

Investigation of the Vaginal and Clitoral Fungi in One-humped Camels (*Camelus dromedarius*)

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Abstract

Background. Bacteria and fungi constitute an important part of the genital tract microenvironment and participates in the mucosal homeostasis. While the genital tract microflora has been studied for several animal species, this is not the case for the one-humped camel (*Camelus dromedarius*).

Objective. The aims of this survey were to investigate the prevalence of vaginal and clitoral fungi in one-humped camels (*Camelus dromedarius*) in different region of Iran.

Materials & Methods. Samples were taken from dairy camel farms in the provinces of Khorasan Razavi, Semnan, Tehran, Qom and Isfahan located in Central and North-East Iran. Two hundred and twenty paired samples were taken from vaginal and clitoral mucosa of 110 camels and cultured on Sabouraud dextrose agar and on CHROMagar™ *Candida* followed by identification of cultured fungi. Data were analyzed using Genmod procedure.

Results. Six different isolates were recovered in this study. Fungi were cultured and isolated from 31% of the camels. *Cladosporium* spp. and *Aspergillus flavus* were the most isolated prevalent. The most frequently recovered species samples was *Aspergillus*. The presence of fungi was evaluated in different ages of the camels, but there was no correlation ($P>0.05$).

Conclusion. It is concluded that fungal contamination of the camel external organs of reproductive system is very low in comparison with other domestic animals. It is likely due to the intrinsic immunity of one humped camel (*Camelus dromedarius*).

Key words: *Aspergillus flavus*, camel, Clitoris, *Cladosporium* spp., fungi, vagina.

Introduction

In the Middle East and some other regions, the one-humped camel (*Camelus dromedarius*) is used as a local source of milk, meat and wool. Also, camel racing is a multi-dollar industry in some regions, which emphasizes the overall high value of camel breeding (Noakes et al., 2019). As for other production and sport animals such as cattle and horses, successful reproduction of camels plays a crucial role in maintaining a continuous and good production and subsequent income for the breeders.

Presence of a normal structure and functional genital tract is a prerequisite for normal fertility in female camels. It is well-known that genital tract infection at the time of breeding reduces the fertility due to lack of fertilization or embryonic death (Noakes et al., 2019; Gideon et al., 2014; Enany et al., 1990). Investigation on fungal contamination of reproduction system has received less attention in animals. However, most of the studies has been established in the field of genital system in animals such as: dairy cows (Talebkhani Garoussi et al., 2008. Massoud Talebkhani Garoussi 2007.), buffaloes (Singh et al. 1992), horses (Azarvandi et al., 2017a. Azarvandi 2017b. Roz'an ski et al., 2013a. Roz'an ski et al., 2013b), cats (Garoussi et al., 2016) and dogs (Cleff et al., 2005. Khosravi et al., 2008). Fungal agents can effect on placenta and / or fetus or both and cause abortion in domestic animals (Antoniassi et al., 2013; Fourichonet al., 2000). However, some investigations have been conducted in the field of bulk milk tank of dairy farms (Hosseinabadi et al., 2022). Fungal agents will be able to invade the genital system and cause reproductive disorders under abnormal conditions (Noakes et al., 2019. Rebhun et al., 1995). However, identification of an abnormal microflora requires knowledge on the normal flora. Several bacteriological studies have been carried out on the reproductive system of camels (Ali et al., 2010; Pal, 2015) but, the studies conducted on fungal infections of one humped camel reproduction are few (Shokri et al., 2010. Hajinejad-Bamroud et al., 2020. Moradi et al., 2021. Rasooli et al., 2023) although fungi generally constitute an important part of the vaginal microenvironment in animals (Azarvandi et al., 2017a; Azarvandi et al., 2017b; Garoussi et al., 2016; Hopper, 2015; Talebkhani Garoussi et al., 2008; Talebkhani Garoussi et al., 2007; Youngquist and Threlfall, 2006; Heuwieser et al., 2000).

To obtain a basic understanding of the mucosal fungi-of the caudal genital of female one-humped camels, we cultured paired swabs of the vaginal and clitoral mucosa of 110 animals to determine the fungi and to study if the fungi depended on the age of the animal.

Materials and methods

Study population

Samples were taken from dairy camel farms in the provinces of Khorasan Razavi, Semnan, Tehran, Qom and Isfahan located in Central and North-East Iran from March 2018 to August 2019 (Fig. 1). These provinces represent the major camel rearing regions in Iran. The study population consisted of around 550 clinically healthy female one-humped camels of which 20% were included randomly using a lottery approach according to Thrusfield and Christley (2018). The age of the sampled camels was determined based on dental characteristics (Hillson, 2009).

The camels were kept in semi-intensive dairy systems with access to pasture. A typical diet consisted of alfalfa hay, corn silage and concentrate in various proportions using totally mixed ration. The average milk production was 5 kg/day with dual milking. Around 45% of the camels were non-lactating at the time of sampling. However, the pregnant camels within the herds were diagnosed using ultrasonography, transrectal palpation and also persistent raised tail (called tashweel) (Noaks et al., 2009; Enany et al., 1990; Merkt et al., 1990; Chen and Yuen, 1984). Vaccination against foot and mouth disease, *Clostridium chauvoei* and *Cl. septicum* was performed on a regular basis. All herds used natural breeding.



Fig. 1 The map of the study area in 5 provinces of Iran

Sampling and fungal culture

The external genitalia were thoroughly washed with a 5% povidine iodine solution and the vulva opening was cleaned with a sterilized tampon prior to sample collection. The vaginal walls were manually separated in order to allow a deep insertion of a simple sterile cotton swab without contamination. The cranial part of the vaginal mucosa was gently swabbed before the cotton swab was removed. Then a second swab was taken from the clitoral mucosa using the same technique. The swabs were transferred to a sterilized Stuart transport media (Difco Laboratories, Detroit, MI, USA) and transported on ice to the Mycology Research Centre, Tehran.

Swabs were spread onto Sabouraud dextrose agar with chloramphenicol (Merck Co., Darmstadt, Germany) and incubated at 32 °C for 48 h under aerobic conditions and on CHROMagar™ Candida (CHROMagar, Paris, France) and incubated at 35 °C for 48 h in the dark. Sugar

fermentation and assimilation tests with the RapID™ Yeast Plus System (Thermo Fisher Scientific, Lenexa, KS, USA) according to the manufacturer's instructions was used for presumptive identification of isolated yeasts. Final identification was done by the germ tube formation test, urease test, and culturing on CHROM agar and corn meal agar medium with Tween 80.

Statistical analysis

Data were analyzed using Genmod procedure including function link logit in the model. Differences at $P < 0.05$ were considered statistically significant.

Results

Culturing revealed growth of fungi from 31% of the examined camels. In 11% of the camels, fungi were found both on the vaginal and clitoral mucosa, while fungi were only isolated from the clitoral or vaginal mucosa from 11% and 9%, respectively (table 1).

Table 1. Isolation of fungi from the clitoral and vaginal mucosa of one-humped camels

Location	Culture results		Total (%)
	Positive (%)	Negative (%)	
Clitoris	12 (11)	26 (24)	38 (34.5)
Vagina	10 (9)	28 (25)	38 (34.5)
Clitoris and vagina	12 (11)	22 (20)	34 (31.3)

Total	34 (31)	76 (69)	110
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Several fungal species were found and, in many cases, they were present as a monoculture (table 2). The fungi of the vaginal and clitoral mucosa seemed to be similar, but varied between animals. No single species was isolated consistently from all or most camels. When analyzing the culture results (culture positive vs. negative) against age of the animals, no correlation was found (table 3).

Table 2. Fungal species isolated from the clitoral and vaginal mucosa of one-humped camels

Fungal species	Location
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	C (%)	V (%)	C +V (%)
Yeast fungi			
<i>Candida</i> spp.	2 (6)	-	2(6)
Filamentous (mold) fungi			
<i>Mono-culture</i>			
<i>Aspergillus niger</i>	1(3)	2(6)	2(6)
<i>Aspergillus flavus</i>	2(6)	2(6)	1(3)
<i>Penicillium</i> spp	2(6)	-	2(6)
<i>Alternaria Alternaria</i>	2(6)	1(3)	2(6)
<i>Mixed culture</i>			
<i>A.niger, A. flavus</i>	3(9)	2(6)	1(3)
<i>Cladosporium</i> spp. <i>A. flavus</i>	-	3(9)	2(6)
Total	12(35)	10(29)	12(35)

C: Clitoris

V: Vagina

Table 3. Fungal culture results from the clitoral and vaginal mucosa compared to age of the examined one-humped camels

Culture	Age (years) (%)					Total (%)
	<5	≥5; <10	≥10; <15	≥15; <20	≥20	
Positive	1 (1)	5 (5)	7 (6)	13 (12)	8 (7)	34 (31)
Negative	7 (6)	11 (10)	25 (23)	22 (20)	11 (10)	76 (69)
Total	8 (7)	16 (15)	32 (29)	35 (32)	19 (17)	110 (110)

Discussion

A range of different fungal species were isolated from the vaginal and clitoral mucosa of the examined one-humped camels. Generally, all species were isolated from both locations indicating that these fungi normally could be inhabited the mucosa of the posterior genital tract. As all camels included in the study were healthy with regard to the genital tract, the isolated fungi probably represent a part of the normal vaginal fungi in camels. However, some fungi are opportunistic pathogens such as: *Aspergillus spp.*, *Candida spp.* and may be involved in the development or progression of an infection. In animals, the aspergilli can cause abortion (Constable et al., 2017). *Aspergillus spp.* and *Mucor spp.* have been isolated from the uterus of one-humped camels with endometritis (Refaat et al., 2020; Tibary et al., 2006). As endometritis could be originated of an ascending transcervical migration of pathogens, therefore, the normal vaginal mucosal fungi could cause post-partum uterus fungal infection. Usually, fungi are not very important in endometritis of camels or other animals, but sometimes they become important (Karstrup et al., 2017). Some of the *Aspergillus spp.* that were isolated in this study

such as *Aspergillus niger* and *Aspergillus flavus* are well-known causes of aspergillosis (Sugui et al., 2015), including mycotic placentitis in cattle and horses (Hopper, 2015; Knudtson and Kirkbride, 1992; Murase et al., 2015; Orellana-Guerrero et al., 2019; Youngquist and Threlfall, 2006). The vaginal and clitoral fungi may therefore serve as a source of genital tract infection under certain circumstances.

Yeast seems to commonly inhabit the mucosa of the caudal genital tract of camels with the highest load in the vestibulum and the numbers decreasing towards the uterine horns (Shokri et al., 2010). *Candida* spp. are particular prevalent, with *Candida zeylanoides* being the most common in the vagina (Shokri et al., 2010; Sobel, 1988). In the present study, *Candida* spp. were cultured from the vaginal mucosa in 3% of the camels and from the clitoris in 15% and therefore not as prevalent as expected from the study by Shokri et al. (2010) Presence of filamentous fungi has not been reported previously, but these occur in both the vaginal and clitoral mucosa at an equal prevalence (table 2).

In the present study, the fungal isolates showed progressive involvement of different structures of external organs of female camle reproduction system, thus confirming the locally invasive nature of different isolates in agreement with previous reports which infection did not disseminate (Garoussi et al., 2016). Therefore, it is possible that this fungal group demonstrates a tropism towards this anatomical site in camels with different ages (Table 3). Interestingly, *A.niger*, *A. flavus*. (No.3, 9%) and *Cladosporium spp.* *A. flavus* (No. 3, 9%) the most frequent fungal species located in cervical and vaginal tissues, respectively (Table 2). They may have the potential to spread through the male genital system in camels. *Candida* can be localized on the mucous membranes and the skin. It is distributed worldwide in different animals and is most commonly caused by species of the yeast-like fungus, *C. albicans*, *C. Krusei* and others (Garoussi et al., 2016).

This survey showed that the amount of fungal contamination of external organs of reproductive system in female camels is much lower than mares (Azarvandi et al., 2017a, b). It can probably be caused by the resistance of the camel's innate and general immune system against fungal infection.

Despite the importance of camels, scientific knowledge on their reproduction and reproductive pathology is rather limited. Establishing of basic knowledge is therefore important, but efforts should also be applied to improve the reproductive efficiency and to increase our knowledge on infections causing reproductive failure such as embryonic losses, abortion and subfertility with the aim to prevent such conditions.

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Disclosure of interest

No competing interests have been declared.

Ethical approval

All the protocols and procedures were approved by the animal experimentation ethics committee of Faculty of Veterinary Medicine, University of Tehran under the recommendations for the proper care and use of laboratory animals (ethics certificate no. 28903. 1.2).

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بررسی فلور قارچی واژینال و کلیتورال شترهای یک کوهانه (*Camelus dromedarius*)

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چکیده

زمینه مطالعه: باکتری‌ها و قارچ‌ها بخش مهمی از محیط دستگاه تناسلی را تشکیل می دهند و در یک پارچگی مخاطی شدخیل هستند. در حالی که میکرو فلور دستگاه تناسلی برای چندین گونه حیوانی مورد مطالعه قرار گرفته است، این مورد برای شتر یک کوهانه (*Camelus dromedaries*) صادق نمی‌کند.

هدف: مطالعه، بررسی شیوع فلور قارچی واژینال و کلیتورال در شترهای یک کوهانه (*Camelus dromedaries*) در مناطق مختلف ایران می‌باشد.

مواد و روش کار: نمونه ها از مزارع شترهای شیری در استان‌های خراسان رضوی، سمنان، تهران، قم و اصفهان واقع در مرکز و شمال شرق ایران اخذ گردید. تعداد 220 نمونه جفتی از مخاط واژن و کلیتورال 110 نفر شتر گرفته شد. نمونه‌ها بر روی محیط Sabouraud dextrose agar و CHROMagar™ Candida کشت شد و سپس قارچ های کشت شده مورد شناسایی قرار گرفت. داده های بدست آمده با استفاده از روش آماری Genmod تجزیه و تحلیل شدند.

نتایج: شش جدایه مختلف در این مطالعه اخذ گردید. قارچ از 31 درصد شترها کشت و جداسازی شد. کلاکسپوریوم و اسپرژیلوس فلاوس شایعترین جدایه‌ها بودند. فراوان‌ترین نمونه‌های بازیابی شده اسپرژیلوس بود. حضور قارچ در سنین مختلف شترها مورد ارزیابی قرار گرفت، اما ارتباطی وجود نداشت. ($P>0.05$)

نتیجه‌گیری نهایی: نتیجه گرفته می‌شود که آلودگی قارچی اندام‌های خارجی دستگاه تناسلی شتر در مقایسه با سایر حیوانات اهلی بسیار کم است. این مساله احتمالاً به دلیل ایمنی ذاتی شتر یک کوهانه (*Camelus dromedarius*) است.

کلمات کلیدی: اسپرژیلوس فلاوس، شتر، کلاکسپوریوم، قارچ، تولید مثل.