ABSTRACT

Yeasts are commensal organisms found in the skin, genital and gastrointestinal tracts, and other mucosa in mammalians. Candida spp has been frequently isolated from domestic and sylvan animals. Yeast isolation from vaginal mucus is frequent in women, especially of Candida genus. In canine females, however, studies about vaginal fungal microbiota are rare and the connection with estrous cycle is unknown. The purpose of this study was to isolate yeast of Candida genus from healthy canine females and identify the isolated species, establishing their connection with estrous cycle phases. Two hundred twenty-four samples taken from vaginal mucus of 14 canine females were analyzed in this study. Candida spp was observed in 83 (37%) from these samples; nine samples were taken in proestrus, 14 in oestrus, 31 in dioestrus, 24 in anoestrus and five samples were obtained during pregnancy. Candida parapsilosis the most frequently isolated species (21.7%), followed by C. guillermondii (8.4%), C. kefiri (6%) and C. albicans (4.8%). We concluded that Candida spp is a common component of healthy canine females’ vaginal microbiota, and isolation varies according to the estrous cycle phase.

Key words: Candida spp, canine females, estrous cycle

INTRODUCTION

Yeasts of genus Candida are components of the microbiota of healthy beings and are described as causes of opportunistic mycoses around the world. These yeasts are widely distributed in the environment and frequently colonize skin and mucous membranes such as oral cavity and genital and gastrointestinal tracts of mammalians (9,12,20,21,32). In dogs, yeasts belonging to Candida genus prefer constantly humid areas, which favor tissue maceration, as occurs in mucous membranes, mucocutaneous junctions, intertriginous areas, nail substructure inter-fingers areas, ear canal and lateral face of the ear (20,33,36). Physiologic changes like estrous cycle and pregnancy are also considered predisposing factors for Candida spp proliferation (6,10,38).

Hormonal changes that occur during estrous cycle in canine females cause morphological, clinical, cytological and vaginal modifications (8,18,24,25). These changes may influence the development of microorganisms in the vaginal mucus (27). Studies establishing a relationship between the estrous cycle and the presence of Candida spp in vaginal microbiota of dogs are inexistent. The purpose of this study was three fold; to
observe the presence of Candida genus yeasts in vaginal microbiota by healthy canine females; to identify the isolated species; and to verify their possible connection with estrous cycle phases.

MATERIALS AND METHODS

The presence of Candida genus yeasts on vaginal mucus of 14 healthy canine females was analyzed during ten months. The samples were collected in distinct estrous cycle phases. Estrous phases were determined through examination of vaginal smears, together with physical and vaginal examination of the bitches (8,25). Animals without true breed, from one to six years old, previously dewormed, were lodged in a research kennel. The dogs were fed with animal feed (Nhoc Supra, Alisul Alimentos S.A., Fábrica São Leopoldo, RS, Brasil) according to their body weight, and water was offered “ad libitum”. Through the experimental period the animals did not receive any medicament.

Samples from each canine female were taken biweekly to determine estrous cycle phase and for yeast isolation. The samples (n= 217) were obtained with curettes and sterile swabs with the aid of a vaginal speculum. Two pregnant females of the experimental group were collected seven times during the pregnancy and at post-partum. Immediately after collection, the samples (n=224) were streaked, on Sabouraud dextrose agar (Sanval Com. Ind. Ltda., São Paulo, SP, Brasil), and incubated at 37ºC for up to seven days. The yeasts were characterized macro and micromorphologically. The samples obtained from the swabs were submitted to Gram stain and to cytological examination for observation of vaginal epithelium cells morphology and determination of the estrous cycle phase.

Yeast colonies presenting macro and micromorphologic features of Candida genus were classified according to criteria and identifications tests preconized by Ahearn (2) for species determination.

The distribution of Candida spp frequencies in estrous cycle phases was analyzed using Fisher's test through Statistix 7.0 programm (Statistix 2000).

RESULTS AND DISCUSSION

Candida spp were isolated in 24 (21.1%) out of 114 samples. Candida spp were isolated from five (71.4%) out of seven samples collected from 10 pregnant bitches (Table 1). The isolation of Candida spp was more frequent in dioestrus (62%) than in anoestrus (p<0.0001). However, the difference between results obtained for proestrus and oestrus was not significant. Candida spp isolation in oestrus (p=0.0097) and proestrus (p=0.0326) was higher than in anoestrus (Table 1).

From Candida spp isolated from the 83 samples, only forty (48.2%) were characterize to species level. We observed that C. parapsilosis was present in 18 (21.7%) samples, C. guillermondii in seven (8.4%), C. kefir in five (6%), C. albicans in four (4.8%), C. krusei in three (3.6%) and C. glabrata in three (3.6%). C. parapsilosis was isolated in all estrous cycle phases, with higher frequency in dioestrus followed by oestrus; other Candida species were more frequent in dioestrus and anoestrus. Candida spp isolation was lower in proestrus (Table 2).

In this work C. parapsilosis was the most frequent (21.7%) among characterized species (Tab. 2). This species is considered of less pathogenic, but in the last years its importance in immunosupressed human patients has incilased (5,9,16,26). In women, C. albicans is the species that most frequently causes vaginal candidiasis, followed by C. glabrata. C. tropicalis, C. kefir and C. krusei (10,19,38). In this study the frequency of C. albicans was low (8%), which explains the absence of vaginitis caused by Candida in the studied bitches.

The incidence of Candida spp (37%) (Table 1) in the vaginal cavity of healthy canine females was similar to that reposted in the same anatomic site for healthy women (20 to 48%) (1,23,31,38), while higher frequencies were mentioned in vaginitis cases (6,10,38). Despite isolation of Candida from sites like ear canal (3,7), skin (14,30) and nasal, oral and anal mucus of canine females (13), the literature does not mention vaginitis caused by Candida cited in bitches, nor presence of Candida in the vaginal mucus of healthy animals.

Table 1. Candida spp isolation from 224 vaginal cavity samples from 14 canine females in distinct estrous cycle phases* (Researchskennel from Veterinary Hospital, Federal University of Pelotas, RS, 2003).

<p>| Table 1. Candida spp isolation from 224 vaginal cavity samples from 14 canine females in different estrous cycle phases* (Researchskennel from Veterinary Hospital, Federal University of Pelotas, RS, 2003). |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Estrous Cycle Phases</th>
<th>Proestrus n (%)</th>
<th>Oestrus n (%)</th>
<th>Dioestrus n (%)</th>
<th>Anoestrus n (%)</th>
<th>Pregnancy n (%)</th>
<th>Total n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>9 (42.9)%</td>
<td>14 (63.8)%</td>
<td>31 (62)%</td>
<td>24 (21.1)%</td>
<td>5 (71.4)%</td>
<td>83 (37)%</td>
</tr>
<tr>
<td>Negative</td>
<td>12 (57.1)%</td>
<td>18 (56.2)%</td>
<td>19 (38)%</td>
<td>90 (78.9)%</td>
<td>2 (28.6)%</td>
<td>141 (63)%</td>
</tr>
<tr>
<td>Total</td>
<td>21 (100%)</td>
<td>32 (100%)</td>
<td>50 (100%)</td>
<td>114 (100%)</td>
<td>7 (100%)</td>
<td>224 (100%)</td>
</tr>
</tbody>
</table>

n= absolute number; (%)= percentage; *p<0.05; a, b different exponents at the same line vary on minimum p< 0.05.
The high incidence of *Candida* spp during dioestrus (62%) may be linked to high progesterone levels in this phase (17) *Candida*-specific cell-mediated immunity, acquired by exposure to *Candida* as a commensal early in life, has been considered the predominant host defense mechanism against mucosal *Candida* infections (11), acting as cellular immunity suppressor, and promoting the expression of a gene that favours synthesis of an epithelial receptor able to bind certain fungi (38). Studies suggest that estrogen, but not progesterone, is important in the reproductive-hormone-associated susceptibility to vaginal *C. albicans* infection (10,11). The high estrogen levels in proestrus (18,28,35) can account for *Candida* isolation in this phase. Fungi development is enhanced by this hormone which increases glycoproteic epithelial complexes exposition acting as receptors for fungi agents (38). Estrogen or also stimulate glycogen collection (15), and the decrease of the vaginal pH due to its metabolization to lactic acid supports to lactobacillus and yeasts growth (22,27). Immunoglobulin A (IgA) and IgG are the predominant Ig classes found in vaginal washes, suggesting that they represent the dominant Ig classes in the female genital tract (29). Decrease of these immunoglobulins in proestrus due to high estrogen levels is also considered as a factor that could influence yeast growth (37). Sex steroid hormones regulate the movement of mucosal IgA antibody and its transporter, polymeric immunoglobulin receptor, from uterine tissues into secretions (34). Biological models demonstrated that estrogen and progesterone levels are high in oestrus, increasing uterine and reducing vaginal immune function, what could justify *Candida* spp isolation in this phase (37). Basal hormones concentration (4,8,35), and alkaline vaginal pH are adverse conditions to fungi development (27) and could, probably, account for little *Candida* spp isolation in anoestrus.

Yeast positive vaginal cavity samples obtained from two pregnant bitches are, possibly, associated with immune suppression that occurs in the female reproductive tract at the time of implantation and throughout pregnancy (37). However, the low number of pregnant animals in this study does not allow us to conclude that yeasts are normally present in vaginal cavity in pregnant bitches. In spite of differences on reproductive cycles, the frequencies obtained for *Candida* genus yeasts in pregnant canine females (71.4%) were similar to those of pregnant women (68%)(1).

### CONCLUSIONS

Our results indicate that *Candida* spp yeasts are component parts of vaginal microbiota of healthy canine females. The isolation frequency was higher in dioestrus and lower in anoestrus. The isolated species were: *C. parapsilosis, C. guillermondii, C. kefri, C. albicans, C. glabrata* and *C. krusei*, with *C. parapsilosis* being the most frequent. The species isolated in pregnant bitches were *C. albicans, C. guillermondii* and *C. parapsilosis*.

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### RESUMO

**Isolamento de Candida spp da microbiota vaginal de fêmeas caninas hígidas durante o ciclo estral**

As leveduras são organismos comensais da pele, trato genital e gastrointestinal, e de outras mucosas de mamíferos. O gênero *Candida* vem sendo isolado frequentemente de animais domésticos e silvestres. O isolamento de *Candida* spp da mucosa vaginal de mulheres é frequente, porém na espécie canina são...
escassos os estudos referentes à microbiota fúngica vaginal, especialmente do gênero *candida*, não se tendo conhecimento de sua relação com o ciclo reprodutivo. O presente trabalho teve como objetivo isolar leveduras do gênero *Candida* em fêmeas caninas hígidas e identificar as espécies isoladas, relacionando-as com as diferentes fases do ciclo estral. Foram analisadas 224 amostras obtidas da mucosa vaginal de 14 fêmeas caninas. *Candida* spp foi observada em 83 (37%) amostras, destas, nove (42,9%) foram obtidas no proestro, 14 (43,8%) no estro, 31 (62%) no diestro, 24 (21,1%) no anestro e cinco (71,4%) de gestantes. Nas amostras caracterizadas, *C. parapsilosis* foi a espécie isolada com maior frequência (21,7%), seguida de *C. guillermondii* (8,4%), *C. kefir* (6%) e *C. albicans* (4,8%). Com base neste estudo pode-se concluir que *Candida* spp faz parte da microbiota vaginal de fêmeas caninas hígidas, e que o isolamento é influenciado pelo ciclo reprodutivo.

**Palavras-chave:** *Candida* spp, fêmeas caninas, ciclo estral

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