

## Preputial Bacterial Flora and Antibiotic Susceptibility in Wrestling Dromedary Bulls in Aydin Region of Turkey

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**Abstract:** In this study, twenty clinically healthy male camels (*Camelus dromedarius*) wrestling in traditional competitions were used to investigate preputial aerobic bacterial flora and antibiotic susceptibility. The aerobic bacteria included *Serratia liquifaciens*, *Staphylococcus aureus*, *Streptococcus* sp., *Klebsiella ozaenae*, *Pseudomonas* sp., *Shigella* sp., *Enterobacter cloacae*, *Flavobacterium* sp., *Actinomyces* sp., *Acinetobacter* sp., *Acinetobacter calcoaceticus* and *Bacillus* sp. were the most common organisms and identified from the preputial samples. In this study, antibiotic susceptibility and resistance were also studied. All of the isolates that were identified in this study were susceptible to ciprofloxacin (100%), danofloxacin (89.2%) and resistant to cloxacillin (100%), sulbactam-ampicillin (85.7%) and cefuroxim sodium (64.2%).

**Key words:** Wrestling, dromedary camel, prepuce, bacterial flora, antibiotics, Turkey

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### INTRODUCTION

Camel husbandry is vital for numerous pastoralist groups in Africa and Asia cause of their ability to survive under harsh environmental conditions and traditional camel racing which is popular in the Arabian Gulf countries and northern Africa (Abbas and Agab, 2002). There are an estimated 1,200 camel wrestlers in Turkey, bred specially for the competitions. Camel breeding is most common in the Aegean region of Turkey, but is also found in the Marmara and Mediterranean regions of that country.

The reproductive efficiency of camels under natural conditions is generally considered as low (Al-Qarawi, 2005). The reasons for these low reproductive rates could be related on the female dromedary due to delay in the onset of puberty, long gestation period (13 months) and lactation-related anoestrus period (8-10 months), high incidence of genital tract abnormalities and genital infections (Al-Eknaah, 2000; El-Wishy, 1987; Musa *et al.*, 1993; Sghiri and Driancourt, 1999; Tibary *et al.*, 2006).

As in many other domestic animal species, the uterus becomes contaminated by a variety of organisms from the caudal genitalia or the environment during breeding, parturition or genital manipulations. In most females, infectious organisms are eliminated by natural uterine defense mechanisms. However, in a proportion of the

females, these defense mechanisms fail partially or completely, allowing establishment of an infection (Tibary *et al.*, 2006). These infectious organisms are responsible for a great amount of diseases that may lead to temporary or permanent infertility in the male and female camels. Various bacterial diseases of camel's reproductive system have been reported by researchers (Refai, 1990; Tibary and Anouassi, 2000). In the pregnant female, infectious diseases may lead to abortion as well as prenatal-neonatal loss and stillbirths (Tibary *et al.*, 2006). The camel prepuce is flattened from side to side and triangular in shape, when viewed laterally. In the absence of sexual arousal, the small preputial opening is directed caudally and because of this they urinate towards the rear, but when erection of the penis occurs, the cranial prepuccial muscles pull the prepuce and also the penis, forward from their backward position. The penis is attached to the prepuce at birth and does not become free until 2-3 years of age (Skidmore, 2000). The most contamination of uterine infections in camelids is during breeding (overbreeding, nonhygienic condition etc). During mating, the penis penetrates the cervical canal and in some cases enters deep into the uterine cavity. Repeated microbial insults to the uterus impair its ability to severe intrauterine infection (Tibary and Anouassi, 2001). Venereal infections can be passed from bulls to she-camels and vice versa. The bull often does not show any clinical signs of contamination, but infection is traced

back through symptoms shown by she-camels he has mated (Al-Qarawi, 2005). In camel breeding, one male could be used for mating in female population included 50-80 females. Therefore, an important component of camel stud farm management is considered the detection, prevention and control of viral, bacterial and protozoan venereal diseases. Promising efforts have been made to introduce artificial insemination into camel breeding will be a useful step towards eradicating the diseases (Arthur, 1992; Wernery and Wernery, 1992). In this present study, it was aimed to investigate the preputial bacterial flora and detect the antibiotic susceptibility in camels in Aydin region of Turkey.

### MATERIALS AND METHODS

**Animals and samples:** The study was carried out in five villages included camel breeders for wrestling in Aydin region. The materials were included 20 clinically healthy between 5-8 year-old male camels. Samples for bacteriological examination belong to male camels were collected under aseptic conditions.

Before taking of samples, the animals were restricted by tie of hind legs or taken to dissertation for the limitation of movement. The hairs around preputium were cut in order to prevent contamination. The entry of preputial cavity was uncovered by another person with disinfected glove hand. Contact and friction of swab to mucosa was provided directly into the cavity without touching other parts of skin. Then, the swab was pulled back without contact to environment and was put inside its sterile container and transported to the laboratory. All samples were transported to Adnan Menderes University, Faculty of Veterinary Medicine, Microbiology Laboratory in cold circle.

**Isolation and identification studies:** The preputial swab samples were inoculated to 5-7% defibrinated sheep blood added Blood Agar, Nutrient Agar, MacConkey Agar, Eosin Methylene Blue Agar, Salmonella-Shigella Agar. Petri dishes were incubated at 37°C for 24-48 h aerobically. Growing colonies were examined with Gram staining method. Suspicious colonies were identified with biochemical tests and carbohydrate fermentation tests (Holt *et al.*, 1994; Koneman *et al.*, 1997; Lassen, 1975).

**The antibiogram studies of identified bacteria cultures:** The antibiotic susceptibility tests for identified microorganisms were applied with multidiscs containing Danofloxacin, Ciprofloxacin, Oxytetracycline, Cefuroxime Sodium, Sulbactam- Ampicillin, Amoxicillin-Clavulanic Acid, Erythromycin and Cloxacillin by Kirby-Bauer Disc Diffusion Method (Bauer *et al.*, 1966).

### RESULTS AND DISCUSSION

Table 1 show that the several microorganisms were isolated from preputial sac. Out of 20 preputial samples belong to male camels of different age groups, *Serratia liquifaciens* (n = 7), *S. aureus* (n = 4), *Streptococcus* sp. (n = 4), *Klebsiella ozaenae* (n = 2), *Pseudomonas* sp. (n = 2), *Shigella* sp. (n = 2), *Enterobacter cloacae* (n = 2), *Flavobacterium* sp. (n = 1), *Actinomyces* sp. (n = 1), *Acinetobacter* sp. (n = 1), *Acinetobacter calcoaceticus* (n = 1) and *Bacillus* sp. (n = 1) were isolated and identified. After the testing of antibiotic susceptibility, all of the isolates that were identified in this research were susceptible to ciprofloxacin in the ratio of 100%, susceptible to danofloxacin in the ratio of 89.2%, resistant to cloxacillin in the ratio of 100%, resistant to sulbactam-ampicillin in the ratio of 85.7%, resistant to cefuroxime sodium in the ratio of 64.2%.

Although, Artificial Insemination (AI) is important in camel breeding to enable more efficient use of genetically superior males as in other species, AI is not used commonly in camels in Turkey. For that reason, microbiological studies must be performed to help prevent the spread of venereal diseases cause of bacteria, virus and protozoan. The present investigation was carried out to study the aerobic bacterial flora and antibiotic susceptibility of the prepuce.

Venereal diseases can be spread through natural service and influence male and female fertility in camels. These are usually transmitted by an infected male mating with susceptible females. However, an uninfected bull can spread the disease by mating with an infected female and subsequently breeding a susceptible female within a short period of time. More unusual cases can occur by the use of infected semen from custom collection of bulls and the failure to test the bull and/or semen for venereal diseases (Overbay, 1999). Various bacterial diseases found in reproductive system of camel have been reported by some workers (Tibary and Anouassi, 2001). For that reason males and females should be examined for presence of genital tract infection before breeding season to avoid genital infection.

Interpretation of microbiological results of uterine swabs is very difficult given the wide range of bacteria that can be isolated. Yagoub (2005), reported that *Staphylococcus aureus*, *E. coli*, *Klebsiella* sp., *Corynebacterium* sp., *Proteus* sp., *Salmonella* sp. and *Streptococcus* sp. were the main isolated bacterium from cases of several uterine infections of female camels. The highest rate of isolation of *Staphylococcus aureus* from cases of orchitis and wounds of the external genitalia of male camels might indicate the wide distribution of this

Table 1: Antibiotic susceptibility of the isolated microorganisms

Isolated microorganisms	Antibiotics																							
	Danofloxacin			Ciprofloxacin			Oxytetracycline			Cefuroxim sodium			Sulbactam-ampicillin			Amoxicilline-clavulanic acid			Erythromycin			Cloxacilline		
	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
<i>Serratia liquifaciens</i> (n = 7)	-	-	7	-	-	7	2	-	5	6	1	-	7	-	-	3	1	3	6	-	1	7	-	-
<i>S. aureus</i> (n = 4)	-	-	4	-	-	4	-	-	4	3	-	1	3	-	1	-	1	3	2	-	2	4	-	-
<i>Streptococcus</i> sp. (n = 4)	1	-	3	-	-	4	-	-	4	-	-	4	1	3	-	-	4	1	-	3	4	-	-	-
<i>Klebsiella ozaenae</i> (n = 2)	1	-	1	-	-	2	-	-	2	1	-	1	2	-	-	-	2	-	-	2	2	-	-	-
<i>Pseudomonas</i> sp. (n = 2)	-	-	2	-	-	2	2	-	-	-	1	1	2	-	-	-	2	-	-	2	2	-	-	-
<i>Shigella</i> sp. (n = 2)	-	-	2	-	-	2	-	-	2	2	-	-	2	-	-	-	1	1	-	1	2	-	-	-
<i>Enterobacter cloacae</i> (n = 2)	-	-	2	-	-	2	1	-	1	2	-	-	2	-	-	1	1	2	-	1	2	-	-	-
<i>Flavobacterium</i> sp. (n = 1)	-	-	1	-	-	1	-	-	1	1	-	-	1	-	-	-	1	-	-	1	1	-	-	-
<i>Actinomyces</i> sp. (n = 1)	1	-	-	-	-	1	-	-	1	-	-	1	1	-	-	-	1	-	-	1	1	-	-	-
<i>A. calcoceticus</i> (n = 1)	-	-	1	-	-	1	1	-	-	-	-	1	1	-	-	-	1	1	-	-	-	-	-	-
<i>Bacillus</i> sp. (n = 1)	-	-	1	-	-	1	-	-	1	1	-	-	1	-	-	1	-	-	-	1	1	-	-	-

S: Susceptible I: Intermediate R: Resistant

organism in nature and consequently cause many diseases. Tibary *et al.* (2006) also reported that *E. coli* and *Streptococcus equi* subspecies zoepidemicus were the major infectious organisms in endometritis and metritis in camelids. Some of these germs are part of the normal vaginal flora whereas, others are opportunistic and can become pathogenic if the right conditions are present (Tibary and Anouassi, 2001).

Truly sexually transmissible bacteria that is, those transferred into the uterus of the she-camel causes endometritis (Refai, 1990; Wernery and Wernery, 1992; Youssef, 1992). In beside of this, Arthur *et al.* (1985), found that the main reproductive diseases and abortion in camels were due to, *Serratia* sp., *Klebsiella* sp., *Pseudomonas* sp., *Streptococcus* sp., *Staphylococcus* sp. and *Corynebacterium* sp. In this study, *Serratia liquifaciens* was the most common organism and isolated in ratio of 35%, both of *Streptococcus* sp. and *Staphylococcus aureus* major organisms were isolated in ratio of 20% and *Pseudomonas* sp. and *Klebsiella ozaenae* were isolated in ratio of 10%, respectively.

Wernery and Wernery (1992) showed that a wide variety of organism cultivated from uteri of camels with and without endometritis. They were identical to the species isolated commonly from the uteri of mares and cows. They were found that *Staphylococcus* sp., *S. aureus*, *Streptococcus* sp., *Bacillus* sp., *E. coli*, *Clostridium sporogenes* were isolated from uterine with or without endometritis. *Campylobacter fetus*, *Pseudomonas aeruginosa*, *Klebsiella ozaenae*, *Salmonella* sp., *Serratia marcescens* and *Micrococcus* sp. were isolated from camels with endometritis. In the study, we isolated as *S. aureus*, *Streptococcus* sp., *Klebsiella ozaenae*, *Pseudomonas* sp. from preputial samples.

As it is known, these bacterial pathogens are opportunistic and they cause severe infections in appropriate conditions. When keeping in view that, this study carried out in only twenty male camels breeding in

same region, these microbiological results could be evaluated as conspicuously and may be useful in further fertility studies carried on camels.

Another finding of this study is about that the antibacterial resistance for preputial flora in camels. Qureshi *et al.* (2002) reported that microbiological examination of uterine discharge revealed a mixed bacterial infection with *E. coli*, *Bacillus* and *Streptococci*, which were all sensitive to nortloxacin, gentamicin and amoxicillin. In this present study, all of the isolates were susceptible to ciprofloxacin and danofloxacin in the very high ratio. However, there was highly resistance to cloxacillin, sulbactam- ampicillin and cefuroxim sodium. In based on these results, it was concluded that the genital microorganisms has various susceptibility to different antibacterial and it should be regarded during genital infection prophylaxis and therapy in camel practice.

## CONCLUSION

The present study shows that pre-breeding bacterial examination is important for male and female fertility and it should be performed on all male camels to detect the high-risk stud bulls, minimize the uterine contamination and eradication of venereal diseases in region.

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