

Human and domestic animal infections caused by *Candida albicans*

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A study covering 317 patients (154 men, 69 dogs, 21 buffaloes, 63 cattle, 6 horses and 4 goats) of different age groups, clinically suspected to have fungal infections, was undertaken to investigate mycologically, the role of *Candida albicans*, an opportunistic polymorphic yeast. The various clinical disorders encountered during the study period in man were otomycosis (39), dermatitis (34), stomatitis (28), onychomycosis (18), vulvovaginitis (15), urinary tract infection (16) and respiratory tract infection (4); while in animals were clinical and subclinical mastitis (55), stomatitis (36), dermatitis (49) and otitis (23). *C. albicans* was diagnosed as the sole pathogen in 31 patients (20 men, 7 dogs, 2 buffaloes, 2 cattle). The organism was repeatedly demonstrated in the clinical specimen both by direct microscopy as well as cultural isolation. This appears to be the first record from India, which delineates the occurrence and etiologic significance of *C. albicans* in stomatitis of buffaloes. It is recommended that the role of *C. albicans* should be prudently investigated in the various clinical disorders of men and domestic animals.

Key words: Clinical disorders, opportunistic yeast, Narayan stain, Pal sunflower seed medium

INTRODUCTION

The high mortality rate associated with bacterial infections has declined with early administration of empirical antibiotics, while fungal infections have become increasingly important in causing morbidity and mortality in immunocompromised patients (Fidel *et al.*, 1999). Mycotic infections as such are worldwide but are more prevalent in tropical and subtropical countries. India is a fairly large sub-continent with a remarkably varied topography, situated within tropical and subtropical belt of the world, which incidentally are the regions incriminated with high incidence of mycotic infections (Rao, 1962). Candidosis (Candidiasis, Thrush), the

commonest global fungal disease of men and animals including birds is principally caused by *Candida albicans* with varied clinical manifestations. The organism occurs as a commensal in human and animals (Pal, 1997). Though it is not the basic etiologic agent, its presence in majority of cases has some significance. Further, the colonization rate of *C. albicans* increases with severity of illness and duration of hospitalization (Odds, 1988). Under certain conditions, however, the saprophytic existence of this organism changes to that of pathogen with associated development of clinical *Candida* infection (Jean-Marcel, 1997). Work has been done in India, with reports on the incidence of different *Candida* species prevalent in various host species, but these are in isolated units. A due consideration to all the abovementioned facts prompted the authors to investigate the role of *C.*

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albicans in various clinical disorders of men as well as domestic animals at Anand, India.

MATERIALS AND METHODS

A total of 317 aseptically collected clinical samples from animal and human subjects, which were suspected to have fungal infections, constituted the materials for this study. The samples of animal origin were procured from Veterinary Polyclinic and Livestock Research Station of Anand Agriculture University as well as Municipal Veterinary Polyclinic at Anand while samples from human patients were procured from District Civil Hospital, Private General Hospitals, E.N.T. clinics, Gynecological clinic and pathological laboratory. The name, age, sex, address, occupation, symptoms, history, predisposing factors etc. were recorded for each patient on the proforma designed for this study. The samples were in the form of swabs, scrapings, biopsies, sputum, hard wax, tracheal exudates, urine and milk as appropriate to the clinical feature of infection. The distribution of these samples in various disorders of men and animals is shown in Table 1.

thoroughly homogenized biopsy samples and then stained with Gram's and Giemsa stain. A part of the skin scrapings was used for direct microscopy in 10 per cent potassium hydroxide. All the milk samples were centrifuged at 12,000 rpm at 4°C for 10 min. Smears were prepared from the sediment and stained with Gram's and crystal violet stain (Simaria and Dholakia, 1986). The remaining material of each sample was liberally inoculated on to the slants and plates of Sabouraud dextrose agar with chloramphenicol (SDA) and Pal sunflower seed agar (PSA) in duplicates (Pal, 1997). The former medium was incubated at 37°C while PSA plates and slants were kept at room temperature for one week. These media were daily examined for fungal growth. The growth was sub-cultured on SDA slants for detailed study. The morphology of yeast isolates was studied in the PHOL stain (Pal, 1997) and Narayan stain which contained 4.0 ml of glycerine, 0.5 ml of 3 per cent methylene blue and 7.0 ml of dimethyl sulphoxide (Pal, 2001). The chlamydospore production (Chlamydospore Agar, HI-MEDIA[®]), germ tube formation, pH tolerance, cultural morphology on PSA

Table 1 : Distribution of clinical samples according to various disorders of man and domestic animals.

Clinical disorder	Type of sample	Number of specimens						Total
		Man	Dog	Buffalo	Cattle	Horse	Goat	
Otitis	Ear swabs	34	16	5	2	--	--	62
	Hard wax	5	--	--	--	--	--	
Stomatitis	Oral swab	14	31	5	--	--	--	64
	Sputum	8	--	--	--	--	--	
	Biopsies	6	--	--	--	--	--	
Dermatitis	Skin scrapings	26	15	4	6	6	4	83
	Biopsies	6	1	--	--	--	--	
	Pus swabs	2	6	3	4	--	--	
Onychomycosis	Nail scrapings	18	--	--	--	--	--	18
Vulvovaginitis	Vaginal swab	15	--	--	--	--	--	15
Mastitis	Milk	--	--	4	51	--	--	55
Urinary Tract Infection	Urine	16	--	--	--	--	--	16
Respiratory Tract Infection	Trachea exudate	4	--	--	--	--	--	4
Total		154	69	21	63	6	4	317

*Forty eight milk samples were from cows with apparently normal udder and 3 from cows with clinical mastitis.

Except milk, all other clinical samples were processed and examined mycologically by the various methods elaborated by Al-Doory (1980). Each sample was divided into two parts, one of which was used for microscopical examination and the other for cultural examination. For microscopical examination, smears were made from swabs, sputum, hard wax, tracheal exudates, urine as well as

and sucrose assimilation tests were performed on the isolates.

RESULTS

Out of 317 patients suspected with fungal infections investigated, only 31 (9.77 per cent) were positive for *C. albicans*. The details of cultural

Table 2 : Cultural isolation of *Candida albicans* from various disorders of man and domestic animals.

Clinical disorder	Cultural isolation of <i>C. albicans</i>						Total
	Man	Dog	Buffalo	Cattle	Horse	Goat	
Otitis	2 (5.13%)	1 (6.25%)	--	--	--	--	2 (3.22%)
Stomatitis	5 (17.85%)	4 (12.90%)	1 (20.0%)	--	--	--	10 (15.63%)
Dermatitis	2 (8.00%)	2 (9.09%)	--	--	--	--	4 (5.41%)
Onychomycosis	2 (11.11%)	--	--	--	--	--	2 (11.11%)
Vulvovaginitis	7 (46.67%)	--	--	--	--	--	7 (46.67%)
Mastitis	--	--	1 (25.00%)	2 (3.92%)	--	--	3 (5.45%)
UTI	1 (6.25%)	--	--	--	--	--	1 (6.25%)
RTI	1 (25.01%)	--	--	--	--	--	1 (25.01%)
Total	20 (13.60%)	7 (10.14%)	2 (9.52%)	2 (3.17%)	--	--	31 (9.72%)

lation are given in Table 2. The direct microscopy of stained smears revealed numerous pseudohyphae and yeast cells morphologically indistinguishable from *Candida* organism (Fig. 1 and Fig. 2). All the isolates grew in pure and luxuriant form after 3 to 4 days of incubation, producing whitish-creamy, pasty, smooth and slightly larger colonies with yeasty odour on the plates of both SDA and PSA. These isolates failed to produce a hyphal fringe on PSA and thereby differentiated from *C. dubliniensis* (Al-Mosaid *et al.*, 2003).

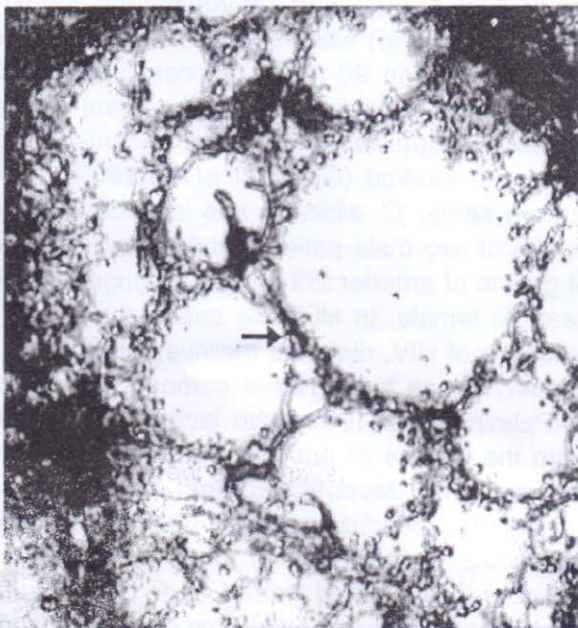


Fig. 1 : Photomicrograph of *C. albicans* in the sediment smear of a sample from a 5-year-old H.F. crossbred cow with subclinical stitis. Gram's stain (X 960).

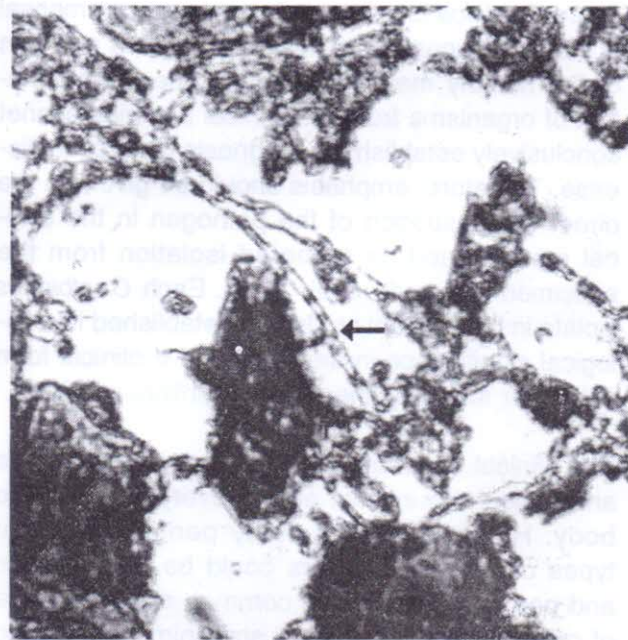


Fig. 2 : Photomicrograph of *C. albicans* in the vaginal smear of a 37-year-old woman with vulvovaginitis showing pseudohyphae, yeast cells and necrosed epithelial cells. Gram's stain (X 960).

Wet mounts of each isolate in PHOL and Narayan stain revealed numerous pseudohyphae and yeast cells. The isolates showed thick walled chlamydospores, germ tube formation, grew well with pH 1.55 and assimilated sucrose.

DISCUSSION

In recent years, opportunistic fungal infections are gaining a greater significance in human medicine as a result of possibly large number of

immunocompromised patients (Pal *et al.*, 1990). However, such fungi may also produce infection in immunocompetent host (Pal and Torres-Rodriguez, 1990). In these opportunistic infections, due to microorganisms from endogenous or exogenous sources, an increasing diversity of opportunistic yeasts is implicated. The yeasts most commonly isolated from clinical specimens, in decreasing order of occurrence, include *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *Saccharomyces* spp., *C. krusei*, *C. guilliermondii*, *Rhodotorula* spp., *Trichosporon* spp. and *Cryptococcus neoformans* (Crist *et al.*, 1996). However, *C. albicans* remains the most common species isolated, representing about 45.8 and 60 per cent of all clinical isolates of yeasts (Berg *et al.*, 1984; Grillot, 1996).

Since *C. albicans* is known to occur as commensal in the mouth, gastrointestinal tract, vagina and skin of the healthy men and animals, the mere isolation of organisms from the clinical samples cannot conclusively establish the diagnosis of mycotic disease. Therefore, emphasis should be given on the direct demonstration of the pathogen in the clinical material and its repeated isolation from the specimen (Pal and Desai, 1998). Each *C. albicans* isolate in the present study had established its etiological significance in their respective clinical form only after following the above mentioned criteria.

The clinical spectrum of *C. albicans* is very wide and appears to involve almost every organ of the body. However, during study period only eight types of clinical disorders could be encountered and perhaps indicate the common clinical feature of candidiasis in the human and animal population under consideration. Moreover, during this study other potential pathogens e.g *Aspergillus* spp. and non-*albicans Candida* spp. were also encountered, but their study is out of the scope of this paper. Therefore, the discussion of the present study is based on the few such disorders only where *C. albicans* was present as a sole pathogen.

The otitis due to fungi (otomycosis) is a superficial, subacute or chronic infection of the outer ear canal, usually unilateral and is characterized by inflammation, pruritus, pain, and scaling. The findings of present study regarding otomycosis in the man are in general agreement with observations

of earlier workers (Erkan and Soyuer, 1991; Mugliston and O'-Donoghue, 1985), who recovered 1.62 and 1.72 per cent *C. albicans*, respectively. It is pertinent to mention in this context that *C. albicans* is more commonly found in temperate zones and *Aspergillus niger* in tropical countries. Although, our observations could be supported by the above mentioned statement, Jaiswal (1990) from India and Sheikh *et al.* (1993) from Iran reported *C. albicans* as predominant etiological agent of the otomycosis. Further, the significance of *C. albicans* in otitis externa of man has been recently reported from India (Jadhav *et al.*, 2003). In case of animals, Fraser (1961) reported *C. albicans* as a primary cause of canine otitis and also mentioned the rarity of primary invasion of fungus in cases of animal otomycosis. In the present study, *C. albicans* was isolated from an 8-year-old German shepherd dog. The similar finding was reported by Azmi *et al.* (1990) who cultured this etiological agent from 4 German shepherd dogs of 3-5 years age having otitis.

Oral candidiasis is also an important sign for clinical diagnosis and an indicator of the evolution of immunodeficiency among HIV carriers (Dupont, 1991). Our study proves the veracity of this statement, as the oral swabs from two HIV infected woman yielded pure, luxuriant and heavy growth of *C. albicans*. As far as HIV patients are concerned, the findings of our study are comparable to that of Pires *et al.* (1996) who reported that oral candidiasis develops in 90 to 95 per cent symptomatic HIV infected individuals. Oropharyngeal carriage of *Candida* spp. in HIV infected patients in India had been studied (Gugnani *et al.*, 2003). In the current study, *C. albicans* was isolated from oral swabs of two male patients and biopsy specimen of growth of anterior 2/3 portion of tongue in a 20-year-old female. In all these cases, there was no evidence of HIV, diabetes mellitus, tuberculosis or cancer. These findings are comparable to those of Palmin *et al.* (1999) who isolated *C. albicans* from the lesions of oral cavity of immunocompetent patients. Mycotic stomatitis in animals is caused by *Candida* spp. in most of the cases (Radostits *et al.*, 2007). In the present study, *C. albicans* was isolated from 4 dogs and 1 buffalo showing symptoms of excessive salivation, stomatitis, halitosis and anorexia. In case of dogs the findings of present study are same as those of

Mckeevar and Klausner (1986) who reported candidal stomatitis in 4 dogs. The literature reveals a great paucity of reports on candidal stomatitis in buffaloes. The demonstration of *C. albicans* in the oral lesions of buffalo perhaps constituted the first report from India.

The findings of current investigation regarding candidal dermatitis in man are closely associated with that of Van Gelderen and Silva (1985) who reported *C. albicans* as an etiological agent of 13.3 per cent cases of cutaneous candidiasis. In India, *C. albicans* has been diagnosed as a prime cause of cutaneous mycoses (Stephen and Rao, 1975; Verma and Singh, 1972). As per the opinion of Jungerman and Schwartzman (1972) cutaneous candidiasis in dog is rare. Our findings are in accordance with Chittawar and Rao (1982) who reported dermatitis in 3 dogs due to *C. albicans* out of 39 positive cases.

Vulvovaginal candidiasis is a universal problem affecting millions of women. Approximately, three quarters of all adult women suffer at least one attack of candidal vaginitis (Berg *et al.*, 1984). According to Hurley (1977) as well as Morton and Rashid (1997), over 80 per cent of yeast isolated from the genital tract of symptomatic and asymptomatic women throughout the world are *C. albicans*. These observations corroborate with the findings of present study, since all the yeasts initially isolated were confirmed as *C. albicans*. The proportion of *C. albicans* positive cases in the present study is much higher than reported by others (Valenza *et al.*, 1998).

Onychomycosis is the sporadic fungal infection of nail. The more localized form of candidal onychomycosis is inflammation of subcutaneous tissues at the base of the finger or toenails. This is characterized by prominent swelling, redness and pain (Emmons *et al.*, 1977). Our observations are in general agreement of Soares *et al.* (1983) who isolated *C. albicans* from 13 patients out of 102 suffering from nail disorders.

Candiduria refers to the presence of *Candida* spp. in the urine. It may be one of the most challenging of the candidal infections. The challenge comes from the fact that finding *Candida* spp. in the urine can be either completely insignificant (e.g., due to

contamination or asymptomatic colonization) or be a marker of a very serious entity such as invasive renal parenchymal disease related to disseminated candidiasis (Kauffman *et al.*, 2000; Myerowitz *et al.*, 1977). In the present study *C. albicans* was isolated from urine samples of only 6.25 per cent samples from men. However, higher incidence rates of about 39 per cent (Lakshmi *et al.*, 1993) and 47 per cent (Mujica *et al.*, 2004) were reported by earlier researchers.

The findings of present study regarding respiratory tract infection due to *C. albicans* in men are closely associated with that of Azoulay and co-workers (2005) who reported *Candida* colonization amongst 26.6 per cent critically ill patient receiving mechanical ventilation. In India, *C. albicans* has been diagnosed as a prime cause of bronchopulmonary candidiasis (Phukan *et al.*, 2000).

Mastitis due to fungi occurs sporadically but sometimes it may result in an outbreak affecting a large number of animals. The disease is of great significance from economic point of view. The incidence of mycotic mastitis appears to be increasing because of extensive rather indiscriminate use of antibiotics for the treatment of mastitis (Gupta *et al.*, 1981). In the present study, *C. albicans* was isolated from 2 cows and 1 buffalo with apparently normal udder. This observation is in general agreement with that of Rehman and Baxi (1983) who reported 5.28 per cent prevalence of candidal mastitis in dairy animals. However, in India higher prevalence of *C. albicans* in subclinical mastitis of dairy animals is reported by others (Chhabra *et al.*, 1998; Sharma and Rai, 1997; Sharma *et al.*, 1977). The observation in the present study regarding the absence of *C. albicans* in cases of clinical mastitis of cows during study under report is in accordance with the findings of Sharma *et al.* (1977) to whom this causative organism was not encountered in the milk samples of 23 cows with clinical mastitis.

The clinical findings of the patients, absence of any other pathogen, direct demonstration of the pathogen in smears and its isolation as a pure and heavy growth indicated that *C. albicans* was incriminated in the above mentioned clinical disorders of men and domestic animals. In the authors view, this appears to be the first systematic report

from this region of India, which unequivocally elucidated the pathogenic role of *C. albicans* in man and animals suffering from various clinical disorders such as otitis, stomatitis, dermatitis, onychomycosis, vulvovaginitis, mastitis, urinary tract infection (UTI) and respiratory tract infection (RTI). The knowledge of the epidemiology of various clinical forms of candidiasis is very important to reduce the suffering of human and animals due to this opportunistic yeast which is the major cause of nosocomial infection.

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