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Review Article

Cryptococcosis: An Enigmatic Mycosis of Humans and Animals

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Abstract
Zoonoses with multiple etiologies affecting immunocompromised hosts have become a matter of concern presently, among the physicians and veterinarians. Among the zoonoses, cryptococcosis is considered a common and important mycozoonosis of global significance mainly affecting HIV/ AIDS patients. Cryptococcosis is primarily caused by *Cryptococcus neoformans and Cryptococcus gattii,* which are found in the soil contaminated with avian droppings or eucalyptus trees and decaying woods. *Cryptococcus neoformans* can survive in the saprobic environment for about 20 years. The respiratory tract is recognized as the principal mode of entry of the pathogen, and the source of infection is exogenous. It is believed that humans and animals acquire cryptococcal infection from saprobic reservoirs. Cryptococcosis occurs in sporadic and epidemic form resulting in high morbidity and mortality in the susceptible hosts. The disease is most often found in cats but has also been reported in cattle, dogs, horses, sheep, goat and other animals. Cryptococcosis is the first manifestation of HIV infection in 26 to 45% of patients, and recent data indicate that *C. neoformans* appears to potentiate HIV infection. Cryptococcal meningitis alone kills approximately 624,000 people in the world annually. The direct demonstration of the pathogen in the clinical specimens and its isolation in pure and luxuriant form remains the "**gold standard**" to the diagnosis of cryptococcosis. The routine application of **Pal's** sunflower seed medium and **Narayan** stain in public health and microbiology laboratories will certainly help in the study of this enigmatic mycosis in humans as well as in animals.

INTRODUCTION

Cryptococcosis, also known as Busse Buschke's disease, European blastomycosis, Torula meningitis, Torulosis, is an acute, subacute or chronic infectious mycotic zoonosis of world-wide significance [1]. One hundred years ago, Sanfelice in 1894 discovered *Cryptococcus neoformans* from peach juice in Italy and in the same year, Busse isolated the yeast from a sarcoma like lesion of tibia and other cutaneous lesions in a 31-year-old woman in Germany [2].The first description on animal cryptococcosis was in a horse by Frothingham in 1902.Sheppe [3] in 1924 recorded the first case of pulmonary cryptococcosis in man. The first report of cryptococcosis in cat and dog was described by Holzworth [4] and Seibold and co-workers [5] in 1952 and 1953, respectively. Pal is credited to elucidate the etiological significance of *C. neoformans* in mastitis of dairy goat and buffalo in 1975 and 1980, respectively [6, 1].

Cryptococcus neoformans, an encapsulated, basidiomycetous yeast, is the important causative agent of human and animal cryptococcosis [1,6,7,]. The source of this organism is mainly pigeon excreta; however, other avian species excreta are also implicated as a source of this yeast [1]. *Cryptococcus gattii* is mainly present in tree bark and hollows, while pigeons and other birds are considered the most important reservoir for *Cryptococcus neoformans* [7].

The pathogen has been reported to infect many species of animals and causes a wide range of manifestations such as meningoencephalitis, pneumonia,

osteomyelitis, abscesses in various internal organs, and ocular disorders [8, 9.] It does not spread via direct contact but is transmitted by inhalation of the aerosolized organism from the soil or feces [10].

Cryptococcus neoformans is the commonest fungal pathogen to infect the human central nervous system (CNS) [11]. *Cryptococcus neoformans* causes life-threatening infections as meningoencephalitis primarily in immunocompromised hosts, generally associated with AIDS [8, 7], and organ transplant recipients next to candidosis. Cryptococcosis is increasing because of an ever rising population of immunocompromised patients, especially those with acquired immune deficiency syndrome (AIDS). The incidence of cryptococcosis in AIDS patients is much higher than in non-AIDS patients [1, 7].The objective of the paper is to present an overview on various aspects of cryptococcosis which is considered as an enigmatic mycotic disease of global significance.

ETIOLOGY

There are 37 species of the genus *Cryptococcus* which are ubiquitous in nature and only two species namely *Cryptococcus gattii* and *C. neoformans* are medically significant [12,7]. However, occasional infections due to other species such as *C.adeliensis*, *C.albidus, C.curvatus* ,*C.flavescens* ,*C.humicolus, C.laurentii, C.luteolus, C.macerans, C.magnus, C. uniguttulatus* and *C.uzbekistanensis* have been recorded in man and animals [13,14,15, 8,9].*C. gattii* and *C. neoformans* have been divided into serotypes [9]. The serotypes are classified on the basis of immunologic reactivity of the cryptococcal capsule with immune sera [12].Serotypes B and C belong to *C. gattii*, and *C .neoformans* has serotypes A, D and AD. The organism is non-motile, Gram-positive, non-fermenting, basidiomycetous, encapsulated yeast. It is recovered from the pigeon droppings, soil, bat guano, wood, parrot excreta, munia bird droppings, other avian excreta, fruits, vegetables, wooden canary cages, unpasteurized milk, *Eucalyptus* trees etc. [2, 16,17,18,19,20]. Pal [21] is credited to report that *C. neoformans* can survive for more than 20 years in pigeon droppings.

HOST

Natural infection has been recorded in men as well as in a variety of animals such as alpaca, anaconda, baboon, bat, bear, buffalo, cat, cattle, cheetah, cockatoo, dog, donkey, dolphin, ferret, fox, gazelle, goat, guinea pig, horse, kiwi, koala, llama ,macaque, macaw, mink, monkey, mouse, palm civet, parrot, pig, pigeon, sheep, shrew, tapir, toad, [22,23,24,25,21,8,7], llama, gazelle, wallabies, non-human primates and other wild marine animals [9]. Most of the infected animals with the exception of cattle and buffaloes had systemic diseases which terminated fatally. The disease in buffaloes and cattle, on the other, had been mostly confined to mammary gland and adjacent lymph nodes [1, 8].

In one study, cryptococcosis was reported as the most important systemic fungal disease to affect mammalian hosts in Australia. *C. gattii* is an important cause of cryptococcosis in animals and people in Australia compared with other regions of the world [26]. The cryptococcal infections in Australian wildlife are both intriguing and exciting, as *C. gattii* accounts for over 90% of cases as determined by culture and/or immunohistochemistry. Of the 72 cases of cryptococcosis investigated in Australian wildlife, the etiologic agent was reported to be *C. gattii* in 44 cases [27].

PATHOGENESIS

The pathogenesis of cryptococcosis is determined by three broad factors—the status of the host defenses, the virulence of the strain of *C. neoformans*, and the size of the inoculum.

The infection occurs through the inhalation of yeast cells or basidiospores. Following inhalation, the yeast spores are deposited into the pulmonary alveoli, where they must survive the neutral-to-alkaline pH and physiologic concentrations of carbon dioxide before they are phagocytized by alveolar macrophages. In the lung, the fungus proliferates in the alveolar space, and in immunocompetent subjects the infection is normally contained in this organ. However, in immunocompromised subjects, dissemination of the yeast cells from the lung to the brain can occur intracellularly (within host cells) and extracellularly (in the blood stream), leading to the development of a life-threatening disease [28]. Rarely, primary cutaneous infection may occur by accidental inoculation of the fungus into the skin following trauma [8].

EPIDEMIOLOGY

Cryptococcosis is a well-known cosmopolitan, highly infectious, enigmatic mycozonosis [1, 7]. The disease is usually sporadic in occurrence but outbreaks of cryptococcal mastitis in cows and cutaneous cryptococcosis in sheep are also documented in literature [1, 7]. The unusual outbreaks of cryptococcosis has also been recorded in cats, dogs, ferrets and a bird [25]. It causes considerable morbidity and mortality in humans as well as animals [29, 11]. The disease occurs both in

immunocompetent as well as immunocompromised hosts. However, the infection is encountered more frequently in immunocompromised patients particularly suffering with AIDS. Since the discovery of HIV in 1981, cryptococcosis has become an important life threatening disease in AIDS patients [30, 6, 8].

In temperate regions of the world, *C. neoformans* is the most often causes clinical disease. On the other hand, in tropical and subtropical regions (where eucalyptus trees are found), *C. gattii* has been reported as a cause of cryptococcosis in people and animals [31]. It seems to occupy a specific ecological niche, having been isolated only from the bark and leaf litter of eucalyptus trees [32]. In nature, *C. gattii* and reside in separate environmental niches, which have been identified as potential sources of pathogenic strains. *C. neoformans* has been isolated from a variety of avian species in addition to pigeons, including chickens, parrots, sparrows and sites contaminated by pigeon excrement and at a lesser concentration in the soil [33,1,8,7]. *C. neoformans* var *gattii* has been isolated from *Eucalyptus* trees particularly E. *camaludulensis* and *E. globulus* originated from Australia and are extensively grown in many countries [32, 7].

C. neoformans has a worldwide distribution and preferentially infects immunosuppressed individuals, especially those suffering with AIDS [1, 8, 7].On the contrary, *C .gattii* causes 70 to 80 % infections in immunocompetent hosts [7]. In sub-Saharan Africa, 15%-30% of all patients with AIDS develop cryptococcal disease. However, in some areas, such as Zimbabwe, 88% of patients with AIDS have cryptococcal infection as their AIDS-defining illness. The disease due to *C. gattii* is mainly reported from tropical and subtropical regions such as Australia, Papua New Guinea and South America. However, *C. gattii* infection can also occur in temperate climate [34].The global incidence of cryptococcal meningoencephalitis in AIDS patients is recorded 2-33% [35].

The avian droppings are considered the main reservoir of *C. neoformans*. It is important to mention that *C. neoformans* can survive for 20 years or longer in dry and old pigeon droppings in dark and humid sheltered site which is not exposed to direct sunlight [21, 7]. There are evidences to believe that man and animal usually acquire the infection from the environment where the fungus grows luxuriantly. It is estimated that 1gram of dry pigeon excreta may contain up to 50 million viable cells of *C. neoformans*. Such natural sites may become a point source of infection to man and animals [7].The zoo attendants, pet bird keepers, bird enthusiast, pigeon breeder and persons engaged in the cleaning of historical buildings, old monuments etc. are more likely to expose to cryptococcal infection [1]. Pulmonary mycosis in a pigeon handler due to *C. neoformans* var. *neoformans* has been reported from India by Pal in 1993[36].The organism was isolated from the sputum of the patient and also from the pigeon droppings on Pal's sunflower seed medium [36]. Nosanhchuk and co-investigators [37] described the zoonotic transmission of *Cryptococcus neoformans* from pet cockatoo to an immunocompromised patient. Similarly, zoonotic transmission of *C.neoformans* from a magpie to an immunocompetent patient is recorded by Lagrou and co-worker [38].

Marine mammals have also been infected. Vectors can disperse the spores from an endemic area to a previously unaffected area [9]. Healthy persons with a history of exposure to pigeons or bird feces and laboratory workers exposed to an aerosol of the organism have a higher rate of positive delayed hypersensitivity skin reactions to cryptococcal antigen or cryptococci. Occasionally, laboratory accidents result in transmission of *C. neoformans*, but pulmonary and disseminated disease is rare in this setting. Accidental cutaneous inoculation with *C. neoformans* causes localized cutaneous disease [29, 1, 8].There are also reports of nosocomial transmission of cryptococcal infections in humans [40]

The disease in animals is usually sporadic in occurrence but outbreaks of cryptococcal infections are also documented in literature [25, 8]. Clinical cryptococcosis is reported most often in cats. It is particularly common in cats that are immunosuppressed by feline leukemia virus or feline immunodeficiency virus infections [39]. Cryptococcosis may also be more common in immunosuppressed dogs. The prognosis is guarded, especially in cases with CNS disease. Untreated infections are usually fatal [7].

TRANSMISSION

Transmission seems to be mainly by inhalation, but *C. neoformans* can also enter the body through the skin [1, 21,7]. Infections seem to be acquired mainly from the environment. Cryptococcosis can also result from the reactivation of a latent infection. Cryptococcal mastitis in cattle is usually associated with the treatment of the mammary gland for another condition. The organism may be introduced into the teat in contaminated syringes, cannulas or antibiotic preparations. It can also enter the mammary gland if the teat ends are not adequately cleaned before treatment [1]. Vertical transmission was recently described, when a HIV-positive mother with peripartum cryptococcal meningitis infected her newborn [28]. Nosocomial transmission has also been

reported by Wand and co-investigators [40].

CLINICAL MANIFESTATIONS

Humans

In case of man the disease is rarely observed in children. Adults between the ages of 30-60 years are commonly affected. The incubation period of disease is usually 2-4 weeks. The lung is invariably the portal of entry and initial site of infection [8]. The infection may subsequently disseminate to other organs of the body including the skin and brain. The clinical features of cryptococcosis depend on the tissue involved. The pulmonary cryptococcosis is characterized with cough, chest pain, fever, hemoptysis, sputum production, malaise, dyspnea, pneumonia, night sweats, and weight loss [2, 8]. Although *C. neoformans* most often infects patients via the pulmonary route, less than 15% of patients present with a clinical picture of pneumonia.

Patient affected with CNS cryptococcosis shows the signs and symptoms of subacute meningitis or meningoencephalitis, such as headache, fever, lethargy, coma,personality change and memory loss [2, 8]. Meningitis may lead to permanent neurologic damage. The mortality rate is about 12%. Skin lesions in cutaneous cryptococcosis may present as nodules, papules, vesicles, tumors, ulcers, abscesses, cellulites, subcutaneous swelling, purpura and superficial granulomas (1, 8].

Animals

Cryptococcal mastitis is observed in cow, buffalo, goat and sheep. The affected animals show swelling of one or more quarters, supramammary lymph nodes, reduced appetite, loss of milk yield and grayish white secretion from the affected teats. The affected quarters are usually swollen and firm. The milk may be viscid, mucoid and grayish-white, or it may be watery with flakes in case of cows [8]. Pulmonary disease and mastitis have been described in sheep and goats. In one goat, *C. neoformans* was associated with an alopecic, exudative skin lesion on the head. Syndromes that have been reported in horses include meningoencephalitis, pulmonary disease,upper respiratory disease affecting the frontal sinuses and para-orbital area, and abortions. Obstructive growths in the nasal cavities are the most common presentation. Cryptococcosis is very rare in birds; mycotic rhinitis and sinusitis have been described. However, the organism can be found in their feces especially in pigeons [9].

Upper respiratory disease (unilateral or bilateral chronic rhinitis or sinusitis) is the most common form of cryptococcosis in cats. The symptoms may include sneezing, snoring or snorting, dyspnea, or a mucopurulent or serosanguineous nasal discharge. Polyp-like masses may protrude from one or both nostrils (Fig.1). The cervical lymph nodes can be enlarged and ulcerative or proliferative lesions are occasionally seen on the tongue, gingiva or palate. Pulmonary symptoms are uncommon [1, 8].

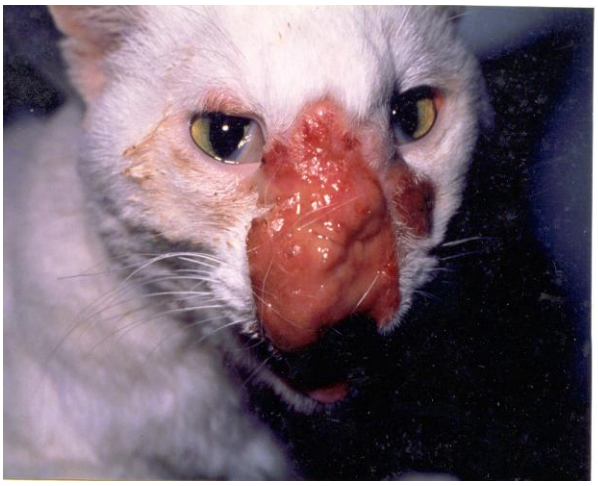

Fig 1. Cutaneous cryptococcosis in a 3-year-old female domestic cat. Note the presence of mucoid, granulomatous mass on the nose.

Source: Pal and Dave, 2006 [1]

DIAGNOSIS

Clinical signs are not very characteristic to warrant the diagnosis of cryptococcosis. Therefore, mycological examination is recommended to provide a more definitive diagnosis. Radiography, CT scan and MRI may help to locate the lesions in the organ [12, 21]. Clinical specimens such as CSF, sputum, skin exudates, nasal exudates, urine, pus, tissue aspirate etc. should be examined in India ink or nigrosin for the presence of thick, wide, circular, encapsulated budding yeast cells[1,8]. Wet preparation and impression smear are also useful to detect the pathogen. Affected tissue biopsies should be macerated with a sterile clean scalpel and be treated with 10% potassium hydroxide (KOH) solution before examination. Pal's sunflower seed medium should also be used as it is highly specific and sensitive for *C. neoformans.* In this medium, the diagnosis of cryptococcosis can be easily and rapidly confirmed by observing light to dark brown colonies of *C. neoformans* [41] (Fig.2). The organism can also be recovered on Sabouraud medium at 37^0C [8].

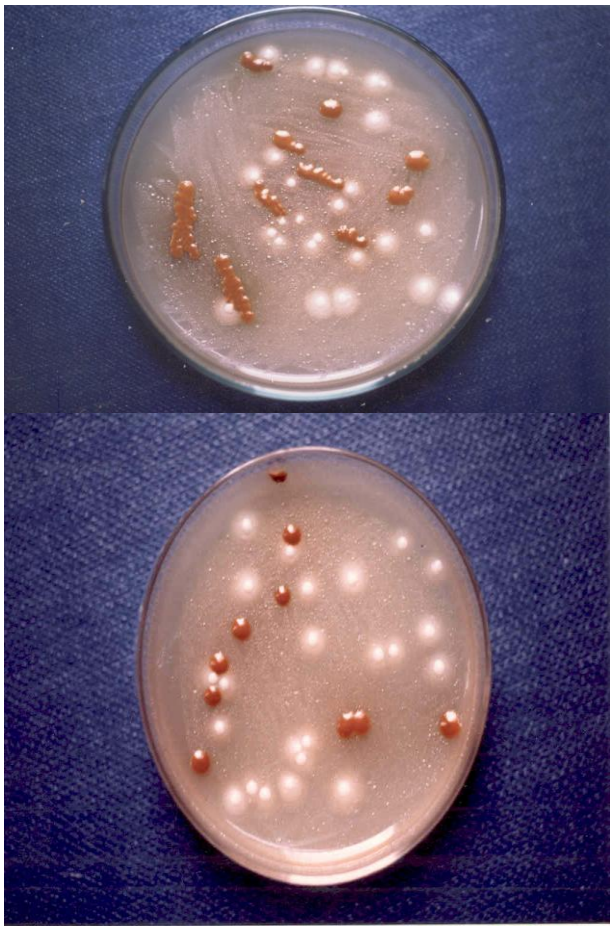

Fig 2. Several smooth, shining, brown colored colonies of *Cryptococcus neoformans* on Pal's sunflower seed medium at 25^0C after 8^{th} days, isolated from the clinical specimens of a 79-year-old woman who had lung cancer as an underlying disease

Source: Pal and Dave, 2006 [1].

The morphology of the cultural isolates could be studied on "NARAYAN" stain [42]. Microscopically, most clinical isolates appear as thick, spherical, budding, encapsulated (1-30 μm) yeast cells in both tissue and culture.

Immunological test mainly latex agglutination is employed for demonstration of cryptococcal antigen in the serum, CSF, urine and broncho-alveolar lavage (BAL). Molecular techniques (PCR, RAPD) have been used in the diagnosis particularly from epidemiological point of view [1]. Animal pathogenicity is performed into the Swiss albino mice by inoculating the culture through intracerebral or intraperitoneal route. The inoculated mice usually die within 7-10 days [43]. The smear prepared from mouse brain revealed many encapsulated cells of *C.neoformans* when stained by periodic acid-Schiff (PAS) technique (Fig.3).The comparison of various methods employed for the diagnosis of cryptococcosis in AIDS patients is given in Table 1. The differentiation of various species of *Cryptococcus neoformans* is based on cultural and biochemical characteristics [8].

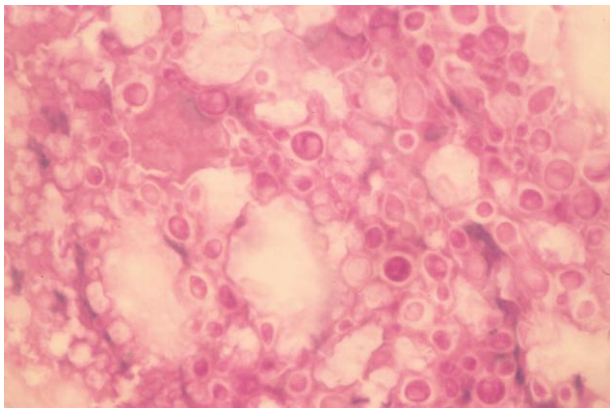

Fig 3. Impression smear from the mouse brain ten days after inoculation of *Cryptococcus neoformans* isolate from an AIDS patient, showing aggregates of *Cryptococcus neoformans* with typical enlarged capsules throughout the tissue .Periodic acid-Schiff × 250.

Source: Pal and Dave, 2006 [1].

Table 1. Comparison of different techniques for the diagnosis of cryptococcal infection.

Technique	Specimen (CSF)	Percent positive
Direct microscopy in India ink or Nigrosin stain	27*/31	87.0
Culture on Pal's medium at 30^0C	29/31	93.5
L. A. for cryptococcal antigen	28/31	90.3

* Numerator denotes number of CSF specimens positive and denominator number of samples examined.

Source: Pal, 2007[8].

TREATMENT

The treatment depends on the involvement of organ and the host's immune status. All patients who are symptomatic should be treated. Attempts should be made to improve the immune status of the host during therapy. Ideally, corticosteroids and other cytotoxic drugs should be stopped [1, 8]. Outcomes of treatment of cryptococcosis are quite varied. Long-term administration of antifungal drugs is the crux of therapy (average of 8.5 months) and relapses occur frequently. Patients with the CNS form of cryptococcosis will require lifelong treatment maintenance. It is recommended that treatment continue for one month after resolution of clinical signs in combination with decrease in antigen titer by at least two orders of

magnitude or until serum cryptococcal antigen is undetectable. The prognosis is much worse if the patient has the neurologic form of disease or is immunocompromised [8].

In case of animals, cryptococcosis can be treated with amphotericin B, flucytosine, itraconazole and fluconazole. Amphotericin B and flucytosine are often used in combination. A combination of ketoconazole and itraconazole has been effective in some experimentally infected cats, including animals with neurologic disease [1]. Amphotericin B (AMB), alone or in combination with other antifungal drugs, has been previously used to treat cryptococcosis. However, this drug is usually reserved for life-threatening and previously unresponsive cases due to its toxicity. Several disadvantages of AMB make it less desirable than other drugs. Intravenous treatment is difficult and causes many adverse reactions [11, 2,1].

In case of humans, ketoconazole (100-200 mg, PO) is found effective for non-meningeal cryptococcosis in patients without severe immunosuppression. Cryptococcal meningitis has been successfully managed with intraconazole (50-400 mg, PO). Fluconazole has excellent pharmacokinetics in the CSF and is fungistatic *in-vitro* and suppress the infection *in-vivo* [8].Further research on the development of safe, effective and cheap drugs should be undertaken for the better management of this fatal mycosis both in man and animals.

PREVENTION AND CONTROL

In most cases, there is no practical means of prevention other than to avoid exposure to the soil, particularly soil contaminated with abundant bird droppings, and the environment around eucalyptus trees. Cryptococcal mastitis in cattle is usually associated with the treatment of the mammary gland for another condition. Care should be taken not to contaminate syringes, cannulas or antibiotic preparations with *C. neoformans* from the soil or other sources. The teat ends should also be adequately disinfected before treatment [9].

Cryptococcosis is difficult to eradicate because of the paucity of epidemiological data on the prevalence of infection in specific areas, sporadic nature and occurrence during severe immunosupression [29, 1, 8]. However, the immunocompromised persons should not visit avian habitats or dusty environment [43, 8]. HIV-infected persons cannot completely avoid exposure to *C. neoformans or C. gattii*. Limited epidemiologic evidence suggests that specific activities, including exposure to bird droppings, lead to an increased risk for infection [29].

There is the possibility of antifungal prophylaxis. The first trial to evaluate prophylaxis with fluconazole to prevent cryptococcosis among patients with AIDS suggested a positive benefit [44]. Active immunization in the form of a vaccine is an ideal strategy for prevention among high-risk patients. A cryptococcal GXM-tetanus toxoid conjugate vaccine has now been developed which appears to be highly immunogenic and to elicit high-affinity IgG antibodies that appear to show protection in murine models [45]. It is emphasized that additional studies on the development of potent, safe and low cost vaccine to prevent the disease in humans as well in animals will be rewarding.

CONCLUSION

Cryptococcosis is an important, infectious, enigmatic, global, opportunistic mycosis of human as well as animals. The disease is chiefly caused by *C. neoformans* which occurs as a saprobe in wide variety of environmental substrates. The pathogen affects humans as well as several species of animals. The infection is mainly acquired through respiratory tract by inhalation of highly infectious yeast cells from saprobic reservoirs. Cryptococcal meningitis is common among patients with immune-suppression. It could be the initial manifestation of HIV infection and should be suspected in any potential HIV infected patient with neurological symptoms especially headache and fever. The generalized form of the disease is invariably fatal, if left untreated. The disease is encountered in immunocompromised and compromised host/individuals. However, maximum cases have been observed in individual with impaired immune system. Early diagnosis and prompt chemotherapy is necessary to reduce the morbidity and mortality due to this life threatening fungal zoonosis. Since **Pal's sunflower seed agar** is easily available, simple to prepare, and cost effective medium for rapid differentiation of *Cryptococcus neoformans* from *Candida, Trichosporon* and other yeasts, it is best recommended for laboratory where biochemical tests are not available for its differentiation from other yeast pathogens.

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