



Canine mycotic stomatitis due to *Candida albicans*

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Summary *Candida albicans*, a medically important opportunistic yeast is described as the etiologic agent of stomatitis in dogs. The oral swabs collected from 34 dogs showing symptoms of stomatitis or gingivitis such as anorexia, halitosis, bleeding within the oral cavity, dysphagia, ptyalism (salivation) and submandibular lymphadenopathy were cultured for isolation of the causative agent. *C. albicans* was isolated from four (11.8%) dogs. The isolates were sensitive to clotrimazole, fluconazole and amphotericin-B but were resistant to nystatin. The routine application of Pal's sunflower seed medium and Narayan stain in microbiological laboratories is highly emphasized. It is recommended that the role of *C. albicans*, as the etiologic agent of canine stomatitis, should be carefully investigated in various clinical related disorders of dogs as well as in other animals.

Key words *Candida albicans*, Dog, Narayan stain, Pal's medium, Stomatitis

Estomatitis en perros por *Candida albicans*

Resumen *Candida albicans* es una levadura oportunista implicada en la estomatitis en perros. Las muestras orales de 34 perros con síntomas de estomatitis o gingivitis, tales como anorexia, halitosis, sangrado de la cavidad oral, disfagia, ptilismo y adenopatía submandibular, se cultivaron para aislar el agente causal. Se aisló *C. albicans* de cuatro perros (11,8%). Los aislamientos fueron sensibles a clotrimazol, fluconazol y anfotericina B, y resistentes a nistatina. Recomendamos la aplicación rutinaria en los laboratorios de microbiología del medio de semillas de girasol de Pal y de la tinción de Narayan e investigar el papel de *C. albicans* en diversas patologías orales en perros y otros animales.

Palabras clave *Candida albicans*, Estomatitis, Medio de Pal, Perro, Tinción de Narayan

Candidosis (candidiasis, thrush), a worldwide fungal disease of man and animals including the birds, is mainly caused by *Candida albicans*, although other species have been also increasingly reported. The organism occurs as a normal microbiota in human and animals. Most *Candida* infections are endogenous in origin [5]. *Candida* spp may overgrow in immunosuppression or prolonged broad spectrum antibacterial antibiotic therapy. Mucocutaneous candidosis is usually manifested as a nonhealing ulcer covered by a whitish gray plaque in the oral cavity (thrush), gastrointestinal tract or on the genitourinary mucosa [8].

Little information on canine mycotic stomatitis is available in India. Thus, this study investigates the role of *C. albicans* in cases of canine stomatitis at the Out Patient Department of University Clinic, Anand, India. In addition, the efficacy of Pal's medium and Narayan stain for the study of *C. albicans* is also addressed.

The sterile swabs in triplicate were used to collect the clinical material from the oral lesions of thirty-four dogs showing clinical signs of gingivitis and stomatitis such as anorexia, halitosis, bleeding within the oral cavity, dysphagia, salivation and submandibular lymphadenopathy. One swab of each collected sample was used to prepare wet mounts by using lactophenol cotton blue stain and smears. Smears were stained with Gram's and Giemsa stain. The other swabs were used for inoculation onto blood agar plates, nutrient agar, Sabouraud dextrose agar with chloramphenicol (SDA plates) and Pal's sunflower seed agar in duplicates [5]. The former media were incubated at 37 °C while Pal's agar was kept at 25 °C for one week. These plates were daily examined for fungal growth. The growth was subcultured on Sabouraud medium for detailed study. The morphology of yeast isolates were studied in the PHOL (Pal, Hasegawa, Ono and Lee) stain [5], lactophenol cotton blue and Narayan stain [4]. Also these isolates were subjected to biochemical tests for species confirmation.

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The *in-vitro* drug susceptibility test was conducted by disc diffusion method described by May et al. [1]. The discs of four antifungal drugs (Hi-Media, India) viz. amphotericin-B (100 units/disc), clotrimazole (10 µg/disc), fluconazole (10 µg/disc) and nystatin (100 units/disc) were used for this test.

The oral mucous membrane of the affected dogs was often erythematous, friable, ulcerated, necrotic, proliferative or covered by pseudomembrane. Out of thirty four samples subjected to mycological examination, *C. albicans* was isolated from four patients (11.76%). The direct microscopy of stained smears revealed numerous pseudohyphae and yeast cells morphologically indistinguishable with *Candida* spp. The organism grew in pure and luxuriant form on Pal sunflower seed medium and SDA after three to four days of incubation at 25 °C and 37 °C, respectively. All the isolates of *C. albicans* failed to produce a hyphal fringe on Pal's agar and thereby it was differentiated from *C. dubliniensis* [3]. These isolates when observed under microscope in the PHOL, lactophenol cotton blue and Narayan stained mounts revealed numerous pseudohyphae and yeast cells.

The results of *in-vitro* disc diffusion test showed that the isolates of *C. albicans* were sensitive to clotrimazole, fluconazole and amphotericin-B but were resistant to nystatin. The owners of two examined dogs were prescribed with topical application of 1% clotrimazole, but there was no feedback from them. Therefore the efficiency of the clotrimazole was evaluated only in two other dogs. The daily application of 1% clotrimazole on the lesions of the selected dogs for 15 days showed good clinical response as the ulcers healed, excessive salivation stopped, appetite improved and bad breath vanished. Mycological examination of the oral swabs after the chemotherapy was not attempted.

Since the yeast is known to occur as a normal microbiota in mouth, gastrointestinal tract, vagina and on the skin of healthy humans and animals, the isolation alone of the fungus from clinical samples cannot conclusively confirm the diagnosis of candidosis. Hence, emphasis should be given on the direct demonstration of the pathogen in the clinical material and its repeated isolation from the specimen [6]. Both of these criteria were followed in every case of positive *C. albicans*. The findings in this study are comparable with that of McKeever and Klausner [2] who diagnosed candidal stomatitis in 4 dogs which had plaques on the oral mucous membrane and showed symptoms of halitosis (bad breath) and excessive salivation. Similar observations on oral candidosis due to *C. albicans* in puppies with mouth lesions were also reported by Refai et al. [7].

The clinical findings of the investigated patients, absence of any other pathogen, direct demonstration of the pathogen in smears and its isolation as a pure and heavy growth and good response with 1% clotrimazole indicated that *C. albicans* was more likely incriminated as the etiology of stomatitis in the dogs. The good growth on Pal's sunflower seed medium confirmed that this medium could be routinely used in microbiology laboratories for the *in-vitro* culture of yeast pathogens. The growing role of this pathogenic yeast should be investigated in the clinical disorders of dog accompanied by symptoms of stomatitis.

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