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DIVERSITY OF BACTERIA AND FUNGI IN THE PREPUCE OF CAMELS (*Camelus dromedarius*)

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ABSTRACT

A total of 42 infertile male dromedary camels (4-12 years old, 500-800 kg) and 7 young male camels (control, 2-2.5 years old) were enrolled in the present study. All camels were sedated and preputial swabs were collected and immediately immersed in 1 ml sterile solution of 0.9% NaCl. Preputial swabs were transferred refrigerated within 1 hour to the bacteriological laboratory. After preputial swabbing, semen samples were collected from infertile camels using an electro-ejaculator and evaluated for routine semen quality parameters using the conventional methods. Statistical analyses were conducted by Student's *t*-test and Chi-square (X^2). Results revealed that semen parameters were reduced in infertile camels with preputial contamination. The bacterial count in 1 ml swab sample was $148x10^3$ and $0.24x10^3$ in infertile mature camels and control immature camels, respectively. Nine bacterial species isolated from 49 camels' preputial swabs comprised 41 gram-positive species (83.7%) and 8 gram-negative species (16.3%). The swabs of infertile camels had colonies species of *Staphylococcus*, *Streptococcus*, *Corynebacterium*, *Bacillus*, *E. coli*, *Pseudomonas aeruginosa*, *Actinomyces*, *Fusobacterium*, *Clostridium perfringens* at percentages of 35.72% (n=15), 21.43% (n=9), 11.91% (n=5), 9.52% (n=4), 7.14% (n=3), 4.76% (n=2), 4.76% (n=2), 2.38% (n=1), 2.38% (n=1), respectively. *Candida* colonies appears in concurrence with bacterial colonies in 28.57% (n=12). In control camels, the examined samples had colonies of *Streptococcus* spp., *Bacillus* spp. and *E. coli* spp. at proportions of 42.86% (n=3), 42.86% (n=3), 14.28% (n=1), respectively. *Candida* spp. observed in 5 colonies (71.43%) of control camels. In conclusion, *Staphylococcus*, *Streptococcus* and *Bacillus* were the frequently isolated bacterial spp. from prepuce of camel.

Key words: Bacterial diversity, camels, fungus, prepuce

The reproductive efficiency of camels under natural conditions is generally considered as low (Al-Qarawi, 2005) and genital infection is considered as most important reason (Tibary *et al,* 2006). It has been demonstrated that the preputial sac can act as a reservoir of organisms and it thus responsible for causing ascending uro-genital infection (Agartan *et al,* 2005). The preputial microbial community plays a key role in maintaining health and altered microbial communities have been associated with a variety of reproductive diseases (Nelson *et al,* 2010; Sandal and Inzana, 2010; Chaban *et al,* 2012). The source of camel preputial contamination is usually soil, faeces and female genital tract (Wickware *et al,* 2020), and microorganisms gain entry through preputial orifice (Paray *et al,* 2018). The camel often does not show any clinical signs of contamination, but infection is traced back through symptoms shown in female camels it has mated (Al-Qarawi, 2005). A long duration of semen collection and collection in a sitting position (El-wishy, 1988) increase the risk of contamination of prepuce of camel Bacterial contamination leads to a series of alterations including reduced sperm motility, morphology, various semen quality parameters (Najee *et al,* 2012; Perumal *et al,* 2013), and subsequent reduced fertility (Ochsendrof and Fuchs, 1993; Griveau *et al,* 1995). This study investigates the frequency of isolated bacteria and fungi in camels' prepuce.

Materials and Methods

Experimental animals

A total of 42 infertile male dromedary camels (4-12 years old, 500-800 kg) admitted to the

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Veterinary Teaching Hospital, King Faisal University and 7 young male camels (control, 2-2.5 years old) belonging to the Camel Research Centre, King Faisal University (25°23′N 49°36′E) were selected in the present study. The control males had no contact with females before or during the experiment (5 months) between November 2020 to and March 2021.

Samples collection

Infertile camels were restrained in sternal position with ropes, sedated intravenously with a combination of xylazine (Rompun®, Bayer; 0.15 mg/kg) and ketamine (Alfasan® 10%, Holland; 2.5 mg/kg) (White *et al,* 1982). Then, sedated camels were turned to lateral recumbency and their prepuce was cleaned externally with sterile saline solution (0.9% NaCl). A sterile swab was inserted and rotated in the prepuce, and immediately immersed in a sterile 5 ml plastic tubes containing 1 ml sterile physiological saline solution (0.9% NaCl). Preputial swabs were collected from the control males as in the infertile camels. All collected swabs were transferred refrigerated within 1 hour to the bacteriological laboratory in the Ministry of Agriculture, Al-Ahsa, Kingdom of Saudi Arabia. After preputial swabing, semen samples were collected from all infertile camels using an electro-ejaculator (Tingari *et al,* 1986). Semen samples were evaluated for routine semen quality parameters such as volume (mL), viscosity (Ghoneim *et al,* 2010), pH, per cent motile sperm, sperm concentration (x10^6/ml), and per cent sperm abnormalities (Table 1) by the same trained researcher using Sperm Vision 3.5 (Minitube of America, Inc.).

Table 1. Semen parameters of infertile mature camels (mean ± SEM; range).

Semen parameters	Infertile mature camels (n=42) (Range)	Reference*
Volume (ml)	6.32 ± 0.74 (7 – 25)	6.56 ± 1.73
Viscosity (0 – 5)	3.57 ± 0.25 (0 – 5)	4.25 ± 0.37
pH	8.11 ± 0.06 (6.8 – 8.4)	7.84 ± 0.14
Motility (%)	8.00 ± 2.11 (0 – 20)	43.13 ± 9.77
Sperm concentration (x10^6/ml)	37.07 ± 13.41 (1 – 100)	366.15 ± 47.55
Sperm abnormalities (%)	50.54 ± 4.65 (15 – 80)	26.00 ± 2.95

*Adapted from Waheed *et al* (2015).

Microbiological evaluation

Bacterial identification

Each swab sample was plated on 5% sheep blood agar, Mac-Conkey agar and *Salmonella-Shigella* agar and incubated at 37°C in 5% CO_2 for 24 h. Growing colonies were examined with Gram staining methods. Suspected colonies were identified with biochemical and carbohydrate fermentation tests using bioMérieux's API identification products (Chesbrough, 2004).

Counting colony forming units per millilitre (CFU/ml) of sample

The quantitative estimation of the microbial contamination of prepuce was performed by the standard plate count method (Shukla, 2011). An aliquot (0.05 ml) of the swab sample was diluted with 1:10 PBS (0.1M phosphate buffer containing 0.15M NaCl, pH 7.3), producing a serial-dilution. Plates of Columbia-blood-agar were inoculated with 100 µl of the diluted swab sample (101-105) and incubated at 37°C for 48 h. The bacterial colonies were counted with the help of a colony counter. The colonies were counted in relation to the swab sample aliquot of 1ml and expressed as CFU/ml.

Mycological examinations

Swab samples were examined for fungus and yeast by seeding the samples in Sabaroud's dextrose agar. The plates were incubated at 25°C for 5 days and checked for fungal growth from the fourth day onward. Fungal identification relied on the morphologic and physiologic features (de Hoog *et al,* 2000). *Yeasts* were sub-cultured to obtain pure cultures for identification. For definitive identification of *yeasts*, the carbohydrate assimilation pattern was defined by ID32 (Biomerieux, Bagno a Ripoli, Italy).

Statistical analysis

The data are presented as the means ± SEM and percentages. Analyses were conducted by Student's *t*-test and Chi-square (X^2) using INSTAT software 3.1 (2017).

Results and Discussion

The bacterial count in 1 ml swab sample was 148x10^3 and 0.24x10^3 in infertile mature camels and control immature camels, respectively (Table 2). Nine bacterial species isolated from 49 camels' preputial swabs comprised 41 gram-positive species (83.7%) and 8 gram-negative species (16.3%). The different types of colonies were *Staphylococcus* spp., *Streptococcus* spp., *Corynebacterium* spp., *Bacillus*

spp., *E. coli* spp., *Pseudomonas aeruginosa* spp., *Actinomyces* spp., *Fusobacterium* spp., *Clostridium perfringens* spp. and *Candida* spp. as shown in table 2. The swabs of infertile camels had colonies species of *Staphylococcus*, *Streptococcus*, *Corynebacterium*, *Bacillus*, *E. coli*, *Pseudomonas aeruginosa*, *Actinomyces*, *Fusobacterium*, *Clostridium perfringens* at percentages of 35.72% (n=15), 21.43% (n=9), 11.91% (n=5), 9.52% (n=4), 7.14% (n=3), 4.76% (n=2), 4.76% (n=2), 2.38% (n=1), 2.38% (n=1), respectively. *Candida* colonies appear in concurrent with bacterial colonies in 28.57% (n=12). In control camels, the examined samples had colonies of *Streptococcus* spp., *Bacillus* spp. and *E. coli* spp. at proportions of 42.86% (n=3), 42.86% (n=3), 14.28% (n=1), respectively. *Candida* spp. observed in 5 colonies (71.43%) of control camels (Table 2).

Table 2. Bacterial load, types and frequency in dromedary preputial samples.

Isolates	Infertile mature camels (N=42)	Control immature camels (N=7)
CFU/ml (mean ± SEM)	$148^{a} \pm 56.96x10^{3}$	$0.24^{b} \pm 0.12x10^{3}$
Staphlyococcus spp. (%)	11 (26.19%)	—
Staphlyococcus spp. + *Candida* (%)	4 (9.52%)	—
Streptococcus spp. (%)	5 (11.91%)	—
Streptococcus spp. + *Candida* (%)	4 (9.52^{a}%)	3 (42.86^{b}%)
Corynebacterium spp. (%)	5 (11.91%)	—
Bacillus spp. (%)	3 (7.14^{a}%)	2 (28.57^{b}%)
Bacillus spp. + *Candida* (%)	1 (2.38^{a}%)	1 (14.28^{b}%)
E. coli spp. (%)	3 (7.14%)	—
E. coli spp. (%) + *Candida* (%)	—	1 (14.28%)
Pseudomonas aeruginosa (%)	2 (4.76%)	—
Actinomyces spp. + *Candida* (%)	2 (4.76%)	—
Fusobacterium + *Candida* (%)	1 (2.38%)	—
Clostridium perfringens (%)	1 (2.38%)	—

Means and percentages with dissimilar superscripts in the same row are significantly different at P<0.05.

In the present study, semen parameters were reduced in infertile camels. These reduced values were accompanied with preputial contamination in these camels. This relationship indicates that preputial microbial load might be considered as a relevent cause of infertility in dromedaries. Previous studies achieved the same findings (Ochsendrof and Fuchs, 1993; Griveau *et al,* 1995; Najee *et al,* 2012; Perumal *et al,* 2013). Published data on the presence and distribution of bacteria and fungi in the camel prepuce are scarce. The current study revealed that the bacterial count in 1 ml semen was 0.24 and 148 $x10^{3}$ CFU in control immature and infertile camels, respectively. The young bulls had significantly lower total bacterial counts than older animals (Reddy *et al,* 1971). However, Brown *et al* (1974) found no correlation between the age and total bacterial count. The saprophytic flora of the prepuce of healthy bulls includes numerous bacterial species (Thibier and Guerin, 2000). The collected preputial samples were colonised by species of *Staphylococcus*, *Streptococcus*, *Corynebacterium*, *Bacillus*, *E. coli*, *Pseudomonas*, *Actinomyces*, *Fusobacterium* and *Clostridium*. Species of *Staphylococcus*, *Streptococcus*, *Corynebacterium* and *Bacillus* were the frequently isolated bacteria. Twelve bacterial spp. were identified in dromedary camels' prepuce that were *Serratia liquefaciens*, *Staphylococcus aureus*, *Streptococcus* spp., *Klebsiella ozaenae*, *Pseudomonas* spp., *Shigella* spp., *Enterobacter cloaceae*, *Flavobacterium* spp., *Actinomyces* spp., *Acinetobacter* spp., *Acinetobacter calcoaceticus* and *Bacillus* spp. (Serin *et al,* 2010). *Streptococcus* spp., *Pseudomonas* spp. and *Staphylococcus* spp. are the most common bacteria isolated from the uterus of infertile camels (Wernery and Wernery, 1992; Ali *et al,* 2010; Almohasen, 2011). Then, camel preputial contamination might lead to uterine infection and *vice versa*. It has been demonstrated that the camel prepuce can act as a reservoir of organisms and it thus might be responsible for infertility (Agartan *et al,* 2005). *Staphylococcus aureus* was the most frequently isolated aerobic microorganism in specimens obtained from the prepuce of dogs (Ling and Ruby, 1978). The present study was done during the camels' rutting season with with a marked peak in sexual activity. This activity might lead to preputial contamination (Zhao, 2000; Fatnassi *et al,* 2014). In the present study, *Candida* spp. was colonised in 26.5% of the collected samples. *Candida* is a common mycoflora present in the genitalia of healthy female camels (Shokri *et al,* 2010). In camels mating, the penis penetrates the cervical canal, and in some cases, it enters deep into the uterine cavity (Serin *et al,* 2010). During the camels' rutting season, the atmospheric concentration of fungus spores is twice as high as in the non-rutting season (Bartzokas, 1975). In stallions, *yeasts* were isolated in 9.1% of the preutial samples (Rota *et al,* 2011). In conclusion, species of *Staphylococcus*, *Streptococcus* and *Bacillus* were the frequently isolated bacteria from camels' prepuce. The present study recommends the washing of the camel's prepuce before mating.

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