MASTITIS IN CULLED FEMALE CAMELS IN THE SUDAN

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المستخلص

تم بحث الأسباب الباكتيرية لألتهاب الضرع بالجمال حيث فحصت 660 من النوق مختلفة الأعماربمسلخ تمبول بمنطقة الجزيرة بالسودان للكشف عن اصابتها بالتهاب الضرع السريرى شملت انواع الرشايدى البطانى,العربى,الكسلاوى,العنافى والكنانى. جمعت31عينة لبن و165 عينات نسيجية من الضر وع المصابة. كل عينات اللبن كانت ايجابية لأختبار كالفورنيا وكانت اعلى نسبة للأصابة بنوع الرشايدى بلغت 77% بينما كانت ادنى نسبة (25.5%) بالنوع العربى من غرب السودان و القضارف. كانت نسبة الأصابة عاليه فى النوق التى زادت عن 14 عاما.اوضحت نتائج الفحص البكتيرى عن عزل 114 نوع من الباكتيريا تم تعريفها بواسطة الاختبارات البكتيرية التقليدية اضافة الى اختبار هميديا السريع (Himedia strips system) . الباكتريا من نوع المكورات العقدية *Staphylococcus* spp عزلت من 40 عينة (35.8%) تليها المكورات السبحية *Streptococcus* spp في 29 (25.4%) عينة والمكورات الصغيرة *Micrococcus* spp في 24 (21%) عينة. الباكتيريا الأخرى التى وجدت بعزلة واحدة (0.9%) شملت *Bacillus cereus*, *Acinetobacter* spp. *Mannhaemia haemolytica* and *Klebsiella pneumonia*.

Abstract

Bacterial causes of mastitis in female camels were investigated. A total of 660 she-camels were examined for clinical mastitis. The camels examined were Butana, Arabic, Kassala, Rashaidi, Anafi and Kennana ecotypes. Thirty one milk samples and 165 tissues specimens affected udders were collected from animals of different age groups at Tumbool slaughter house, Al-gazera State, Sudan. All milk samples were positive for California Mastitis Test. A highest prevalence of 77.8% clinical mastitis was found in the Rashaidi ecotype and the lowest of 25.5% was found in western Arab and Al Gadarif ecotypes. A high prevalence was also observed in camels Over 14 years. One hundred and fourteen bacteria isolated from the udder tissues and identified by conventional bacteriological tests and by the rapid test (Himedia strips system). Forty of the isolates (35.8%) were *Staphylococcus* spp. followed by 29(25.4%) *Streptococcus* spp and *Micrococcus* spp24(21%). Other bacteria recovered were *Bacillus cereus*, *Acinetobacter* spp. *Mannhaemia haemolytica* and *Klebsiella pneumonia*, each represented one isolate (0.9%).

Key words: (culled female camel, mastitis, Bacteria, Sudan.)

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Introduction

The Sudan ranks the second in camel population in the world and in the country they were estimated to be 37,724,000 heads distributed in different States; Kurdofan (36.81%) Darfur (23.70%) Kassala (13.47%) Red Sea (7.01%) El Gedarif (5.18%) Blue Nile (4.48%) El Gazera (2.59%) Sinnar (2.45%) River Nile (2.40%) Northern State (1.03%) White Nile (0.74%) and Khartoum State (0.14%) (Anon, 2005).Tumbool market is a famous market for camel meat in the Sudan, about 4-5 thousand heads are slaughtered annually in Tumbool abattoir (Anon, 2009). The most common causes of mastitis are bacteria, fungi, yeasts and certain viruses (Suheir, 2004). Traumatic injuries are predisposing factors resulting from trampling by feet or lacerations that caused by barbed wire on teats or mammary gland. These lesions may be superficial or deep lacerating into the cistern with release of the milk through the wound. The wounds may be exposed to secondary bacterial infection which results in mastitis. An accidental trampling of the teat may lead to amputation (Radostitis *et al*, 2000). Previous studies in Jordon, Ethiopia, Kenya and in kingdom of Saudi Arabia revealed that the most common bacteria isolated from mastitic camel milk were *Staphylococcus* (*Staph.*) *aureus Streptococcus* (*Str.*) *agalactiae, Str. dysagalactiae, Corynebacterium* (*Coryne.*) *pyogens , Coryne. pseudotuberclosis, Pseudomonas* (*Ps*) *aeroginosa, E. coli* and *Micrococcus* (*M*) pp. in Hawari and Hassawi, 2008, Abdelgader *et al*, 2005, Younan, 2002 and Hafez *et al.*, 1987. Moreover, *Staph. aureus, Rhodococcus equi*, *Acinetobacter* spp., *Staph.* spp., *Pasteurella haemolytica*, *E. coli*, and *Str. agalactia* were isolated from mastitis of camel milk in Somalia and Ethiopia (Mohammed *et al*, 2005; Birhanu *et al*, 2008). . In eastern and western Sudan, the predominant bacteria isolated from milk samples of mastitis she-camels were *Staph. aureus, Str. agalactia, Micrococcus* spp., *E. coli, Aerococcus, Staph. epidermidis, Coryne.* spp. and *Bacillus* spp. Amel, 2003; Sanna, 2005; Ismail, 2006. In addition, *Brucella* spp. and *Bordetella parapertusis* were isolated from mastatic she-camels (Agab, 1993; Suheir, 2004). The objectives of the present study were to identify the bacteria associated with mastitis in camels, study the effect of ecotype and age in the occurrence of the disease and determine the predisposing factors of the disease.

Materials and Methods

A total of 660 female camels (31 were lactating) from different Sudanese ecotypes and culled for different reasons were examined for clinical mastitis at Tambool slaughterhouse. This survey was carried out regularly twice weekly during 2009.

Collection of samples:

Thirty one Milk samples were collected from camels after cleaning the teats with a cotton wool moisturized with 70% alcohol. The first eject of milk was stripped off and about 15ml of milk was drawn in a labeled sterile McCartney bottle and placed in an ice box and transported to the laboratory and tested with California Milk Test. Specimens of udder tissues were collected in sterile plastic bags using sterile surgical scalpel blade and placed in ice box and sent to the Veterinary Research Institute for bacteriological examination.

California Mastitis Test (CMT):

The test was performed by placing equal volumes of CMT reagent (Kebra test) and milk sample in CMT tray, mixed for 10 seconds and the result was scored according to the degree of agglutination (FAO, 2006).

Conventional bacteriological examination:

Few drops of each milk sample were streaked onto the surface of a blood agar plate and incubated at 37ºC for 24 hrs and the growth was subcultured for bacterial isolation and identification. Each fresh udder was taken aseptically, cut deeply; about $1cm^3$ was impressed on the surface of the blood agar medium and streaked. The same portion of the specimen was placed into a bottle containing nutrient broth medium and both cultures were incubated at 37ºC for 24 hrs. A loopfull of pus from an abscess was streaked onto a plate of blood agar medium. Each hard abscess was scraped at periphery using sterile scalpel blade, placed into nutrient broth medium and incubated at 37ºC for 24-48 hrs for bacterial isolation. The purified isolates from

the milk samples and tissue specimens were identified according to their cultural and biochemical characteristics (Barrow and Fellthem, 1993; Quinn *et al.*, 1994).

Rapid biochemical tests:

KB004 Histaph- identification kits for identification of *Staphylococcus* spp. were used in this a study according to the manufacturer's instructions (Hi Media, India). Each *Staphylococcus* isolate to be identified was cultured on nutrient agar medium, 1-3 well isolated colonies were picked and a homogenous suspension was prepared in 3 ml sterile saline to an optical density of 0.1 at 620nm. According to manufacture instructions, each *Streptococcus* isolate to be identified was grown onto a blood agar medium, a single isolated colony was picked and inoculated into 5 ml brain heart infusion broth and incubated at 37ºC for 6 hours to a turbidity equal to 0.1 OD at 620nm. The kit was opened aseptically and each individual well was inoculated with a loopfull of the suspension. The inoculated strip was incubated at 37ºC for 18-24 hours.

Following incubation, reagents were added to Voges- Proskaur, PYR and alkaline phosphatase wells, the results were interpreted in a data sheet and read.

Results

The 31(100%) milk samples were found positive for CMT, the color of milk varied from creamy or apparently normal in 11 she-camels, pinkish or hemorrhagc in three animals and the 17 milk samples were watery and included clots. Tow form of mastitis found acute 33 (%20)and chronic132 (80%). The 165 tissues 75(45.5%) were positive to bacterial growth. Gram positive bacteria were 107(93.86%) while the Gram negative ones represented 7 (6.14%). Among *Staphylococcus*. isolates, *Staph. aureus* were 12(30%) Among *Str*. isolates *Str. pyogene* were8(27.6%), *Str. agalactiae* 6(5.3%) and the least were *St. ubris* and *Str. anginosus* 2(1.7%) (Table, 1). Of the examined camels 87.5% over 14 years had mastitis and in 5-7 years of age 12.1% (Table 2). The higher incident of the disease was found among Rashaidi ecotype, (87.5%) and the lowest(17.6%) in Butana ecotype (Table, 3).

Table (1) Frequency of bacterial spp isolated from She-camel udders at Tumbool Slaughterhouse

Bacterial isolate	Frequency
Staphylococcus spp.	40 (35.08%)
Staphylococcus aureus sub sp *aureus*	12
Staph.epidermidis	7
Staph.hemolyticus	5
Staph.casyoliticus	5
Staph.carnosus	4
Staph.chromogens	3
Staph.hycus	2
Staph.lentus	2
Streptococcus spp.	29 (25.43%)
Streptococcus pyogens	8
Strep.agalactia	6
Strep.dysaglactia	5
Strep.pnemoni	3
Strep.anginosus	3
Strep.ubris	2
Strep.mutans	2
Corynebacterium psudotuberclosis	2 (1.57%)
Corynebacterium pyogens	3 (2.63%)
Micrococcus spp.	24 (21.05%)
Aerococcus spp.	2 (1.57%)
Bacillus cereus	1 (0.87%)
Acinetobacter	1 (0.87%)
E.coli	3 (2.63%)
Mannheimia haemolytica	1 (0.87%)
Klebsiella pneumonia	1 (0.87%)
Enterococcus spp.	5 (4.38%)
Proteus spp.	2 (1.57%)
Total	114

Table (2) Relationship between age group of she-camel and mastitis cases at Tumbool Slaughterhouse during Jan-Dec 2009

Age group (years)	No of She-camel examined	No mastitis cases	%from each age examined
5-7	215	26	12.09
8-10	380	84	22.10
11-13	57	48	84.21
14and above	8	7	87.50
Total	660	165	

Table (3) Relationship between ecotype of She-Camel (*Camelus dromedaries*) and Mastitis cases at Tumbool Slaughterhouse during Jan-Dec 2009

Ecotype/location	No examined	No cases	Mastitis %
Butana*1	278	49	17.6
Arab/west*2	141	36	25.6
Arab/Gedarif*3	102	26	25.5
Kassala*4	101	28	27.7
Rashidi	18	14	77.8
Anafi	6	2	33.3
Kinani	14	10	71.4
Total	660	165	

*1- Shukria, Lahaween, Batahen
*2- Darfor and kordufan camels
*3- Bright and brown Butana heavy camels
*4-Bniamer

Discussion

All examined animals were culled due to untreated problems such as generalized mange, bone fractures, off food due to stomatitis or contagious ecthyma, bilateral blindness, contagious skin necrosis, ray neck syndrome, sterility, udder undulation and old age. The results of the study revealed the prevalence of mastitis to be 25.00% in culled female camels slaughtered at Tumbool slaughterhouse and this was higher than that reported by other investigators in the Sudan Obied *et al.*, (1996); Salwa, (2005) and in Ethiopia Abdelgader 2005 and lower than that found in another study Bakhiet *et al.*, (1992). This study indicated that the prevalence of mastitis was higher among the Rashaidi and Kennana ecotypes (77.8%, 71.4%), respectively because they are known to be dairy camels. The prevalence among Arabic ecotype found in Darfur, Kurdofan, Butana plains and Kassala ranged from 17.6% to27.7%. Although, the Anafi ecotype ranks the second (33.3%), but only 6 she-camels were examined which were not true representatives to the population. This was likely because Anafi is a racing ecotype camel of a high value and mastitis Anafi female camels are not usually culled. The results of the study revealed that milk samples of all lactating she- camels (N=31) were positive for CMT and cultures, and this was in agreement with the results of other studies which stated that there was a relationship between the CMT score and the number of bacterial isolates Amel (2003) Hawari and Hassawi, (2008). The bacteriological findings were comparable to the results of other Investigator in Kurdofan and Darfur States, Sudan (Salwa, 1995; Amel, 2003; Suheir 2004) and worldwide (Amena, 2011; Benkerroum *et al* 2003; Birhanu *et al*, 2008; kapur *et al.*, 1982). Of the 165 specimens from mastitis udders 90(54.5%) were negative for bacterial growth. This was in agreement with the results of other studies by Hawari and Hassawi (2008), Bakhiet *et al, (*1992) and Abdurahman *et al* (1995) and that could be due to the fact that in a chronic form of mastitis, fibrosis occurred in 45.45% or could be due to agents other than bacteria, viz. viruses, mycoplasmas and fungi.

References

Abdel Gadir, A. E. A; Hildebrandt, G; Kleer, J; Molla, B; Kyule, M. and Baumann, M. **(2005).** Prevalence and risk factors of camel (*Camelus dromedarius)* mastitis based on bacteriological examinations in selected regions of Ethiopia. *J. Cam. Prac. Res.*, **12**: 33-36.

Abdurahman, O. A. Sh. **(1995).** The detection of subclinical mastitis in the camel (*Camelus bactrianus*) using somatic cell count and California mastitis tests. *Vet. Res. Comm*., **20**: 9-14.

Agab, H. **(1993).** *Epidemiology of camel diseases in Eastern Sudan with emphasis on brucellosis.* M.V.SC. Thesis University of Khartoum, Khartoum, Sudan.

Amel, M. A. M**. (2003).** *Bacteria and fungi isolated from she-camel mastitis in Red Sea area of the Sudan.* M. V. Sc Thesis. University of Khartoum. Khartoum, Sudan.

Amena, M. I; Al-Humiany, A. A; Ahmed, M. A. M. And Hoda, M. Z. **(2011).** Epidemiological and microbiological studies on mastitis in she-camels. *Inter. J. Microbiol. Res*., **2**: 18-27.

Anon **(2005).** Annual Bulletin of Animal Resources Statistics. Ministry Animal Resources and Fishers, Khartoum, Sudan.

Anon **(2009).** Annual report of Tumbool Camel research center. Tumbool, Sudan.

Bakhiet, M. R; Agab, H. and Mamoun, I. E. **(1992).** Camel mastits in western Sudan. *Sudan J. Vet. Sc. Anim. Husb*.**31**:58-59.

Barrow, G.L. and Feltham, P.K.A. **(1993).** *Cowan and Steel's Manual for the Identification of medical Bacteria*, Third Edition Cambridge, press U.K.

Benkerroum , N; Boughdadi, A; Bennani, N. and Hidane, K. **(2003).** Microbiological and quality assessment of Moroccan camel's milk and identification of predominating lactic acid bacteria. *World. J, Microbiol. Biotechnol.*, **19**: 645-648.

Birhanu, H; Etsay, K. and Hailay, K. **(2008).** Assessment of bacteriological quality of raw camels' milk in Ab-Ala, north eastern Ethiopia. *Livestock, Res. Rural Develop*., **20**: 1-13.

FAO **(2006).** Food Agriculture Organization of the Nation (www.fao.org).

Hafez, P. M .;Razig, S.A. and El-Amarosi, S.**(1987).** Studies on mastitis in farm animals in El-Hassa. Analytic studies. *Assiut Vet Med.J.*,19:140-145.

Hawari, A. D. and Hassawi, D. S. **(2008).** Mastitis in one humped she-camels (*Camelus dromedarius*) in Jordan. *J. Biological Sc.*, 8:958-961.

Ismail, M. A. A. **(2006).** *Epidemiological studies on camel mastitis in North Kordofan State*. M. Sc. Thesis. University of Khartoum. Khartoum Sudan.

Kapur, M. P; Khanna, B. M. and Singh, R. P. **(1982).** A peracute case of mastitis associated with *Klebsiella pneumaniae* and *Escherichia coli*, *Indian. Vet. J.*, **59**: 650-651.

Mohammed, A.; Molla, B; Ruiz Bascaran, M. and Abera, B. **(2005).** A cross-sectional study of mastitis in camels (*Camelus dromedarius*) in Somali Region, Southeastern Ethiopia. *Bull. Anim. Hlth. Prod Afri.* **53**: 195-201.

Obeid, A. I; Bagadi, H. O. and Mukhtar, M. M. **(1996).** Mastitis in *Camelus dromedarius* and the somatic cell content of camels' milk. *Res. Vet. Sci*., **61**: 55-58.

Quinn, P. J; Carter, M. E; Markey, B. and Carter, G. R. **(1994).** *Clinical Veterinary Microbiology*. London, England. WOLFE publishing.

Radostits, O.M.; Gay, C.C.; Blood, D.C and Hincheliff, K.W**.(2000).** In: *Veterinary Medicine*. Diseases of cattle, sheep, pigs, goats and horses. Ninth ed. W.B. Saunders, London.

Salwa, M. S. **(1995).** *Studies on Camel Mastitis: Aetiology, clinical picture and milk composition.* M. V. Sc Thesis. University of Khartoum.

Sanna, O. Y. **(2005).** Bacterial Diseases of the Reproductive System of Camels (*Camelus dromedarius*) in Eastern Sudan. *J. A. V. A*. **4**: 642-644.

Suhier, I. A. **(2004).** *Some bacterial species, mycoplasma and fungal isolate associated with Camel Mastitis.* M. V. Sc Thesis. University of Khartoum, Khartoum, Sudan.

Younan, M. **(2002).** Parenteral treatment of *Streptococcus agalactiae* mastitis in Kenyan Camels (*Camelus dromedarius*). *Revue Elev. Med. Vet. Pays Trop*. 55 :177-181.