Streptococcus dysgalactiae</i> | 02 | 05 | 02.63 |
| <i>enterococcus faecalis</i> | 03 | 07.5 | 03.95 |
| <i>Streptococcus uberis</i> | 02 | 05 | 02.63 |
| <i>Mycoplasma arginine</i> | 02 | 05 | 02.63 |
| <i>Acholeplasma laidlawi</i> | 02 | 05 | 02.63 |
| Yeast | 03 | 07.5 | 03.95 |
| Mould | 02 | 05 | 02.63 |
| Total | 40 | 100% | 52.63 |

slightly lower incidence when CMT was applied as screening sub-clinical mastitis test.

There was strong correlation between CMT score and the bacteriological results which is in line with the results reported by Obeid *et al.* (1983), Abdelrahman *et al.* (1995) who respectively found 49.97%, 45%, 56% and 43.5%. However, these findings are lower than that (67.3%) reported by Amel (2003).

The fact that *Staph. aureus* was the main cause of sub-clinical camel mastitis, confirms the results obtained by Abdulrhman *et al.*

(1995) and Amel (2003). Similar findings have been reported by Abdelgader (2001) who attributed subclinical camel mastitis to 24.7% *Staph. aureus*. More *Staph. aureus* involvements (33.18% and 44.2%) have been reported by Amel (2003 and Salwa (1995), respectively.

Abdulrahman *et al.* (1995) who respectively found 49.97%, 45%, 56% and 43.5%. These findings are lower than those reported by Amel, 2003 (67.3%).

Table 4: Frequency of microorganisms isolated from camel milk collected from Khartoum (Omdurman) city.

| Species | No. isolates obtained | isolates % | Prevaence of each isolate in relation to total isolates% |
|-----------------------------------|-----------------------|-------------|--|
| <i>Staphylococcus aureus</i> | 07 | 25.9 | 9.21 |
| <i>Staphylococcus epidermidis</i> | 02 | 07.4 | 2.63 |
| <i>Staphylococcus hyicus</i> | 03 | 03.7 | 3.95 |
| <i>Staphylococcus simulans</i> | 01 | 11.1 | 1.32 |
| <i>Staphylococcus hominis</i> | 01 | 03.7 | 1.32 |
| <i>Staphylococcus klossii</i> | 01 | 03.7 | 1.32 |
| <i>Staphylococcus lentus</i> | 03 | 11.1 | 3.95 |
| <i>Streptococcus agalactiae</i> | 02 | 07.4 | 2.63 |
| <i>Streptococcus dysgalactiae</i> | 01 | 03.7 | 1.01 |
| <i>Streptococcus uberis</i> | 01 | 03.7 | 1.32 |
| <i>Enterococcus faecalis</i> | 03 | 11.1 | 3.95 |
| Yeast | 02 | 07.4 | 2.63 |
| Total | 27 | 100% | 35.54 |

Staphylococcus aureus has been identified as the main cause of sub-clinical camel mastitis, this confirm the results obtained by Abdurhman *et al.* (1995) and Amel (2003). Similar findings were reported by Abdelgader (2001) who isolated 24.7% *Staph. aureus*, this was lower than that isolated by Amel (2003) (33.18%) and Salwa (1995) 44.2%.

The prevalence of streptococcus species proved to be the second, 22 isolates (22.22%), of those *Strep. agalactiae*, *Strep. dysgalactiae*

and *Strep. uberis* were identified. The number of isolates reported in this study was higher than that obtained by Obeid

Table 5: Frequency of microorganisms isolated from camel milk in Port Sudan

| Species | No. of isolates obtained | Isolates from port Sudan % | Prevaence of each isolate in relation to total isolates % |
|-------------------------------------|--------------------------|----------------------------|---|
| <i>Staphylococcus aureus</i> | 1 | 11.11 | 1.32 |
| <i>Staphylococcus epidermidis</i> | 1 | 11.11 | 1.32 |
| <i>Staphylococcus hyicus</i> | 1 | 11.11 | 1.32 |
| <i>Staphylococcus kloosii</i> | 1 | 11.11 | 1.32 |
| <i>Staphylococcus lentus</i> | 0 | 0 | 0 |
| <i>Staphylococcus agalactiae</i> | 2 | 22.22 | 2.63 |
| <i>Staphylococcus dysagalactiae</i> | 1 | 11.11 | 1.32 |
| <i>Mycoplasma arginini</i> | 1 | 11.11 | 1.32 |
| Yeast | 1 | 11.11 | 1.32 |
| Total | 9 | 100% | 11.87 |

(1983) and Salwa (1995), but lower than that recovered by Amel (2003). Although these organisms were isolated from sub-clinical cases, they were also isolated from clinical camel mastitis (Abdurhman *et al.*, 1995) which proved their potentiality to cause mastitis.

Isolation of *Corynebacterium* spp and *Micrococcus* spp supports the results obtained by Amel (2003), but the isolation of anerobic cocci in this study is considered an important finding since no previous reports are available, this is why the role of anaerobic cocci in camel mastitis should be considered.

Bacillus spp., *Bordetella parapertussis* and enterobacteria were isolated in this study in variable numbers. This result was in agreement with the findings of Abdelgadir (2001). In an investigation into the role of Mycoplasmas in camel mastitis, *M. arginini* and *A. Laidlawii* were reported but they used to be isolated from pneumonic cases (Abduljabber *et al.*, 1977; El Faki *et al.*, 2002). The frequency of *M. Arginini* and *A. laidlawii* (Jasper, 1967; Pan and Ogala, 1969) gave controversial views for considering these species as pathogenic to mammary glands, so this result raises the necessity for experimental trials to prove this point. The isolation of mycoplasma associated with

bacterial spp. might reflect their synergistic effect and are considered as important observation. Isolation of *A. laidlawii* in this study does not conclusively emphasize its pathogenic role in camel udder and thus further experimental studies are needed.

Mycotic mastitis in camels is relatively uncommon. The rate of fungal isolation, in the present study is considered higher than that encountered by Babkeer *et al.* (1994) and Salwa (1995), but similar results were reported by Amel (2003).

The results of this study indicated that CMT can detect sub-clinical mastitis in camel and there is relationship between stage of lactation, number of calving and mastitis incidence. *Staph. aureus* was dominant cause of camel sub-clinical mastitis. Further investigations of *M. arginini*, *A. Laidlawii*, anaerobic cocci, moulds and yeast associated with camel sub-clinical mastitis are needed.

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