

Original Article

Investigation of the Vaginal and Clitoral Fungi in One-Humped Camels (*Camelus Dromedarius*)



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ABSTRACT

Background: Bacteria and fungi constitute crucial parts of the genital tract microenvironment and participate in mucosal homeostasis. While the genital tract microflora has been studied for several animal species, this is not true for the one-humped camel (*Camelus dromedarius*).

Objectives: This survey aims to investigate the prevalence of vaginal and clitoral fungi in one-humped camels (*C. dromedarius*) from different regions of Iran.

Methods: Dairy camel farms were sampled in Khorasan Razavi, Semnan, Tehran, Qom, and Isfahan Provinces in central and northeast Iran. Two hundred and twenty paired samples were taken from the vaginal and clitoral mucosa of 110 camels and cultured on Sabouraud dextrose agar and CHROMagar™ Candida, followed by identification of cultured fungi. Data were analyzed using the Genmod procedure.

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

Conclusion: Fungal contamination of the camel's external organs of the reproductive system is very low compared to other domestic animals. It is likely due to the intrinsic immunity of one-humped camel (*C. dromedarius*).

Keywords: *Aspergillus flavus*, Camel, Clitoris, *Cladosporium* spp., Fungi, Vagina

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Introduction

In the Middle East and other regions, the one-humped camel (*Camelus dromedarius*) is a local source of milk, meat, and wool. Camel racing is a multi-dollar industry in some regions that 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 continuous and good production and subsequent income for breeders.

A normal structure and a functional genital tract is a prerequisite for normal fertility in female camels. Genital tract infection during breeding reduces fertility due to lack of fertilization or embryonic death (Noakes et al., 2019; Gideon et al., 2014; Enany et al., 1990). Investigating fungal contamination in the reproductive systems of animals has received less attention. However, most studies have been conducted in genital systems of animals, such as dairy cows (Dabiri et al., 2020; Talebkhan Garoussi et al., 2008; Talebkhan Garoussi, 2007), buffaloes (Singh et al., 1992), horses (Azarvandi et al., 2017a; Azarvandi 2017b; Różański et al., 2013a; Różański et al., 2013b), cats (Garoussi et al., 2016), and dogs (Cleff et al., 2005; Khosravi et al., 2008). Fungal agents can affect the placenta, fetus, or both and cause abortion in domestic animals (Antoniassi et al., 2013; Fourichon et al., 2000). However, some investigations have been conducted in bulk milk tanks on dairy farms (Hosseiniabadi et al., 2022). Fungal agents can invade the genital system and cause reproductive disorders under abnormal conditions (Noakes et al., 2019; Rebhun et al., 1995). However, identification of abnormal microflora requires knowledge of the normal flora. Several bacteriological studies have been conducted 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) although fungi constitute a crucial part of the vaginal microenvironment in animals (Azarvandi et al., 2017a; Azarvandi et al., 2017b; Garoussi et al., 2016; Hopper, 2015; Talebkhan Garoussi et al., 2008; Talebkhan Garoussi et al., 2007; Youngquist & Threlfall, 2006; Heuwieser et al., 2000).

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

Materials and Methods

Study population

From March 2018 to August 2019, dairy camel farms were sampled in Khorasan Razavi, Semnan, Tehran, Qom, and Isfahan Provinces located in central and northeast Iran (Figure 1). These provinces represent major camel-rearing regions in Iran. The study population included 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 pastures. A typical diet consisted of alfalfa hay, corn silage, and concentrates in various proportions using a mixed ration. Average milk production was 5 kg/day with dual milking. Approximately 45% of camels were non-lactating at the time of sampling. However, pregnant camels within the herds were diagnosed using ultrasonography, transrectal palpation, and persistently raised tail (called tashweel) (Noaks et al., 2009; Enany et al., 1990; Merkt et al., 1990; Chen & Yuen, 1984). Vaccination against foot and mouth diseases, *Clostridium chauvoei*, and *Clostridium Septicum*, were performed regularly. All herds used natural breeding.

Sampling and fungal culture

The external genitalia was thoroughly washed with a 5% povidone-iodine solution, and the vulva opening was cleaned with a sterilized tampon before sample collection. The vaginal walls were manually separated to allow the deep insertion of a simple sterile cotton swab without contamination. The cranial part of the vaginal mucosa was gently swabbed before removal of the cotton swab. Then a second swab was taken from the clitoral mucosa using the same technique. The swabs were transferred to a sterilized Stuart transport medium (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 were performed using the RapIDTM Yeast Plus System (Thermo Fisher Scientific, Lenexa, KS, USA) according to the manufacturer's instructions for the presumptive identification of



Figure 1. The map of the study area in 5 provinces of Iran isolated yeasts. Final identification was performed using the germ tube formation test, urease test, and culturing on CHROM agar and corn meal agar medium with Tween 80.

Statistical analysis

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

Results

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

Several fungal species were found, and in many cases, they were present as monoculture (Table 2). The fungi

of the vaginal and clitoral mucosa seemed to be similar but varied between animals. No single species was consistently isolated from all or most camels. No correlation was found when analyzing the culture results (culture positive vs. negative) against the animals' age (Table 3).

Discussion

A range of different fungal species was isolated from the vaginal and clitoral mucosa of the examined one-humped camels. All species were isolated from both locations, indicating that these fungi can normally inhabit the mucosa of the posterior genital tract. As all camels included in the study were healthy in 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. and *Candida* spp., and may be involved in the development or progression of an infection. In animals, *Aspergillus* 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 can originate from ascending transcervical migration of pathogens, therefore, normal vaginal mucosal fungi can cause post-partum uterine fungal infection. Usually, fungi are not vital in the endometritis of camels or other animals, but they sometimes become crucial (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 & Kirkbride, 1992; Murase et al., 2015; Orellana-Guerrero et al., 2019; Youngquist & Threlfall, 2006). Therefore, vaginal and clitoral fungi may serve as sources of genital tract infections in certain circumstances.

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

Location	No. (%)		
	Culture Results		
	Positive	Negative	Total
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

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

Fungal Species		No. (%)		
		Location		C +V (%)
		C (%)	V (%)	
Yeast fungi	<i>Candida</i> spp.	2(6)	-	2(6)
	<i>A. niger</i>	1(3)	2(6)	2(6)
Filamentous (mold) fungi	<i>A. flavus</i>	2(6)	2(6)	1(3)
Mono-culture	<i>Penicillium</i> spp	2(6)	-	2(6)
	<i>Alternaria Alternaria</i>	2(6)	1(3)	2(6)
Mixed culture	<i>A. niger</i>	3(9)	2(6)	1(3)
	<i>A. flavus</i> <i>Cladosporium</i> spp. <i>A. flavus</i>	-	3(9)	2(6)
Total		12(35)	10(29)	12(35)

C: Clitoris; V: Vagina.

Yeast commonly inhabits the mucosa of the caudal genital tract of camels with the highest load in the vestibulum, and the numbers decrease 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. was cultured from the vaginal mucosa in 3% of the camels and from the clitoris in 15% of camels and, therefore, not as prevalent as expected from the study by Shokri et al. (2010). The presence of filamentous fungi has not been previously reported, but they 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 in the female camel reproduction system, thus

confirming the locally invasive nature of different isolates consistent with previous reports that infection did not disseminate (Moradi et al., 2024. Garoussi et al., 2016). Therefore, this fungal group may demonstrate tropism towards this anatomical site in camels of different ages (Table 3). *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). Therefore, they may spread through the male genital system in camels. *Candida* can localize to mucous membranes and skin. It is distributed worldwide in different animals and is most commonly caused by species of yeast-like fungus, *Candida albicans*, *Candida Krusei*, and others (Garoussi et al., 2016).

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

Culture	No. (%)					Total	
	Age (y) (%)						
	<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)	

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

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

Ethical Considerations

Compliance with ethical guidelines

The Animal Experimentation Ethics Committee of the Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran, approved all protocols and procedures under the recommendations for proper care and use of laboratory animals (Code: 28903. 1.2).

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Authors' contributions

Conceptualization, visualization, and funding acquisition: Massoud Talebkhan Garoussi; Methodology, and software: Hosein Kochakzadeh Omran; Investigation: Mehran Dabiri, and Massoud Talebkhan Garoussi; Validation, and formal analysis: Jørgen Steen Agerholm; Writing the original draft: Hosein Kochakzadeh Omran and Massoud Talebkhan Garoussi; Supervision, review and editing: Massoud Talebkhan Garoussi, and Ali Reza Khosravi.

Conflict of interest

The authors declared no conflict of interest.

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مقاله پژوهشی

بررسی فلور قارچی واژینال و کلیتورال شترهای یک کوهانه

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جیکیده

زمینه مطالعه: باکتری‌ها و قارچ‌ها بخش مهمی از محیط دستگاه تناسلی را تشکیل می‌دهند و در یک پارچگی مخاطی دخیل 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: هدف مطالعه، بررسی شیوع فلور قارچی واژینال و کلیتورال در شترهای یک کوهانه (*Camelus dromedaries*) در مناطق مختلف ایران می‌باشد.

روش کار: نمونه‌ها از مزارع شترهای شیری در استان‌های خراسان رضوی، سمنان، تهران، قم و اصفهان واقع در مرکز و شمال شرق ایران اخذ گردید. تعداد ۲۲۰ نمونه جفتی از مخاط واژن و کلیتورال ۱۱۰ نفر شتر گرفته شد. نمونه‌ها بر روی محیط Sabouraud dextrose agar و CHROMagar™ *Candida* agar و سپس قارچ‌های کشت شده مورد شناسایی قرار گرفت. داده‌های بدست آمده با استفاده از روش آماری Genmod تجزیه و تحلیل شدند.

نتایج: شش جدایه مختلف در این مطالعه اخذ گردید. قارچ از ۳۱ درصد شترها کشت و جداسازی شد. کلاسپوریوم و آسپریلوس فلاوووس شایع‌ترین جدایه‌ها بودند. فراوان ترین نمونه‌های بازیابی شده آسپریلوس بود. حضور قارچ در سنین مختلف شترها مورد ارزیابی قرار گرفت. اما از تاباطی وجود نداشت ($P>0.05$).

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

کلیدواژه‌ها: آسپریلوس فلاوووس، شتر، کلاسپوریوم، قارچ، تولید مثل.

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