

Etiology and Bacterial Antimicrobial Susceptibility of Endometritis in Camels (*Camelus dromedarius*)

Abstract

The study aimed to investigate the bacterial etiology of endometritis and in vitro antibiotic sensitivity of the isolates in dromedary camels admitted to a clinic. Uterine swabs were collected from 45 animals diagnosed as suffering from endometritis out of 95 infertile female camels. The samples were subjected to bacteriological isolation and identification. All the isolates were tested for *in-vitro* antibiotic sensitivity. Bacterial species isolated from these samples were *Arcanobacterium pyogenes*, with 33.33% (n=11), *Staphylococcus aureus*, 27.27% (n=9), *Proteus mirabilis*, 24.24% (n=8), *Bacillus spp.*, 9.09% (n=3), *Streptococcus agalactiae* 3.03% (n=1) and *Kocuria kristinae* 3.03% (n=1). *A.pyogenes*, *Staph. aureus*, *P.mirabilis* were significantly ($P<0.01$) associated with endometritis in dromedary camels. The antibiotic sensitivity pattern of the isolates indicated that 51.51%, 12.12%, 12.12%, 0.00%, 0.00%, 45.45% and 48.48% were sensitive for nitrofurantoin, erythromycin, oxy-tetracycline, ampicillin, penicillin, chloramphenicol and kanamycin respectively. The isolates were significantly ($P<0.01$) sensitive to nitrofurantoin, chloramphenicol and kanamycin.

Keywords: dromedary camel, endometritis, bacteria, etiology, antibiotics, therapy.

Introduction

Uterine infection is a significant cause of reproductive failure and infertility in camelids (Tibary and Anouassi, 2001; Tibary, 2004; Ali et al., 2010a). Different uterine affections have been described in the camel (Tibary and Anouassi 1997) with some reports of bacterial causative agents. Uterine inflammation has been considered as the most commonly encountered form that lead to infertility in camels (Tibary and Anouassi,

2000), yet reports, from camel-raising regions in the World, on sound techniques for clinical diagnosis of endometritis and its etiologic agents, are few.

Escherichia coli has been isolated from cases with purulent vaginal discharges in female camels (Ali et al., 2010b). Albihi (2003) suggested that *E.coli* was more associated with repeat breeding than with clinical endometritis. *E. coli* and *Proteus* spp have been considered in equine and cattle as nonspecific pathogens associated with endometritis (Ricketts 1981, Arthur et al. 1985), and they were observed in cases with purulent discharges (Ali, et al., 2010b). *Aeromonas hydrophilla* has been obtained from cases with ovarian hydrobursitis in female camels (Al-Eknah and Ali, 2001). In the Kingdom of Saudi Arabia (KSA), Al-Afaleq et al. (2012) reported that different species of microorganisms were isolated from the reproductive tracts of camels. Among these, *E. coli*, *Proteus vulgaris*, *Arcanobacterium pyogenes*, and *Pseudomonas aeruginosa* were the main organisms associated with acute catarrhal endometritis whereas *E. coli*, *A.pyogenes*, *Mannheimia morgana* and *Candida albicans* were mainly associated with purulent endometritis. It was reported that *Streptococcus pyogenes* (31%), *E. coli* (24%) and *Staphylococcus aureus* (20%) were the most common vaginal bacterial isolates in camels in Nigeria (Mshelia et al., 2014).

Uterine culture is a common technique for diagnosis of endometritis pathogens (Riddle et al., 2007). Antibiotic therapy is a common treatment approach for endometritis (Perkin 1999). For clinicians, there is a need for microbiological diagnosis so that adequate treatment of the uterine infection can be performed. However, bacterial isolation and sensitivity test are laborious, time-consuming processes that need special laboratory facilities. The choice of antibiotic based on data from earlier studies is an alternative for microbiological diagnosis. In dromedary camels, most of the uterine bacteriological investigations have focused on slaughterhouse studies and there is lack of information about reproduction history (Zaki and Mousa, 1965; Laila et al., 1987; Enany et al., 1990; Hussein et al., 2006). Hence, studies on clinical diagnosis and confirmation of laboratory diagnosis findings from different camel-rearing areas is vital for animal health, productivity and public health.

The VITEK 2 Automated System (bioMérieux, Marcy L'Etoile, France) is one of the most widely used instruments in clinical microbiology laboratories for the identification of Gram positive and negative bacteria up to species level. Basically it is a colorimetric reading of biochemical profiles of microorganisms. Based on these readings, an identification profile was established and interpreted according to a specific algorithm. Final profile results were compared to the database, generating identification of the unknown organism. Final results were analysed using a software which is an Advanced Expert System (AES) specifically designed to evaluate the results generated by the VITEK 2 system. Testing was repeated wherever suggested by the AES.

Objectives of the present study were to contribute to clinical diagnosis of endometritis in dromedary camels, determine the most common bacterial causative agents and their antimicrobial susceptibility. Results of the intended work could be of interest for the practitioners as a guide to adequate treatment of uterine infection in the dromedary camels.

Materials and Methods

Animals

The animals involved in the present study were 95 infertile female camels (*Camelus dromedarius*) admitted to a clinic (During the period from October 2014 to November 2014). **Animals with history of repeat breeding or abortion are likely to develop uterine infection.** Camels had a history of failing to conceive after more than two services. Camels included were aged from 4 to 20 years. Animals were subjected to a wide range of management systems that differed with regard to housing, feeding, and aspects of breeding.

Gynaecological Examination (GE):

GE comprised external (EE) and internal examination (IE). EE involved base of the tail, perineum and the vulva to detect mucopurulent discharge and dry flakes which indicate presence of infection. Conspicuous oedema of the vulva may be due to overbreeding. IE started by using a tube vaginoscope to evaluate the vaginal cavity for vaginitis or traumatic lesions. Then rectal palpation and ultrasonography were done to rule out pregnancy and detect the size, consistency and contents of the uterus

where abnormal changes such as thickened uterine wall, adhesions or abscesses may suggest uterine inflammation. In a non-pregnant animal, accumulation of fluid in the uterine cavity should be investigated adequately to be managed. Relaying on history, rectal, ultrasound examination (UST-588U-5, SSD-500V, ALOKA, Co., LTD JAPAN) and vaginal examination (Tibary and Anouassi, 1997; Walldridge and Pugh, 1997, Vyas and Sahani, 2000), 45 animals were diagnosed as suffering from endometritis.

Collection of uterine swabs

Preceding to collection of samples, the perineal area were washed twice with povidone-iodine 0.1% and dried with disposable paper towel. A double-guarded swab (Equi-VET® Kruus) was guided manually through the vulva, vagina and cervix to reach the right uterine horn. After the swab had been positioned in the right uterine horn, it was rolled on the endometrial surface for 30 seconds. The aforementioned steps were repeated with the left uterine horn. Then, the swab was retracted into the protecting tube of the double guarded swab and removed from the camel. The swab was transported at ambient temperature in Amies` modified medium to the laboratory and cultured within 24 hours.

Bacterial isolation and identification

Individual swabs were cultured on Columbia agar (CM331; Oxoid, Basingstoke, UK) containing 5% citrated sheep blood and MacConkey agar (Oxoid, Basingstoke, UK). After inoculation, the plates were incubated aerobically and anaerobically for 18 to 24 h at 37 °C and for a further 24 h if bacterial growth had not ensued. If > 90 % of the grown colonies in an incubated agar were similar phenotypically, the result was considered as substantial growth in primary culture. Mixed cultures with dissimilar colonies were discarded as no growth. Presumptive identification was done according to descriptions in textbooks (Koneman et al., 2005). Confirmation of the identification of isolates was done by Vitek 2 technique (bioMerieux, Marcy L'Etoile, France).

Antimicrobial susceptibility test

All the isolates were tested for *in-vitro* antibiotic sensitivity by disc diffusion method (Muneer et al., 1991). Discs containing nitrofurantoin (300 µg), erythromycin (15 µg), oxytetracycline (30 µg), ampicillin (10 µg), penicillin G (10 IU), chloramphenicol (30 µg) and kanamycin (30 µg) per disc (Oxoid) were employed. After the incubation period the diameter of inhibition zone was measured and interpretation of result based on CLSI guidelines was performed (CLSI, 2010).

Data analysis

The data are presented as percentages, and analyses were conducted by Chi-Square test using SPSS 16.0 statistical software (2007).

Results

Overview of the microorganisms that were isolated as well as their frequency of isolation is presented in Table 1. In this study, 45 animals out of 95 infertile female camels were diagnosed as suffering from endometritis. The infected samples recorded in this study were 73.33% (n=33). Negative growth was reported in 26.66% (n=12) of the swabs. *A.pyogenes*, *Staph.aureus*, *P.mirabilis*, *Bacillus* spp., *Strept. agalactiae* and *Kocuria kristinae* were found to be 33.33% (n=11), 27.27% (n=9), 24.24% (n=8), 9.09% (n=3), 3.03% (n=1) and 3.03% (n=1) respectively. *A.pyogenes*, *Staph. aureus*, *P.mirabilis* were significantly ($P<0.01$) associated with endometritis in dromedary camels. Table 2 presents the antibiotic sensitivity pattern of bacterial isolates from cases of endometritis. The antibiotic sensitivity pattern of the isolates indicated that 51.51%, 12.12%, 12.12%, 0.00%, 0.00%, 45.45% and 48.48% were sensitive to nitrofurantoin, erythromycin, oxytetracycline, ampicillin, penicillin, chloramphenicol and kanamycin respectively. The isolates were significantly ($P<0.01$) sensitive to nitrofurantoin, chloramphenicol and kanamycin.

Discussion

Endometritis in camel may interfere with the animal reproductive performance leading to infertility, drop in production and economic loss.

The current report proved that 47 %, of a group of infertile dromedary camels brought for veterinary investigation, were suffering from endometritis. In confirmation of the findings of the present study, Tibary

and Anouassi (2001); Tibary (2004); Wernery and Kumar (1994); Ali et al., (2010a) considered the uterine infection as the most frequent cause of reproductive failure in camelidae. The negative bacterial growth rate (26.66%) recorded in the present study was also reported by Tibary et al. (2006). This phenomenon can be explained by the observation that the uterine swab may miss the focal infections (Ball et al., 1988; Brook, 1984). Besides, in mare, Thomson (1984); Asbury (1986); Brito and Barth (2003) pointed that endometritis may occur in the presence or absence of uterine infection. Causes of endometritis other than bacterial infection have also been described (Kotilainen et al., 1994 and Troedsson et al., 1995). The microorganism isolated in the present study (*A.pyogenes*, *Staph. aureus*, *P.mirabilis*, *Bacillus* spp., *Strept. agalactiae* and *Kocuria kristinae* are also reported by several authors (Nawito, 1973; Enany et al., 1990; Powers et al., 1990). The main reproductive diseases and abortion in Saudi camels are due to *Salmonella* spp, *Proteus* spp, *Escherichia* spp., *Serratia* spp., *Klebsiella* spp., *Pseudomonas* spp., *Streptococcus* spp., *Staphylococcus* spp, and *Corynebacterium* spp. (Arthur et al. 1985). In Egyptian she camels, Ali et al. (1987) isolated *E coli*, *Corynebacterium pyogenes*, *Proteus* spp. and *Pasteurella multocida* from cases of endometritis associated with pyometra.

Antibiotics are commonly used in the treatment of uterine infection in camels (Tibary and Anuassi 2001). However, the efficacy of such therapeutic agents needs to be evaluated occasionally due to continuous emergence of drug resistant bacterial strains. The present study revealed that the bacterial isolates from infected uteri are significantly sensitive to nitrofurantoin, kanamycin and chloramphenicol. Parallel to our results, Ali et al. (2010b) reported that the bacterial isolates from infected camels' uteri are significantly sensitive to chloramphenicol. As well, bacterial isolates from infected cows' uteri are sensitive to nitrofurantoin (Barman et al., 2013).

In conclusion, *A.pyogenes*, *Staph. aureus* and *P.mirabilis*, were the most isolated bacteria from the cases of endometritis in dromedary camels. The bacterial isolates from camel endometritis were sensitive to nitrofurantoin, kanamycin and chloramphenicol.

Table 1. Uterine bacterial isolates from dromedary camels suffered from endometritis

Bacterial isolate	Number of isolates	Percentage
<i>A.pyogenes</i>	11	33.33 ^a
<i>Staph. aureus</i>	9	27.27 ^a
<i>P.mirabilis</i>	8	24.24% ^a
<i>Bacillus spp.</i>	3	9.09% ^b
<i>Strept. agalactiae</i>	1	3.03% ^b
<i>Kocuria kristinae</i>	1	3.03% ^b
Infected samples	33	73.33%
Negative growth	12	26.66%
Total	45	

Percentages with dissimilar superscripts in the same column are significantly different at $P<0.01$

Table 2. Antibiotic sensitivity pattern of bacterial isolates from cases of endometritis

Microorganism isolated	Number of Sensitive Isolates to Antibiotics						
	F	E	OT	Amp	P	C	K
<i>A.pyogenes</i>	4	1	-	-	-	3	3
<i>Staph. aureus</i>	8	2	4	-	-	6	6
<i>P.mirabilis</i>	3	-	-	-	-	5	6
<i>Bacillus spp.</i>	-	-	-	-	-	-	-
<i>Strept. agalactiae</i>	1	1	-	-	-	1	1
<i>Kocuria kristinae</i>	1	-	-	-	-	-	-
Total Number	17	4	4	0	0	15	16
Sensitivity Percentage	51.51 ^a	12.12 ^b	12.12 ^b	0.00	0.00	45.45 ^a	48.48 ^a

Key: F-nitrofurantoin, E-erythromycin, OT-oxytetracycline, Amp-ampicillin, P-penicillin G, C chloramphenicol, K-kanamycin

Percentages with dissimilar superscripts in the same row are significantly different at P<0.01

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