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CANDIDA ZEYLANOIDES: THE MAJOR PATHOGENIC *Candida* SPECIES IN GENITAL TRACT OF FEMALE DROMEDARIES

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ABSTRACT

The goals of this study were to isolate *Candida* flora from healthy female dromedary camel's genitalia and to determine the prevalence of different *Candida* species based on mate and age conditions. The specimens were collected from various sites of genital tract including vestibule, vagina, cervix, uterine body and uterine horns of 40 camels. These were grown on Sabouraud dextrose agar and incubated at 30°C for 7-10 days. A total of 332 *Candida* yeasts were obtained from the genital tract. *Candida zeylanoides* (27.7%) was the most prevalent *Candida* species, followed by *C. tropicalis* (19%), *C. krusei* (14.2%), *C. albicans* (12.6%) and *C. rugosa* (12.1%). The mean number of *Candida* isolates found in the vestibule (40.9%) was significantly higher than the results obtained from other parts ($P<0.05$). In addition, the mean value of CFUs from unmated females (68.3%) was significantly higher than mated females ($P<0.05$). The results showed that *C. zeylanoides* was a common component of healthy female dromedary camels' genitalia and the number of *Candida* species varied between mated and unmated females.

Key words: Camel, *Candida* flora, *Candida zeylanoides*, genital tract

Vulvovaginal candidiasis (VVC) is a common fungal infection that affects animals of all ages. Current literature examining the species distribution of *Candida* isolates involved in VVC demonstrated that some of these fungi were potential pathogens such as *C. albicans* (39.1%), *Geotrichum* (8.7%), *Cryptococcus neoformans* (4.3%), and other yeasts (13%) in cows (Garoussi *et al,* 2007), *C. albicans, C. parapsilosis, C. lusitaniae, Cr. rugosa, C. neoformans, Hansenula anomala, H. polymorpha, Rhodotorula minuta, R. rubra*, and *Torulopsis Candida* in horses (Chengappa *et al,* 1984), *C. parapsilosis* (21.7%), *C. guilliermondii* (8.4%), *C. kefyr* (6%), and *C. albicans* (4.8%) in dogs (Cleff *et al,* 2005).

Camelids are important animal production resources in many areas of the world. The reproductive efficiency of camelidae, particularly dromedary camels, is generally considered low (Kaufmann, 2005; Tibary *et al,* 2005). Microbial organisms are responsible for a myriad of diseases that directly or indirectly affect reproductive success in camelidae (Tibary *et al,* 2006) and knowledge of persistent or transient microflora living in the animal's cervicovaginal environment is relevant to a better understanding of these pathological processes.

Microbial infections of the genital tract may lead to temporary or permanent infertility in the female camels as well as abortion and prenatal and neonatal loss in pregnant subjects (El Wishy, 1987; Tibary and Anouassi, 1997). Verma *et al* (1999) showed that different fungi including *Aspergillus, Penicillium, Acremonium, Cladosporium, Mortierella, Aureobasidium pullulans,* Zygomycetes, and *Candida* species could cause genital infections in cows and buffaloes. Vaginitis, endometritis, and cervicitis due to fungal agents have been described in horses (Blue, 1983; Pugh *et al,* 1986) and dogs (Cleff *et al,* 2005). Different fungi were isolated and identified from vaginal flora in lions (Ismar *et al,* 2004). Scarce information on fungal flora of female genitalia and the impact of fungal genital infections have been described in dromedary camels (Wernery, 2000). Fungi may be an endogenous sources of infections, identifying the mycoflora of the normal genital tract will contribute to our understanding of their role in genital infections. The aim of this study was to investigate the *Candida* flora in genitalia of healthy female dromedary camels.

Materials and Methods

Sampling procedure

Samples were collected from genital tract of 40 non-pregnant dromedary camels in different ages (2-15 years old; mean age: 7.9 years old). These animals belonged to 7 farms in arid regions of

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northeastern Iran. These animals had no previous genital infections noted in their files as well as upon physical examinations. Animals with a recent history of disease, or that had been administered local or systemic drugs or antimicrobial agents in the last 6 months were excluded from this study. The females were classified into mated (a history of sexual interaction) and unmated (no history of sexual interaction) female camels.

In slaughter houses the carcasses were immediately opened. Subsequently, the complete genital tract was removed aseptically, ligated with a nylon suture and transferred under cool condition to Mycology Laboratory, Iran during 8-12 hours after euthanasia. Samples were taken from the internal surfaces of vestibule, vagina, cervix, uterine body, and uterine horns using sterilised cotton swabs for fungal cultures. Two swabs were taken from each site. Special care was taken to ensure that the swab did not come into contact with the other body sites. A total of 400 samples from different parts of genital tract were submitted for fungal analysis.

Sample culture and yeast identification

Samples were inoculated onto Sabouraud dextrose agar (*Merck Co., Darmstadt, Germany*) supplemented with antibiotic (chloramphenicol; 0.005%) and kept at 30°C for 7-10 days before being considered negative. Visual examinations of the fungal colonies were made and their characteristics including texture, pigment and rate of growth on medium were recorded. Colonies were examined under a light microscope to determine the morphological structures of the yeasts on slide mounted in lactophenol-cotton blue. Total numbers of yeast colonies were counted in each positive agar plate and the number of CFU per swab were reported as the mean numbers of yeast colonies in different parts of genital tract. The identification of *Candida* species was confirmed by germ tube test, CHROM agar, urease test, sugar fermentation, and assimilation tests by RAP ID yeast plus system (*Remel Inc.,* USA).

Statistics

The chi-square (x^2) test was used to assess statistical differences between the groups. A P value less than 0.05 was statistically considered significant.

Results

Fungal agents were isolated from all animals in this study. The yeasts isolated and the mean numbers of *Candida* species were illustrated in Table 1. A total of 332 *Candida* isolates were obtained from the specimens.

As shown in Table 1, *C. zeylanoides* (27.7%) was the most frequent *Candida* species, indicating significant difference in comparison with other *Candida* species (P<0.05). The other *Candida* species were found to be *C. tropicalis* (19%), *C. krusei* (14.2%), *C. albicans* (12.6%), *C. rugosa* (12.1%), *C. kefyr* (6.3%), *C. parapsilosis* (5.1%), *C. glabrata* (1.5%), *C. stellatoidea (0.9%), C. guilliermondii,* and *C. famata (0.3%).* We observed that *C. zeylanoides* was present in 22 camels, *C. tropicalis* in 15, *C. albicans* in 9, *C. parapsilosis* in 7, *C. krusei* in 6, *C. glabrata* in 4, *C. rugosa and C. stellatoidea* in 2, *C. kefyr, C. guilliermondii,* and *C. famata* in 1.

As shown in Fig 1, *Candida* isolates were recovered from different parts of genital tract including vestibule, vagina, cervix, and uterine body while no growth of any yeast colonies was observed in uterine horns. The mean number of yeast colonies in the vestibule (40.9%) was significantly higher than other parts (P<0.05). The isolation of *Candida* species from unmated females (68.3%) was significantly higher than that from mated females (P<0.05). In addition, the most frequent yeasts isolated were associated with camels higher than 5 years old.

Discussion

About 100 species of fungi were identified as pathogens of human and animals under predisposing conditions such as abnormal susceptibility, prolonged antibiotic treatment, intrauterine therapy, and traumatic implantation (Tibary *et al,* 2005). Reproductive infections are considered to be the most common cause of reproductive failure in camelidae (Tibary, 2004; Kaufmann, 2005). However, there are limited data on the incidence of fungal vaginitis, cervicitis, endometritis and metritis, as well as the pathogenesis and treatment of reproductive infections in camelidae. The resident fungal flora of the female

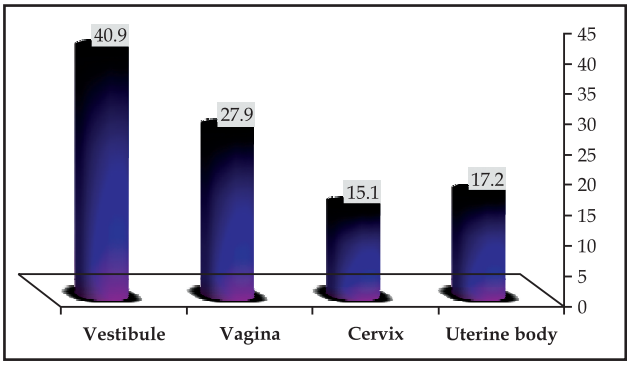

Fig 1. Comparison of the percents of *Candida* species isolated from the different parts of camel's genitalia.

Table 1. Frequency of yeast species isolated from different parts of genital tract of healthy female dromedary camels.

Candida species	Vestibule *No.(%)	Vagina *No.(%)	Cervix *No.(%)	Uterine body *No.(%)	Uterine horns *No.(%)	Total *No.(%)
C. albicans	32(9.6)	1(0.3)	7(2.1)	2(0.6)	0	42(12.6)
C. tropicalis	27(8.1)	8(2.4)	0	28(8.5)	0	63(19)
C. rugosa	5(1.5)	0	8(2.5)	27(8.1)	0	40(12.1)
C. kefyr	0	0	21(6.3)	0	0	21(6.3)
C. zeylanoides	27(8.1)	58(17.6)	7(2.1)	0	0	92(27.7)**
C. krusei	27(8.1)	14(4.3)	6(1.8)	0	0	47(14.2)
C. parapsilosis	11(3.3)	5(1.5)	1(0.3)	0	0	17(5.1)
C. glabrata	1(0.3)	4(1.2)	0	0	0	5(1.5)
C. guilliermondii	0	1(0.3)	0	0	0	1(0.3)
C. famata	0	1(0.3)	0	0	0	1(0.3)
C. stellatoidea	3(0.9)	0	0	0	0	3(0.9)

* No. Mean number of yeast colonies isolated from positive agar plates.
** Significant difference was observed between *C. zeylanoides* and other yeasts isolated (P<0.05).

genital tract is still unknown in dromedary camels and this knowledge would be very useful in assessing the accuracy of treatments.

In this study, a large range of *Candida* flora was isolated from the genital tract specimens, indicating the presence of these organisms in the healthy dromedary camel's genitalia living in arid regions of northeastern Iran.

Our results showed a high prevalence of *Candida* species in genital tract of examined female camels. The predominance of this yeast was expected, since their role as members of female genital flora has been reported as a natural condition of women (Phillips, 2005) and animals such as cow (Garoussi *et al,* 2007), dog (Cleff *et al,* 2005), horse (Chengappa *et al,* 1984), and monkey (Steel *et al,* 1999). Yeasts of genus *Candida* are widely distributed in the environment and frequently colonise skin and mucous membranes such as oral cavity and genital and gastrointestinal tracts of mammalians (Anaissie *et al,* 2003).

Among different *Candida* species, *C. zeylanoides* was the most frequent species isolated from different parts of camel genital canal, in particular in vagina. The isolation of *C. zeylanoides* appears not to have been recorded earlier, although its involvement in fungemia, arthritis, and skin and nail infections in human was well documented (Bisbe *et al,* 1987; Crozier, 2007). The observation in this study differed from those of the other researchers in human and cow that presented *C. albicans* as major yeast flora with frequencies of 85-90% and 39.1%, respectively (Sobel, 1988; Garoussi *et al,* 2007). The explanation for this difference in our population is not clear. However, a study with a larger sample could conclusively establish the possible relationship between *C. zeylanoides* and dromedary camels. This yeast was hypothesised to be the major flora of the genital area. In fact, due to the increased number of isolations and the existence of a relationship among the camels, the real role of it in the normal genital flora of camels was considered to be important in genital mycosis.

Candida isolates were obtained from different parts of genital tract including vestibule (40.9%), vagina (27.9%), cervix (15.1%), and uterine body (17.2%) of healthy non-pregnant camels. The value found in the vestibule was significantly higher than results obtained from other parts (P<0.05). The primary reservoir for infectious agents that colonise into the genital canal is the caudal genital tract including external genitalia such as vulvovestibule, although contamination from faecal matter (pneumovagina, poor perineal conformation, etc.) or due to iatrogenic means (after uterine culture/ cytology/ biopsy or artificial insemination) is also possible. Therefore, it is suggested that uterine body along with vagina and external genitalia should be cultured when fungal infections are suspected or identified.

Another important component of this study was the correlation between the number of *Candida* species and the reproductive status of the animal. The low isolation of yeasts in mated females suggests that their vaginal environment cannot support a complex resident microflora, may be due to the hormonal stimulation, mucous production, leukocyte activity, and pH variation of vaginal environment in oestrous cycle. In conclusion, this study demonstrated that

there were different yeast species in genital tract of female dromedary camels, and *C. zeylanoides* was the most predominant yeast species isolated. The data acquired in this study provide helpful insights into the occurrence of yeast flora in dromedary camel's genitalia and confirm the importance of both clinical examinations and fungal cultures for the diagnosis of reproductive infections.

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