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RESEARCH PAPER

Subclinical Mastitis in Camels in Oman: A Pilot Study

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إلتهاب الضرع تحتَ السَّريريَّ في الإبل في عمان: دِراسَة إِرْتِيَادِيَّة

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Abstract. Camels are important and multipurpose animals in many parts of the world including Middle East. Camel milk may harbor different bacteria. Centuries old tradition of consumption of raw camel milk is still a common practice in Oman. This study was carried out to conduct a microbiological analysis of camel milk samples with subclinical mastitis in the region of Muscat, Sultanate of Oman. A total of 61 camel *(Camelus dromedarius)* milk samples were collected from various animal holdings in and around Muscat. Onsite California Mastitis Test (CMT) revealed 18 (29%) camels positive for subclinical mastitis. Positive milk samples were subjected to routine microbiological workup for bacterial isolation and identification. A total of 7 (47%) *Enterobacter cloacae* isolates, 4 (27%) *Escherichia. coli*, 3 (20%) coagulase negative *Staphylococci spp*. (CNS) and 1 (7%) Micrococcus spp. were identified out of 15 milk samples. Three milk samples did not yield any growth after two repeat attempts. Isolates belonging to *Enterobacteriaceae* were further subjected to antimicrobial sensitivity testing. All E. cloacae samples 7 (100%) were found to be resistant to penicillin, ampicillin, amoxicillin-clavulanic acid, first generation cephalosporins, and the macrolide group of antibiotics whereas 3 (43%) *E. cloacae* isolates were found to be intermediately resistant to the phenicol group of antibiotics. All four *E. coli* (100%) isolates were found resistant to penicillin, ampicillin, amoxicillin-clavulanic acid, first generation cephalosporins, and 2 (50%) showed resistance to macrolides, whereas 1 (25%) isolate was found to be resistant to tetracyclines. In this study, *Enterobacteriaceae* were the most common group of bacteria isolated from camels with subclinical mastitis. *Enterobacter cloacae* and *E. coli* were the predominant organisms.

Keywords: Raw camel milk, *Enterobacter* cloacae, *E. coli*, resistance

المستخلص: تعتبر الإبل حيوانات مهمة ومتعددة الأغراض في أجزاء كثيرة من العالم بما في ذلك الشرق الأوسط. قد يحتوي حليب الإبل على أنواع مختلفة من البكتيريا. لا يزال تقليد استهلاك حليب الإبل الخام ممارسة شائعة في عمان منذ قرون. أجريت هذه الدراسة لإجراء تحليل ميكروبيولوجي لعينات حليب الإبل المصابة بالتهاب الضرع تحت السَّريريَّ في منطقة مسقط ، سلطنة عمان. تم جمع ٦١ عينة من حليب الإبل (*Camelus dromedarius*) من مختلف حيازات الحيوانات في مسقط وحولها. أظهر اختبار كاليفورنيا لإلتهاب الضرع (CMT) أن ١٨ (٢٩٪) من الإبل كانت إيجابية للإصابة بإلتهاب الضرع تحت السريري. تم إخضاع عينات الحليب الموجبة للفحص الميكروبيولوجي الروتيني لعزل وتعريف البكتيريا. أظهرت النتائج ٧ (٤٧٪) عزلات من الأَمْعائِيَّةُ المِذْرَقِيَّة ، ٤ (٢٧٪) من الإشْريكِيَّةُ القولونِيَّة ، ٣ (٢٠٪) من المكورات العنقودية السلبية المخثرة و ١ (٧٪) من جنس البكتريا المكَيَّرَة من أصل ١٥ عينة حليب. ثلاث عينات من الحليب لم تسفر عن أي نمو بكتيري بعد محاولتين متكررتين. تم إجراء إختبار الحساسيه للعزلات التي تنتمي إلى الأَمْعائِيَّات لمضادات الميكروبات. أظهرت النتائج أن جميع عينات ٧ (١٠٠٪) الأَمْعائِيَّةُ المِذْرَقِيَّة كانت مقاومة للبنسلين، الأمبيسلين، حمض أموكسيسيلين–كلافولانيك، الجيل الأول من السيفالوسبورينات، ومجموعة الماكروليد من المضادات الحيوية، بينما ٣ (٤٣٪) من عزلات الأَمْعائِيَّةُ المِذْرَقِيَّة ذات مقاومة متوسطه لمجموعة الفينيكول من المضادات الحيوية. تم العثور على عزلات الإشريكية القولونية الأربعة (١٠٠٪) مقاومة للبنسلين والأمبيسلين وحمض أموكسيسيلين كلافولانيك والجيل الأول من السيفالوسبورين و ٢ (٥٠٪) مقاومة للماكروليدات ، بينما وجدت عزلة واحدة (٢٥٪) مقاومة للتتراسيكلين. في هذه الدراسة ، كانت البكتيريا الأَمْعائِيَّةُ هي المجموعة الأكثر شيوعا من البكتيريا المعزولة من الإبل المصابة بإلتهاب الضرع تحت السريري. كانت بكتريا الأَمْعائِيَّةُ المِذْرَقِيَّة و الإشْريكِيَّةُ القولونِيَّة هي السائدة.

الكلمات المفتاحية: حليب الإبل الخام ، الأَمْعائِيَّةُ المِذْرَقِيَّة ، الإشْريكِيَّةُ القولونِيَّة ، المقاومة.

Introduction

Camels are a mainstay of rural communities in Oman as elsewhere in arid and semi-arid zones in the Middle East and Africa, by virtue of their endurance and productive potential under such agro-ecological environments. In Oman, as in the Arab world, camel *(Camelus dromedaries)* is akin to culture and subsistence livelihood in rural areas where camel is an important source of milk (Barlowska et Al., 2011; Zibaee et al, 2015), which is generally acclaimed for its nutritive and health benefits (Mullaicharam, 2014). Moreover, camel's milk has recently been gaining wider consumption and a place in the market of dairy products. Relevant to that is the relatively substantial population of camel in Oman, estimated at 273,000 (NCSI, 2019), with significant contributions to livelihood of the owners and economy. However, one of the major problems

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impacting camel milk production and health is mastitis, though there is lack of reports in Oman. Mastitis, both clinical and subclinical, has been reported in Gulf countries neighboring Oman, UAE and Saudi Arabia, in addition to Iraq, Kuwait, Pakistan, India, Somalia, Sudan, Kenya and more (Toroitich et al., 2017). Subclinical mastitis does not seem to show clear clinical signs but pathogenic microorganisms are harbored in intramammary tissue and are secreted in the milk. However, milk from an animal positive for subclinical mastitis may appear normal with normal total somatic cell count. This does raise public health concern considering the tradition of consumption of raw and unpasteurized milk in these regions, besides its bearing on camel health and losses in milk yield. However, unpasteurized milk is not recommended for its safety issue. Our literature search indicated that there is lack of studies on subclinical mastitis in camels in Oman. Therefore, the aim of this study was to investigate the status of subclinical mastitis by conducting microbiological analysis of milk of camels to ascertain the extent of positive cases.

A number of studies investigated subclinical mastitis in camel with successful application of California Mastitis Test (CMT) (Saber et al., 2010, Ali et al., 2019). Assessment of milk samples by CMT and somatic cell count (SCC) was shown to tie significantly regarding the detection subclinical mastitis in camels (Abdulrahman, 1996), together with a good correlation of CMT to milk leukocyte count (Obeid, 1983). Applying CMT and SCC to screen for subclinical mastitis in a group of camels led to revealing the predominance of Gram-positive bacteria including *streptococci* spp. and *staphylococci* spp (Saleh, 2011). Moreover, a similar study in camels revealed the predominance of *Enterobacterium* spp., *Staphylococcus* spp. and *Streptococcus* spp. in cases of subclinical mastitis (Al-Sailihi, 2017). Diverse bacterial species were reported to be implicated in subclinical and clinical mastitis in camel. This is apparently related to a location as well as hygiene conditions and their management. A study of subclinical mastitis in camels in a district in Iraq showed that the main isolates involved were coagulase negative *Staphylococci* followed by *Streptococcus spp*. and *E. coli* and *Micrococcus spp*. were in a lesser extent (Al-Rammahi et al., 2018). In another location, Ali et al. (2019) investigated subclinical mastitis in the two districts in Pakistan and observed the high prevalence of S. aureus. In a habitat with close resemblance to Oman, such as that of UAE, isolates from subclinical as well as clinical mastitis of camel were identified to be mainly *Staphylococcus spp*. trailed by *Streptococcus spp*. and *Enterobacterium* spp. (Al-Jaboori et al., 2013).

Materials and Methods

Study Area

In this study, a total of 61 milk samples from healthy she-camels (*Camelus dromedaries*) located in various holdings in and around Muscat, Sultanate of Oman were considered.

Ethics Statement

In this study, verbal consent of camel's owners was obtained prior to the collection of milk samples from their animals. Animals were used just once for milk collection by professional veterinary technologists at the Department of Animal & Veterinary Sciences. This work was not an experimental research on animals and hence approval by the ethical committee at Sultan Qaboos University was not obtained.

Milk Samples Collection

Milk sampling was done by hand stripping just prior to milking using sterile screw caped 50 ml Falcon tubes (Kartell S.p.A and Cellstar tubes, Germany). Milk sample (10 mL) was collected from each quarter in a sterile tube and labeled as per guidelines. (National Mastitis Council., 1990).

California Mastitis Test (CMT)

Milk samples were subjected to onsite CMT test. CMT was carried out using the methods as described by Schalm and Noolander (1957). The CMT solution was obtained from ImmuCell (Portland, USA). Equal volume of milk and CMT working solution were mixed in corresponding testing paddles. The mixture was gently rotated in horizontal position and results were recorded as negative, weak positive (+), distinct positive (++) and strong positive (+++). Milk samples positive for onsite CMT test were transported to the laboratory in an ice box and processed for bacteriological examination within two hours of collection. Animals with history of mastitis or recent treatment with antimicrobials were excluded from this study.

Bacteriological Examination

Bacteriological examination of the samples was carried out following the standard methods as described by Quinn et al. (1999). For microbiological analysis, each milk sample (0.1 mL) was streaked on blood agar (Oxoid, Basingstoke, England) and MacConkey's agar (Oxoid, Basingstoke, England) and subjected to incubation at 37°C for 24-48 hours in both aerobic and anaerobic conditions. In the case of no growth, corresponding milk sample was cultured again to obtain growth. The plates with growth were examined for growth colony morphology, hemolysis, and pigment production.

Identification of Bacterial Isolates

All isolates were subjected to Gram staining, catalase, and oxidase tests. *Micrococci spp*. and *Staphylococci spp*. were identified using Mannitol Salt Agar (Oxoid), and coagulase test using both slide and tube methods along with catalase and oxidase testing. All biochemical tests were done according to standard procedures (NCCLS, 2000).

Analytical Profile Index Test

Gram-negative isolates with oxidase negative and catalase positive reactions were subjected to API® 20E (Biomérieux, France) and were further confirmed using Vitek2 Gram-negative (GN) test cards (Biomérieux, France) in an automated Vitek® 2 instrument. All isolates were kept in Viabank ™ tubes at -70°C for further use. Gram-positive cocci isolates with positive catalase reactions were subjected to API® Staph (Biomérieux, France). *Staphylococci* isolates were subjected to Coagulase test using the tube coagulase method (Quin, 1998).

Antimicrobial Sensitivity Test

Antimicrobial susceptibility and minimum inhibitory concentrations (MICs) were determined using automated Vitek® 2 instrument with AST cards (Biomérieux, France). Breakpoints used as recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2000).

Results

California Mastitis Test and Bacteriological Examination Analysis

A total of 61 camels were examined for subclinical mastitis. Out of 61 animals, 18 (29%) camel milk samples were found to be positive for subclinical mastitis using California Mastitis Test (CMT), whereas only 15 isolates yielded growth during microbiological analysis. Three samples positive for CMT, did not yield any growth even after second culture. Out of 15 positive growths, 7 (47%) isolates were confirmed as *E. cloacae*, 4 (27%) *E. coli* whereas 3 (20%) coagulase negative *staphylococci* and 1 (7%) isolate was confirmed as *Micrococcus spp*.

Antimicrobial Sensitivity Test Analysis

Antimicrobial sensitivity analysis revealed that all *E. cloacae* (100%) isolates were resistant to penicillin, first generation cephalosporins, and macrolide group of antibiotics, whereas 3 isolates (43%) shown intermediate resistance to phenicols. All four *E. coli* (100%) isolates exhibited resistance to penicillin, ampicillin, and amoxicillin, 2 (50%) isolates were resistant to erythromycin and tylosin, 3(75%) were resistant to first generation cephalosporins whereas only 1 (25%) isolate was found resistant to tetracycline.

Discussion

The present study shows the presence of subclinical mastitis in camels in Oman based on CMT and bacterial culture of milk samples. They also reflect a high correlation between CMT and cultural isolates. *Enterobacteriaceae E. cloacae* and *E. coli* were found as the predominant bacteria with few coagulase negative *Staphylococci* and *Micrococcus sp*. *E. cloacae* isolated in this study belongs to the microbiota in the surrounding environment. This is a common commensal in animal digestive tract and is a common member of the human microbiome (Keller et al., 1998). Our results are in slight discrepancy with other reports since we report *Enterobacteriaceae E. cloacae* and *E. coli* as dominant causative agents in subclinical mastitis in camels. Reports on isolates from camel cases from neighboring countries implicate *Staphylococcus* and *Streptococcus spp*. as dominant ones in clinical and subclinical mastitis (Al-Jaboori et al., 2013; Al-Rammahi, 2018), in agreement with other reports (Hadel et al., 2018). Though Al-Jaboori et al. (2013) identified *Enterobacterium spp*. from subclinical mastitis in camels in the UAE, yet the dominant ones were *Staphylococcus* and *Streptococcus spp*. Considering rural management practice, it is likely that cross-infection was established in camels since management and the level of hygiene is not optimal.

To best of our knowledge this is the initial investigation to report subclinical mastitis in camels in Oman, and that the coliform is the dominant type. Not a single camel owner interviewed during sampling was aware of the concept of subclinical mastitis in camels or use of California Mastitis Test (CMT) screening. Camel milk is obtained via hand milking without any pre or post milking dipping. It was even observed camel owners drinking milk right from camel teats using hands, although it is not recommended for safety.

E. cloacae is a biofilm forming organism and this secretes a number of cytotoxins deemed important for its pathogenicity (Mezzatesta et al., 2012). More importantly, due to expression of extended spectrum β-lactamases (ESBL) and carbapenemases, it has turned into third broad spectrum *Enterobacteriaceae* specie causing nosocomial infections along with K. pneumoniae and E. coli (Potron et al., 2013).

In last few decades, *E. cloacae* have been added to the list of most worrying microorganisms due to their ability to acquire resistance. In humans, *E. cloacae* are known to cause septic arthritis, endocarditis, and skin, urinary, respiratory and abdominal infections. It is contracted via skin and gastrointestinal tract (Sanders et al., 1997, Lee et al., 2002). Coliform mastitis is also the most common form of clinical mastitis in cattle in different parts of the world. Among coliforms, *E. coli* is most commonly isolated from animals with mastitis and primary source of these bacteria are cow feces, environment and infections via teat canal (Sumathi et al., 2008, Lipman et al., 1995).

In the present study, *Enterobacteriaceae* was observed to be the predominant isolates from camels with subclinical mastitis. This is in line with other studies conducted in cows with mastitis (Bengtsson et al., 2009, Saidi et al., 2014). However, a study done in subclinical mastitis in camels in Saudi Arabia, *Streptococcus spp*., *Staphylococcus aureus* and other *staphylococci spp*. were found to be the predominant organism (77%) whereas only 12.9% isolates were identified as *E. coli*. (Saleh & Faye, 2011). In another study conducted on subclinical mastitis in camels, the authors found coagulase negative staphylococci (CNS) to be the predominant isolates (35%) and only (10.72%) were confirmed as *E. coli* (Leyla et al., 2017). A study (Al-Rahmmahi et al., 2018) carried on in Al-Najaf on 82 camels, the researchers found Coagulase Negative Staphylococci to be the predominant (17.68%) followed by *Streptococcus* spp. (12.92%). The authors also reported *Staphylococcus aureus* (10.2%), *E. coli* (8.16%) and *Micrococcus* (4.08%). In our study we found 3 isolates (20%) of CNS and 1 isolate of *Micrococcus*.

In the present study, all *E. cloacae* (100%) isolates were found to be resistant to penicillin, first generation cephalosporins, macrolides and 43% were intermediately resistant to phenicols. Currently, there is a lack of consensus regarding exact definition of multidrug resistant organisms in veterinary medicine. In human medicine, for an organism to be reckon as multidrug resistant, it should be resistant to at least one agent in at least three antimicrobial classes. However, according to criteria for assessment of multidrug resistance in bacteria (food.gov.uk), these organisms cannot be considered multi-drug resistant as *E. cloacae* harbors intrinsic resistance to penicillin, first generation cephalosporins and cephamycins.

In our study, all *E. coli* (100%) isolates were resistant to ampicillin, amoxicillin, 3 (75%) were resistant to the first generation cephalosporins, 2 (50%) resistant to macrolides whereas 1 (25%) were found resistant to tetracyclines. Similar trends have been reported in cows with mastitis (Saidi et al., 2014) and camels with subclinical mastitis (Saleh and Faye, 2011).

Camel milk if consumed raw can be a source of infection for humans. A case of *E. cloacae* sepsis has been reported in a preterm infant feeding on mother's milk. Same pathogen was cultured from milk samples obtained from mother over the period of 7 days. However, mother's milk could not be established as a direct cause of *E. cloacae* infection in infant as other sources of infection could not be ruled out (Weem et al., 2015). Further studies warranted to establish the link between consumption of raw camel milk and human infections.

All of the isolates (100%) were found to be resistant to macrolide antibiotics including erythromycin and tylosin. Macrolides have shown good activity especially against gram-positive cocci organisms. However, these antibiotics have limited efficacy against *Enterobacteriaceae* in general. *Enterobacteriaceae* may acquire resistance against macrolide in a variety of mechanisms described elsewhere (Leclercq, 2002; Ojo et al., 2004)

Identification of *Enterobacter cloacae* establishes the fact that the organism is AmpC producer that yields the inducible chromosomal AmpC β-lactamase and therefore it is not necessary to detect AmpC production in these isolates (Gupta et al., 2014). Coagulase negative Staphylococci (CNS) are opportunistic pathogens that may cause infection due to improper teat disinfection. CNS are commonly isolated from bovine milk samples as these organisms are part of normal skin flora and contamination of milk is common. However, infections are usually subclinical in nature.

Raw milk contamination with *Enterobacteriaceae* can occur during milking process, contamination of milk with animal feces or mastitis (Dahmen et al., 2013). As camel milk is obtained exclusively by hand-milking, washing hands and udder of the animal, post milking teat dipping, pasteurization of milk and prevention of contamination during transport of camel milk can help in control of these organisms. The best way forward is the education of camel owners about dangers of drinking raw camel milk that can lead to infection with variety of bacteria and importance of pasteurization to avoid infections.

Consumption of raw camel milk is commonly practiced in Middle East including Oman; therefore, it is important to evaluate the microbiological quality of the camel milk. More number of camels could not be included in the study due to various factors including overall low camel population in the study area as compared to other parts of Oman, noncompliance of camel owners to provide milk samples, small holdings (one or two camels commonly kept along with other livestock animals), and non-availability of camels for sampling at holdings due to long overall grazing time period. It is concluded that camel owner education programs are required to improve the udder health of the camels, regular screening of camels for subclinical mastitis using CMT, and educate them regarding hazards of using raw camel milk.

Conclusion and Recommendation

This is the initial investigation to report subclinical mastitis in camels in Oman, and that the coliform is the dominant type. In the present study, *Enterobacteriaceae* was observed to be the predominant isolates from camels with subclinical mastitis. This is the initial report of isolation of *E. cloacae* and *E. coli* from camel milk samples positive for subclinical mastitis in Oman. In the present study, all *E. cloacae* (100%) isolates were found to be resistant to penicillin, first generation cephalosporins, macrolides and 43% were also intermediately resistant to phenicols. In this study, we could conclude that camel owner education programs are required to improve the udder health of the camels, regular screening of camels for subclinical mastitis using CMT, and educate them regarding hazards of using raw camel milk.

Table 1. Percentage resistance of 11 Enterobacteriaceae species from camel positive for subclinical mastitis.

Enterobacteriace strains	Total	Number of resistant isolates (percentage resistance)																		
		PEN. CET	AMP	AMC.	TIM	STR.	ENR.	ERY.	TYL.	TET.	FFC.	CFP.	FUR.	IPM.	GEN.	NEO.	FLU.	ENR.	MAR.	SXT.	LEX.
E. cloacae	7	7(100)	7(100)	7(100)	0(0)	0(0)	0(0)	7(100)	7(100)	0(0)	3(43)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	7(100)	7(100)
E. coli	4	4(100)	4(100)	4(100)	0(0)	0(0)	0(0)	2(50)	2(50)	1(25)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	3(75)	3(75)
Total	11	11(100)	11(100)	11(100)	0(0)	0(0)	0(0)	9(82)	9(82)	1(9)	3(27)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	10(90)	10(90)

PEN: Penicillin; AMP: Ampicillin; AMC: Amoxicillin + Clavulanic acid; TIM: Ticarcillin/Clavulanic acid; STR: Streptomycin; ENR: Enrofloxacin; ERY: Erythromycin; TYL: Tylosin; TET: Tetracycline; FFC: Florfenicol; CFP: Cefoparazone; FUR: Ceftiofur ; IMP: Imipenem ; GEN: Gentamicin; NEO: Neomycin; FLU: Flumequine ; ENR: Enrofloxacin ; MAR: Marbofloxacin ; SXT: Trimethoprim/Sulfamethoxazole; LEX: Cefalexin; CET: Cefalotin;

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