Clinical Study on Camel Mastitis (Camelus dromedarius) at Butana Region, Sudan

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Abstract
This study was conducted for detailed investigation of mastitis in she-camels reared in the Butana region in the middle of the North-eastern Sudan. Three hundred and nineteen milking camels during both hot and cold seasons were examined. The study included determination of percentage of clinical mastitis according to season, age, stage of lactation and number of calving as well as detection of susceptibility of isolated microorganism to the antimicrobial drugs. This study showed that the overall percentage of clinical mastitis was 9.09%. Three forms of clinical mastitis were diagnosed. There was chronic form with high occurrence (72.41%) followed by acute form (24.14%) and the least was the gangrenous form (3.45%). The highest occurrence of clinical mastitis was found at the ages between 11-15 years and in late stage of lactation (55%). The highest incidence of clinical mastitis was found at the first, second and third calving (65.52%). The predominant isolated organism was Staphylococcus spp. (37.8%) followed by E.coli (18.9%), Streptococcus spp. (13.5%), Bacillus spp. (10.8%), Micrococcus spp. (8.1%), Corynebacterium spp. (5.4%) and Salmonella spp. (5.4%). The antimicrobial susceptibility test of the isolated bacteria generally showed high susceptibility to the most of the used antimicrobial agents.

Keywords: Camel, mastitis, Bacteria, Antibacterial.

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Introduction
According to animal population Sudan ranks the first in the Arab world and second in African. Concerning camels population Sudan is the second largest country worldwide with in camels population estimated at more than four millions(Ministry of animal Resource,2013). Camels in the Sudan are spread in a belt configuration; it extends between latitudes 12°-16° N. (Eisa and Mustafa, 2011). Camel stock (25.7%) is found in the Eastern region of Sudan (Dorosa, 2005).

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 may be the only milk available in places where other milking animals cannot be maintained (Abdurahman, 2006). Camel milk also has valuable nutritional properties as it contains a high proportion of antibacterial substances and higher concentration of vitamin C in comparison with cow milk (Barłowska et al., 2011). Milk can be considered as a good source of minerals, vitamins and characterized by...
higher ratio of lactoferrin. Moreover, camel milk could meet a big part of the daily needs of humans from these nutrients because camel milk has most the essential nutrients (Al-Otaibi and El-Demerdash, 2013).

Little work has been done on mastitis in camels comparing to studies on sheep and cows. During a decade ago mastitis in the camel has been reported from a number of camel-rearing countries of the world (Al-Ani, 2004; Mohammed et al., 2005), but little attention of mastitis as a problem was paid at herd level.

Mastitis has both an extreme zoonotic and economic importance. It is the cause of multiple hazardous effects on human health and animal production (Hegazy et al., 2004 and Al-Majali et al., 2008).

Camel mastitis has been estimated to affect more than 25% of lactating she-camel (Salah and Faye, 2011; Alamin, et al., 2013). It is also known to cause approximately 70% losses in milk production (Fazlani et al., 2011).

Many different bacteria have been isolated from mastitic mammary glands in camels either in the form of pure or mixed infection (Kalla et al., 2008; AL-Tofaily and Al rodhan, 2011 and Alamin et al., 2013). There are various studies which have been conducted worldwide on the isolation and identification of bacterial organisms in mastitic camel milk and their effect on quantity and quality of milk.

Acute or chronic mastitis is one of the important diseases of she-camel in Eastern Sudan. The isolates Staphylococcus aureus, Escherichia coli, Corynebacterium spp. are the main causes of mastitis in camels (Amel, 2003; Suheir, 2004; Sanaa, 2005). The objectives of this study were to investigate the incidence of clinical mastitis in Butana region and to isolate the causative agents together with their susceptibility to antibiotics.

Materials and Methods

Area of Study:
The study was conducted in Butana area which occupies the middle of the North-eastern Sudan in an area extending over 120,000 km² representing one third of the area of Eastern Sudan. It is in a geographical zone which lies approximately between latitude 14-16 N and longitude 33-36 E (Eisa and Mustafa, 2011).

Animals:
One hundred and sixty (160) she-camels in cold season from twelve herds (656 animals) and one hundred fifty nine (159) she-camels in hot season from fifteen herds (758 animals) were examined for mastitis by clinical examination and California Mastitis Test (CMT).

She-camels that showed clinical mastitis had been subjected to clinical examinations such as temperature, plus rate, respiratory rate and general health condition. Clinical examinations of udder had been carried out according to Kelly, (1984) and AL-Tofaily and AI rodhan, (2011). California Mastitis Test method was used as described by Quinn et al., (1999).

Collection of milk samples:
The first stream of milk was allowed to flow out and a volume of 5-10ml was collected into labeled sterile universal bottles and kept in cool box with ice pack or kept to 4-5°C and transported to laboratory immediately.

Bacteriological examinations:
Isolation and identification of microorganisms was done in Microbiology Laboratory, College of Veterinary Medicine, Sudan University of Science and Technology. Samples were collected from mastitic she-camel for bacteriological analysis. Bacterial isolation and identification was done according to standard procedures. A loop full of milk sample was streaked onto a plate of blood agar (5% defibrinated sheep blood) and...
MacConkey agar (Oxoid, Hampshire, England). Plates were then aerobically incubated at 37°C for 18-24 hours. The pure colonies were subjected to the primary and secondary biochemical tests for identification as recommended by Barrow and Feltham, (2003).

**Antibiotic Sensitivity:**
The disk diffusion method as described by Barrow and Feltham, (2003) was used to test the sensitivity of isolated bacteria to the antibiotics: Twenty µg Ampicillin/Sulbactam (AS), 25 µg Co-Trimoxazole(BA), 30µg Tetracycline(TE), 5µg Ciprofloxacin (CP), 5µg Cloxacillin(CX), 10µg Gentamicin(GM), 30µg Chloramphenicol (CH) , and 30mcg Amikacin (AK). The efficacy of various antibiotics on bacteria isolates was ranked on 0 to 2 scales: 2 being sensitive, 1 moderately sensitive and 0 resistant.

**Results**
Among 319 milking camels during summer and winter seasons 29 (9.09%) camel were positive to mastitis. She-camels served were 656 in 12 herds during cold season. One hundred and sixty were found as milking she-camels; among these she-camels 13 cases were diagnosed as clinical mastitis. In hot season among 758 camels in 15 herds, 159 were found as milking camels; among these she-camels 16 cases of clinical mastitis were diagnosed (Table 1).

Table 1: Occurrence of clinical mastitis among 319 milking camels from 28 camel herds in both winter and summer seasons:

<table>
<thead>
<tr>
<th>Categories</th>
<th>Total camels</th>
<th>Herds</th>
<th>Milking she-camels</th>
<th>Clinical mastitis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>Cold</td>
<td>656</td>
<td>13</td>
<td>160</td>
<td>24.39</td>
</tr>
<tr>
<td>Hot</td>
<td>758</td>
<td>15</td>
<td>159</td>
<td>20.97</td>
</tr>
<tr>
<td>Total</td>
<td>1414</td>
<td>28</td>
<td>319</td>
<td>22.68</td>
</tr>
</tbody>
</table>

According to type of mastitis the acute form was diagnosed in 7 cases (24.14%), chronic 21 cases (72.41%) and gangrenous form was found in one case (3.45%) (Table 2).

Table 2: The differed type of clinical mastitis diagnosed in 29 mastitic camels:

<table>
<thead>
<tr>
<th>Categories</th>
<th>She-camels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
</tr>
<tr>
<td>Acute mastitis</td>
<td>7</td>
</tr>
<tr>
<td>Chronic mastitis</td>
<td>21</td>
</tr>
<tr>
<td>Gangrenous mastitis</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
</tr>
</tbody>
</table>

Clinical signs for acute mastitis were severe inflammation and enlargement of supramammary lymph node, redness of the udder and swelling, pains in the affected quarter, the mammary secretions were watery, yellowish or containing blood and clots or flakes (Figure1, 2, and 3). Signs for chronic mastitis were atrophy of the mammary gland and watery secretions with thick white pus (Figure 4). Same cases of chronic mastitis showed obstruction of the teat canal, hypertrophy of the teat were constantly detected (Figure 5). Certain chronic mastitis cases showed firm, atrophied and painless in affected quarter (Figure 6). The gangrenous mastitis showed bluish discoloration of udder (injury by anti-suckling devices) (Figure 7).
Figure 1: A case of acute mastitis (Redness of mammary gland and enlargement of supramammery lymph node)

Figure 2: A case of acute mastitis (Redness - watery and yellowish secretions)
Figure 3: A case of acute mastitis (swelling of affected quarter, with tick infestation)

Figure 4: A case of chronic mastitis with clear thick and white pus

Figure 5: A case of chronic mastitis (Obstruction of teat canal and hypertrophy in affected quarter)
The age of she-camels with clinical mastitis varied between 6 to 23 years. The result showed 10.3% of the cases were at the age ranging from 6-10 years, 65.5% were at the age ranging from 11-15 years and 24.1% were at the age of 16≤ years (Figure 8).
Few cases of mastitis were observed in the first stage of lactation (5 cases, (17%)). Increased cases were observed at the middle stage (8 cases, (28%)) and the highest incidence of cases were diagnosed at in late stage of lactation (16 cases, (55%)) (Figure, 9).

There was a direct relationship between number of calving and clinical mastitis. The incidence of clinical mastitis during the first, second and third calving was in 19 cases(65.52%). During the fourth and fifth calving there was 7 cases (24.14%) and during the sixth calving the number decreased to 3 cases (10.34%) (Figure, 10).
Table (3) showed that the predominant isolated organism was *Staphylococcus* spp. (37.8%) followed by *E.coli* (18.9%), *Streptococcus* spp. (13.5%), *Bacillus* spp. (10.8%), *Micrococcus* spp. (8.1%), *Corynebacterium* spp. (5.4%) and *Salmonella* spp. (5.4%).

Table 3: The percentage of microorganisms isolated form clinical mastitis in she-camel in Butana region.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>No. of isolate</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus</em> spp.</td>
<td>14</td>
<td>37.8</td>
</tr>
<tr>
<td><em>S.aureus</em></td>
<td>10</td>
<td></td>
</tr>
<tr>
<td><em>S.hyicus</em></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td><em>S.lentu</em></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>7</td>
<td>18.9</td>
</tr>
<tr>
<td><em>Streptococcus</em> spp.</td>
<td>5</td>
<td>13.5</td>
</tr>
<tr>
<td><em>St. agalactiae</em></td>
<td>4</td>
<td></td>
</tr>
<tr>
<td><em>St. Dysagalatiae</em></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>4</td>
<td>10.8</td>
</tr>
<tr>
<td><em>Micrococcus</em> spp.</td>
<td>3</td>
<td>8.1</td>
</tr>
<tr>
<td><em>Corynebacterium</em> spp.</td>
<td>2</td>
<td>5.4</td>
</tr>
<tr>
<td><em>Salmonella</em> spp.</td>
<td>2</td>
<td>5.4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>37</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>

Results of antibiotic sensitivity against bacterial isolates were shown in table (4). The antimicrobial susceptibility test of the isolated bacteria generally showed high susceptibility to the most of the used antimicrobial agents. There was high sensitivity to Gentamicin, Ciprofloxacin, Cloxacillin and Amikacin, and moderate...
sensitively to Ampicillin/Sulbactam and Trimoxazole and the greatest resistance was found with Tetracycline and Chloramphenicol.

Table 9: Sensitivity test of the isolated bacteria against different antibiotics (disc diffusion method)

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>No. of isolates</th>
<th>Ampicillin/Sulbactam</th>
<th>Gentamicin</th>
<th>Co-Trimoxazole</th>
<th>Clavuloxin</th>
<th>Chloramphenicol</th>
<th>Tetracycline</th>
<th>Amikacin</th>
<th>Ciprofloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus spp.</td>
<td>14</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Bacillus spp.</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Micrococcus spp.</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>E. coli</td>
<td>7</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Corynebacterium spp.</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

2= sensitive, 1= moderately sensitive and 0= resistant

Discussion
The occurrence of clinical mastitis in she-camel herds in the present investigation was 9.09%. This is in agreement with reports by Hawari and Hassawi (2008), Sibtain et al., (2012) and Abera et al., (2010) these authors found an incidence in the range of 10.2%, 8% and 8.3% respectively. The prevalence in the present study was lower than 25% reported by Alamin et al., (2013) in Kordofan state and 24.7% reported by ALjuboori et al.(2013) in Abu Dhabi, United Arab Emirates and higher than that reported (5.22%) in Iraq by AL-Tofaily and ALrodhan (2011). The highest occurrence of clinical mastitis was found in age between 11-15 years, is confirms the study of Ynte et al., (2003) who demonstrated that she-camels above 9 years were most susceptible for clinical mastitis.

The clinical mastitis found in this study with its three forms as diagnosed by the obvious clinical signs and the visible alteration of milk and udder shape is in agreement with the study reported by Tibary and Anouassi (2000) and AL-Tofaily and ALrodhan (2011). The high incidence of chronic mastitis followed by acute and the least was gangrenous form. This agrees with finding of Sanaa (2005) who reported that the acute and chronic mastitis were one of important diseases of she-camel in Eastern Sudan. AL-Tofaily and ALrodhan (2011) reported in Iraq that lest incidence type of clinical mastitis was gangrenous mastitis.

The present results displayed that the age of she-camels at different seasons had no significant effect on the occurrences of clinical mastitis this is accord with the studies of Abdurahman (2006) and AL-Tofaily and ALrodhan (2011). The highest occurrence of clinical mastitis was found in age between 11-15 years, is confirms the study of Ynte et al., (2003) who demonstrated that she-camels above 9 years were most susceptible for clinical mastitis. Husein et al., (2013) reported that the incidence of mastitis was significantly influenced by age and the lowest prevalence of mastitis in she-camels in age between 5 to 7 years in Jijiga town, Ethiopia.

The present findings indicated that there was correlation between stage of lactation and clinical mastitis, this agrees with the studies of Salwa (1995) and Suheir (2004) who reported the percentage of mastitis increased with progress of lactation.
During this study it was found that at the first, second and third calving had not significantly effect on the occurrence of the clinical mastitis disease. This is in agreement with that study reported by AL-Tofaily and ALrodhan (2011) who recorded the high incidence of clinical mastitis at the first, second and third calving. Salwa (1995) reported that at the first calving the incidence of mastitis reached 20.5%, where during the second and third calving the incidence decreased to 19.2% and then continued to decline till last calving where it reached 1.3%. The present finding are in contrast to study of Suheir (2004) who reported that during fourth and fifth calving the high incidence of mastitis was 43.8% and Omer (1991) who reported the incidence of mastitis increased during fifth calving then continued to decline till the last calving.

In the present study the isolated Gram positive bacteria was at the rate of 81.08%, this is in according with the findings of Hawari and Hassawi (2008), Husein et al., (2013) and Wanjohi et al., (2013) who reported that Gram positive cocci of genera: Staphylococcus, Streptococcus and Micrococcus were most dominant udder pathogen isolated and regarded as an impotent pathogens in camel. Also the Gram negative bacteria was found in the present study lower than reported by AL-Tofaily and ALrodhan (2011) who record 23.8% of the isolates were Salmonella spp, Klebsiella pneumonia and Mannheimia haemolytica. The predominant isolated organism of clinical mastitis in this study was Staphylococcus spp.37.8%. This result agrees with the studies of Kalla et al., (2008) who reported the coagulase positive and coagulase negative Staphylococci were the main pathogenic bacteria occurring in camel mastitis (28.5%).

In the present study Staphylococcus aureus was isolated a rate of 27.03%. This is similar to finding obtained by Abdel Gader (2001) and Alamin et al., (2013) who isolated Staphylococcus aureus at the rate of 24.7% and 22.75% respectively. Our results the isolation rate (27.03) was lower than that reported by ALJubooriet al., (2013) (44.82%). The present result showed that the second isolated bacteria were Escherichia coli (18.92%). This is similar to that reported by Amel (2003) and Kalla et al., (2008) who reported the isolation percentage of 18.18% and 18% respectively. The present results were lower than that isolated by Wanjohi et al. (2013) (60%) from North-Eastern province, Kenya.

The isolation rate of Streptococcus spp. was 13.51%. This is similar to the findings of Sibtain et al.(2012) (15.63%) and lower than that of Suhier (2004) and ALjuboori et al. (2013) where their findings were 22.22% and 21.67% respectively. All the Bacillus cereus isolated were found in mixed culture with Staphylococcus spp. and Streptococcus spp. This result is in agreement with the findings of Alamnet et al. (2013) (9.09%) and higher than that of Suhier (2004) (2.02%). This bacterium was reported by Salwa (1995) and Eyassu and Bekele (2010) as causative agent of all types of she-camel mastitis.

Also Micrococcus spp. isolation rate in this study was 8.11% where the isolation rate by AL-juboori et al. (2013) was 5%. Amel (2003) and Suhier (2004) stated that these organisms are an important causative of the mastitis among camels.

Corynebacterium spp. was isolated (5.41%) in present study. This is similar to that isolated by Suhier (2004) (7.07%). It is lower than AL-juboori et al. (2013) and Sanna (2005) whose findings were 10% and 30.7% respectively. It is higher than Alamin et al. (2013) (3.03%).
Salmonella spp. that isolated in present study was (5.4%), is similar to isolates of AL-Tofaily and ALrodhan (2011) 2(9.52%). The antimicrobial susceptibility test against the isolated bacteria in generally showed high sensitivity to most used antimicrobial agents. The isolates were highly sensitive to Gentamicin, Ciprofloxacin, Cloxacillin and Amikacin, moderate sensitive to Ampicillin/Sulbactam and Trimoxazole and showed greatest resistance to Tetracycline and Chloramphenicol. This results are in agreement with the finding of AL-Tofaily and ALrodhan (2011), Fazlani et al.,(2011) and Alqurashi et al. (2013) and in contrast to the findings of AbdelGadir (2001) who recorded Oxytetracycline,Tetracycline and Chloramphenicol were effective drugs against camel mastitis.

Conclusion
It could be concluded that clinical mastitis in Butana region was mainly chronic and acute forms. The most isolated organisms were susceptible to routinely used antibiotics.

References


