

Research Paper

Clinical and mycological analysis of dog's oral cavity

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Abstract

The oral microbiota of humans and animals is made up of a wide variety of yeasts and bacteria, but microbiota of dogs is not totally described. Although such identification is an important step to establish the etiopathogenesis and adequate therapy for the periodontal disease. The aim of this study was to evaluate and correlate oral alterations with the presence of yeasts in oral cavity of female dogs. After clinical evaluation samples from healthy and from dogs with oral diseases were obtained from three different oral sites by swabs, curettes, millimeter periodontal probes and HA membrane tip in cellulose ester. Yeast identification was performed through macroscopic and microscopic colony features and biochemical tests. Dental calculus was the most prevalent occurrence in the oral cavity of 59 females. However, the isolation of yeasts was significantly higher ($p < 0.05$) in animals suffering from halitosis. Eleven yeast species were identified, namely: *Malassezia pachydermatis*, *Rhodotorula* spp., *Candida albicans*, *C. catenulata*, *C. famata*, *C. guilliermondii*, *C. parapsilosis*, *C. intermedia*, *Trichosporon asahii*, *T. mucoides* and *Cryptococcus albidus*. It could be concluded that the yeasts are part of the microbiota from the different sites of the oral cavity of the female canines studied without causing any significant alterations except halitosis.

Key words: oral microbiota. yeasts. *M. pachydermatis*. *Candida* spp.. halitosis.

Introduction

Trabulsi and Sampaio (2005) observed that the oral cavity microbiota of humans and animals is made up of a wide variety of yeasts and bacteria. As these microorganisms can be responsible for different oral affections and may as well be involved in systemic diseases, they identification has a fundamental importance in human dentistry and medicine. Braga *et al.* (2005) reported the identification of the major microorganisms with a pathogenic poten-

tial in the oral cavity of animals also plays an important clinical role such as to provide support towards adequate diagnosis and therapy in veterinary medicine.

Cleff *et al.* (2007) mentioned that most yeasts mainly those of the *Candida* genus, make up the microbiota of healthy beings and are known to cause opportunistic mycoses all over the world. In dogs, yeasts preferably colonize moist areas such as mucous membranes, mucocutaneous junctions, skinfolds, interdigital areas and ears.

Few studies on yeast-like microbiota and the pathogenic potential of these agents in the oral cavity of dogs are available in the literature. Thus, the aim of this study was to evaluate and correlate oral alterations with yeasts isolation from different sites of canine oral cavity.

Materials and Methods

In order to do this study, 59 mongrel female canines of varying ages were used. The animals were referred to the Hospital de Clínicas Veterinária of the Universidade Federal de Pelotas (HCV-UFPel) for selective ovariectomy. After their discharged from the surgical center, animals were randomly selected and all females were duly identified in medical records. A clinical examination and sample collecting for fungal culture from different sites in the oral cavity of the animals were done.

The clinical examination of the oral cavity was performed in anesthetized patients in the immediate postoperative period, previously to antimicrobial administration. The following variables were analyzed: dental calculus, dental fracture, malocclusion, halitosis and gingival bleeding occurrence. Then the periodontal sulcus was measured by the introduction of a millimeter periodontal (ABC Instrumentos Cirúrgicos LTDA, São Paulo, Brazil) probe through the buccal surface of the 4th right upper premolar (4th RUPM) and the upper left canine (ULC) of each animal.

Oral cavity samples were obtained from different sites, such as gingival mucosa, dental biofilm and periodontal sulcus. Material collecting from the gingival mucosa was done by sterile swab friction and scraping by a sterile curette, while the dental biofilm samples from the upper canine (left or right, randomly) were collected by sterile curette friction. In the periodontal sulcus collecting, two samples from both the 4th RUPM and ULC teeth were obtained by a sterile millimeter periodontal probe and membrane tip (Millipore, Barueri, São Paulo, Brazil) which were introduced in the respective dental sulcus.

All samples were immediately cultivated on Sabouraud dextrose Agar (Acumedia, Lansing, Michigan, USA) added with chloramphenicol and olive oil, incubated at 36 °C for up to a seven-day period, and accompanied daily. Edge, topography, color and texture were the macroscopic features evaluated from yeast-like colonies. Micromorphological identification was done by the Gram technique, followed by 100X objectives immersion visualization.

Another tests as growth in culture medium without the addition of long chain fatty acids to prove lipodependence, urease and catalase tests, as well as germ tube production in horse serum were also performed. Final identification, except for the *Malassezia* and *Rhodotorula* genera, was done by the ID 32C standardized system, bioMérieux (ATB Expression[®], Marcy-l'Etoile, France) for yeasts.

Frequency analysis was performed by the Chi-Square test using the Statistix 8.0 software.

Results

In the oral cavity examination of 59 female dogs, dental calculus (77.97%) was the most prevalent alteration found, followed by dental fractures (30.51%), malocclusion (23.73%) and gingival bleeding (11.86%). A significant relation among these alterations and yeast isolation was not found; however, yeast isolation in halitosis-bearing animals (85.7%) was significantly higher ($p < 0.05$) than that in animals without halitosis (46.2%).

The sulcus mean depth in the evaluated animals was 2 mm; only two 4th RUPM sulcus (3.39%) and one ULC sulcus (1.69%) had a depth over 3 mm, and there was no yeast isolation in these cases.

Yeasts were isolated from 30 (50.85%) animals. There was positive animal variation according to technique and site of collecting. Also, there was variation in number of isolates per animal: in 22 (37.29%) only one microorganism was found and in eight (13.56%), two or three yeasts were found.

Sixty-one yeasts isolates from different sites of oral cavity belonging to the *Malassezia* (50.8%), *Candida* (14.7%), *Trichosporon* (14.7%), *Rhodotorula* (13.1%) and *Cryptococcus* (6.6%) genera were found. From these five genus, 11 species were identified, and the most frequently isolated yeast was *M. pachydermatis*, which was also the only yeast present in all studied sites. From the *Candida* genus, *C. albicans*, *C. catenulata*, *C. famata*, *C. guilliermondii*, *C. parapsilosis* and *C. intermedia* were the species identified; however, there was no growth of this genus in the biofilm and periodontal sulcus samples. Other yeasts such as *Rhodotorula* spp., *T. asahii*, *T. mucoides* and *Cryptococcus albidus* were isolated. *C. albidus* was isolated from the four different collecting procedures, but from only one animal. Fungal growth was obtained in all examined sites (Table 1).

Discussion

According to Braga *et al.* (2005), the knowledge of infectious agents in the oral cavity and the relation of these agents to oral diseases have been studied both in human medicine and dentistry. Nevertheless, in veterinary medicine, minor informations are known about microbiota microorganisms and their relation to periodontal disease, which responds for the greatest number of veterinary care.

Roza (2004) studied the high prevalence of yeast isolation in halitosis-bearing animals founded in our study suggests a relation among yeast presence and a decrease in oral health, once halitosis is one of the alterations found in small animal extra-oral examinations when this cavity is illness-stricken.

Table 1 - Yeasts isolates in different sites and with distinct collecting techniques from 413 samples of females dogs oral cavities.

Yeast	Gingival mucosa (swab/curette)n (%)	Biofilm (%)	4th RUPM ^a n (%)	ULCP ^b n (%)	4th PMT ^c n (%)	ULCT ^d n (%)	Total n (%)
<i>M.pachydermatis</i>	17(27.87)	9(14.75)	1(1.64)	1(1.64)	1(1.64)	2(3.28)	31(50.82)
<i>C. albicans</i>	1(1.64)	-	-	-	2(3.28)	-	3(4.92)
<i>C. catenulata</i>	-	-	1(1.64)	-	1(1.64)	-	2(3.28)
<i>C. famata</i>	1(1.64)	-	-	-	-	-	1(1.64)
<i>C. guilliermondii</i>	1(1.64)	-	-	-	-	-	1(1.64)
<i>C. parapsilosis</i>	1(1.64)	-	-	-	-	-	1(1.64)
<i>C. intermedia</i>	-	-	1(1.64)	-	-	-	1(1.64)
<i>Rhodotorula</i> spp.	3(4.92)	1(1.64)	1(1.64)	1(1.64)	2(3.28)	-	8(13.11)
<i>T. asahii</i>	1(1.64)	2(3.28)	-	3(4.92)	-	2(3.28)	8(13.11)
<i>T. mucoides</i>	-	-	-	-	-	1(1.64)	1(1.64)
<i>C. albidus</i>	1(1.64)	1(1.64)	1(1.64)	-	1(1.64)	-	4(6.56)
Total	26(42.62)	13(21.31)	5(8.2)	5(8.2)	7(11.47)	5(8.2)	61(100)

^acollected from 4th upper premolar by a periodontal sulcus probe. ^bcollected from left upper canine by a periodontal sulcus probe. ^ccollected from the periodontal sulcus of the 4th upper premolar by HA membrane tip in cellulose ester. ^dcollected from the periodontal sulcus of the upper left canine by HA membrane tip in cellulose ester.

The sulcus average depth found in this study suggests that all animals had a healthy periodontium, presenting a sulcus depth within the normality range for dogs which correspond to 3 mm (Roza 2004; Mitchell 2005).

In this study, eleven different yeast species were isolated, whereas Brito *et al.* (2009) identified only four yeast species in the oral mucosa of dogs, including *M. pachydermatis* (n = 69), *C. parapsilosis* (n = 4), *C. tropicalis* (n = 1) and *S. cerevisiae* (n = 1). Ours results are similar when only *M. pachydermatis* is considered. This yeast was isolated from all analyzed sites and in the different collection procedures (50.82%), being the most frequently isolated yeast in the oral mucosa. Several authors have described *M. pachydermatis* as being part of the oral microbiota (Bond and Lloyd, 1997; Bond *et al.*, 2000; Brito *et al.*, 2009). However, Pinter and Noble (1998) mentioned a relation among oral diseases in dogs and this yeast in a stomatitis, pharyngitis and tonsillitis case. *M. pachydermatis* is the most widely studied yeast species in animals, and it is thought to make part of the microbiota of various anatomical sites, mainly the external ear and skin (Nobre *et al.*, 1998; Bond *et al.*, 2000; Cleff *et al.*, 2005). However, Nobre *et al.* (1998) reported that at an imbalance condition between host and microorganisms occurs, this yeast can turn pathogenic due to a cell number increase referred to a predisposing factor.

The *Candida* genus, which represented 14.75% of the isolates in this study, is also considered to be part of the microbiota of animals (Ferreiro *et al.*, 2002; Brito *et al.*, 2009) and humans (Farah *et al.*, 2000). *C. albicans*, isolated in three samples (4.92%) is the main oral candidiasis pathogen in humans. In addition, other species such as *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, *C. krusei*, *C. lusitaniae*, *C. pseudotropicalis*, *C. guilliermondii* and *C. stellatoidea* can also be involved (Farah *et al.*, 2000). However, the low occurrence of *C. albicans* in this study shows that there are probably differences among oral human and canine yeast-like microbiota, as described by Hayashi *et al.* (2008). Stomatitis caused by *C. albicans* have been reported in four dogs (Jadhav and Pal, 2006); this was also the only species isolated from the oral mucosa of cats without oral lesions (Ferreiro *et al.*, 2002). However, in recent studies, such as those by Hayashi *et al.* (2008) and Brito *et al.* (2009), this pathogen was not isolated from the oral mucosa of dogs. The specie *C. parapsilosis* was described by Brito *et al.* (2009) and also isolated in our study.

Species like *C. catenulata*, *C. famata* and *C. intermedia* have not been described yet as part of the oral microbiota of dogs. Radosavljevic *et al.* (1999) reported that this is a very important data since these yeasts have a pathogenic potential to develop systemic infections, especially in immunosuppressed patients.

Yeast isolation in the oral cavity of dogs suggests that these yeasts may be involved in oral pathologies, including dental biofilm formation. This information is supported by

studies that demonstrated the ability of *Candida* species to the adherence and biofilm production (Gasparetto *et al.*, 2005; López-Ribot 2005). In addition, a study by Canizzo *et al.* (2007) demonstrated the similar ability of *M. pachydermatis* isolated from dog ears to produce biofilm in materials such as polystyrene and polyurethane, which increase the survival rate of these yeasts.

The *Rhodotorula* genus identified in eight (13.11%) isolates has already been described as an opportunistic agent, and is thought to be an emerging pathogen (Navarro *et al.*, 2001). *Rhodotorula* spp. has already been isolated in animals as part of the microbiota of the external ear, oral cavity of dogs and the vaginal mucosa of healthy cats and canine females (Bornand 1992; Amaral *et al.*, 1998; Braga *et al.*, 2005; Cleff *et al.*, 2007). However, this yeast was never associated with oral diseases nor in animals neither in humans.

In this study, *T. asahii* was isolated from eight (13.11%) oral cavity samples. Shareef *et al.* (2008) described a human glossitis case associated to this yeast. The *Trichosporon* genus has also been isolated from the bronchoalveolar lavage of healthy dogs (Melchert *et al.*, 2008) and from the external ear of healthy cats (Amaral *et al.*, 1998). Trichosporosis cases have been reported in cats (Greene *et al.*, 1985; Doster *et al.*, 1987).

From the *Cryptococcus* genus, *C. neoformans* is the main pathogenic specie (Mitchell and Perfect, 1995). In this study, *C. albidus* was isolated from all analyzed sites in one healthy animal (3.22%) only, which demonstrates that this yeast was probably present in these sites transiently. This species is encapsulated and thought to be saprophytic, but it is rarely associated to clinical cases, although it has been isolated in system infection cases and pyelonephritis in dogs (Newman *et al.*, 2003; Labrecque *et al.*, 2005; Kano *et al.*, 2008).

Malassezia pachydermatis, *Candida* spp., *Rhodotorula* spp. and *Trichosporon asahii* yeasts make part of the microbiota in the different sites of the oral cavity of the females dogs studied, being present without causing alterations. Yeast isolation was significantly more frequent in halitosis-bearing animals, which suggest some role of these microorganisms in the decrease of oral health.

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