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2. LITERATURE REVIEW

2.1 DISCOVERY OF CRYPTOCOCCUS

In 1894, the Roman-born Italian medical scientist Sanfelice isolated an encapsulated yeast-like fungus from peach juices (Sanfelice, 1894a), which he named *Saccharomyces neoformans* (Sanfelice, 1895). He produced pseudo-tumoural, sarcoma-like lymphoadenic lesions in experimentally infected animals after intraperitoneal inoculation with these yeast-like fungus. The most fascinating phenomenon of capsule production by the fungus in man and animal causing a peculiar tumour-like alteration of tissue prompted Sanfelice to propose the species epithet ‘neoformans’. Subsequently, he isolated a yeast from a lymphnode of an ox, that died from carcinoma of the liver (Sanfelice, 1894b) and he considered this yeast to be a little different from that of *S. neoformans* and named *S. lithogens*, because of its tendency to produce calcification in tissues. It was recently relegated to a synonym of *C. neoformans*. At about the same time in Germany, Busse (1894) and Buschke (1895) separately reported the isolation of the same fungus from a sarcoma-like lesions of the tibia and other lesions in a 31 year old woman and, the fungus was named as *Saccharomyces hominis* (Busse, 1895).

In France, Curtis (1895a) described a yeast species to be the agent causing myxomatous tumours, an inguino-cural gelatinous tumefaction and a lumbar ulcerated abscess in a young french man. After a benign surgical intervention, the patient was discharged from the hospital and died ten month
later with meningitis manifestations without other details (Curtis, 1895b). Curtis named the yeast _Megalococcus myxoides_ and reported preliminary experimental data on mice, rats and guinea-pigs reproducing tumour-like lesions. In the following year, Curtis who knew the publications of Sanfelice and Busse, gave a detailed description of the capsulated yeast with round cells and surprising elongated, oval and bacilliform yeasts and he emphasised the absence of fermentation at 37°C (Curtis, 1896). Curtis obtained experimental lethal infections in rats inoculated subcutaneously with enormous tumour-like lesions as well as involvement of the lungs, spleen and kidneys. Similar lesions were obtained in mice, guinea-pigs and dogs. Granulomatous cellular reactions or the absence of reaction similar to those observed in the patient were described. Curtis named this strain _Saccharomyces subcutaneous tumefaciens_, considering it to be distinct from the _S. neoformans_ of Sanfelice and the _S. hominis_ of Busse, because of the morphological differences and its great affinity for the subcutaneous tissues of experimental animals.

In France, the botanist and mycologist, Vuillemin (1901) examined several cultures and he did not find the ascospore characteristics of the genus _Saccharomyces_ and placed it in the genus _Cryptococcus_, the generic name created for yeasts without endospores by Kützing (1833). Therefore, Vuillemin reclassified the yeasts isolated by Busse and Curtis in the genus _Cryptococcus_ and the isolate of Sanfelice (1894a) as _C. neoformans_ confirmed by Fell _et al._ (1989). Several cases were reported especially in Europe and the disease was confused with blastomycosis and considered to be an European blastomycosis.
In the USA, Stoddard and Cutler (1916) described the clinical aspects of the disease. Ignoring the European literature, the investigators mistook the mucoid capsule of the yeast observed in the USA among European immigrants for histolysis of host tissue and therefore named the fungus *Torula histolytica* without any proof of enzymatic hydrolysis (Drouhet and Segretain, 1949) and the disease was known for a long time as Torulosis or Torula meningitis, until the terms *Cryptococcus neoformans* and cryptococcosis became widely used in medical mycology owing to the efforts of Lodder (1934) and particularly Benham (1935).

Benham clearly differentiated cryptococcosis from the blastomycosis especially from North American blastomycosis by studying more than 40 strains of *Cryptococcus* species including the original cultures from Sanfelice, Busse and Curtis, for her doctoral dissertation on yeasts identification. Benham showed that only one species is responsible for this disease, although there are serological differences among the strains (Drouhet, 1997).

### 2.2 AETIOLOGY OF CRYPTOCOCCOSIS

Although there are 19 known species of the genus *Cryptococcus*, the aetiologic agent in virtually all cases of human cryptococcosis is *C. neoformans* (Rippon, 1988). However rare cases of infection by *C. albidus* and *C. laurentii* have been reported (Krumholz, 1972; Melo *et al.*, 1980; Lynch *et al.*, 1981; Chakrabarti *et al.*, 1995).
2.3 MYCOLOGY OF CRYPTOCOCCUS NEOFORMANS

2.3.1 Morphology

2.3.1.1 Microscopic appearance

Most clinical isolates are spherical or oval, budding, encapsulated yeast cells in both tissue and culture. Rarely short hyphal forms are also observed and filamentous variants have been isolated (Lurie and Shadomy, 1971). The yeast cells vary in size from 5 to 10 μm in diameter and exhibit both single and multiple budding.

The hallmark of C. neoformans is its mucoid polysaccharide capsule (Plate 1) which may be up to two or three times the width of the cell. Considerable variation exists in the size of the capsule, which is determined both by inherent strain differences and conditions of growth. Elevated glucose, CO₂ or temperature enhance capsule formation.

For instance, Bottone et al. (1986) reported the clinical isolate, which was morphologically unusual, the cells and capsules are several times larger in an infected tissue than those commonly described in the literature.

2.3.1.2 Cultural characteristics

Visible colonies of C. neoformans develop on routine mycological media with 36 to 72 hr at 25°C. They are white to cream coloured, opaque and may be several millimeters in diameter (Plate 2). Colonies may develop sectors and that is because of changes in the composition and structure of the
capsules. Colonies are typically mucoid in appearance and indeed the amount of capsule produced can be judged by the degree of colony wetness. The texture is mucoid and the colony may flow to the bottom of the slant. The edges are entire and without pseudomycelium. Occasional isolates produce little or no capsular material. Colonies of such organisms appear dry or glabrous (Rippon, 1988).

2.4 PHYSIOLOGY

Of the several species of Cryptococcus, only C. neoformans is able to grow at 37°C. C. neoformans produces a unique enzyme phenoloxidase, that convert a variety of hydroxy benzonic substrates into brown coloured melanin pigments. This reaction was first observed by Staib (1962), when the organism was cultured on a medium containing crushed seeds of Guizotia abyssinica (niger or bird seed) has been exploited for the rapid identification of C. neoformans (Plate 3).

2.5 VIRULENCE FACTORS

The capsule formation is one of the two best known virulence factors of C. neoformans for man and animals. Drouhet et al. (1950) studied the chemical composition of the capsular polysaccharide of C. neoformans which led to an important identification of a Glucoronoxylomannan (GXM) by paper chromatography. They showed that GXM is a virulence factor related to the dimensions of the polysaccharide capsule of natural sectorial S (smooth, weak by capsulated yeasts) and M (mucoid, broadly capsulated yeasts) colonies. The
Plate 1  Encapsulated cells of *C. neoformans* with negative stain

Plate 2  Pure culture of *C. neoformans* on SDA against black background

Plate 3  Pure culture of *C. neoformans* on Staib's agar with BCE
GXM inhibits the migration of leukocytes and phagocytosis as showed by Drouhet and Segretain (1951).

It has been demonstrated that capsule deficient mutants had little or no virulence in mice (Bulmer et al., 1967; Kozel and Cazin, 1971). The degree of encapsulation in vitro however has never been shown to correlate with the degree of C. neoformans virulence in vivo (Dykstra et al., 1977). Although previous studies on the role of encapsulation in the virulence of C. neoformans were convincing, they did not explain why C. neoformans is the only pathogen in the genus Cryptococcus in which, all members are normally encapsulated.

The formation of melanin pigment is yet another virulence factor. The enzyme responsible for the pigment formation was identified as a membrane bound phenoloxidase enzyme (Polacheck et al., 1982; Polacheck and Kwon-chung, 1988).

Phenoloxidase enables C. neoformans to synthesise melanin from certain catecholamine precursors. Phenoloxidase activity and melanin production have been postulated to contribute to the propensity for C. neoformans to invade the CNS, an area rich in catecholamines (Rhodes et al., 1982).

The end product of the phenoloxidase activity was implicated in certain aspects of the virulence and its experimental pathology on animals studied (Kwon-chung et al., 1982a; Rhodes et al., 1982; Polacheck et al., 1990; Kwon-chung, 1992). Huffnagle et al. (1996) has shown in animal experiments
that a low-melanin producing strain induced production of tumour necrosis factor (TNF) followed by inflammation and resolution of the infection but a high-melanin producing strain did not elicit TNF until a late stage and the infection progressed and became disseminated. Moreover, the pigmented cells are less susceptible to free-radical killing, suggesting that the melanin-like pigments protects against oxidants produced by host effector cells and the pigmented cells are also less susceptible to the antifungal agent, amphotericin B and this may contribute to persistence of infection in human (Wang et al., 1995).

Kwon-chung and Rhodes (1986) showed that in experimental cryptococcosis in animals, the virulence of C. neoformans is highly dependent upon a combination of two factors, the capsule formation and the production of melanin at 37°C.

2.6 VARIETIES AND SEROTYPES

The observations by Bennett et al. (1978) showed that two varieties of C. neoformans, var neoformans and var gattii, differed quantitatively in the assimilation of creatinine on Staib's agar. In addition to the brown colour effect (BCE), there was also a green colour effect (GCE), but only with some strains of C. neoformans. The GCE of the medium was due to strong creatinine assimilation and an alkaline pH value. This observations had led to the development of the two varieties.
Kwon-chung et al. (1978) established the biochemical basis of the creatinine-dextrose-bromothymol blue (CDB) medium for the differentiation between the two varieties. But Muchmore et al. (1980) observed higher rate of false-negative reactions on the CDB agar. Salkin and Hurd (1982) developed another colour medium, glycine-cycloheximide-phenol red (GCP) agar, differentiating the two varieties. Kwon-chung et al. (1982b) suggested that, the false-negative percentage with GCP was as high as with the CDB medium and CDB medium was modified into canavanine-glycine-bromothymol blue (CGB) medium using L-canavanine sulphate for the better result. For the same purpose, D-proline assimilation test was introduced by Dufait et al. (1987).

Evans (1950) identified three serotypes A, B and C on the basis of antigenic differences in the capsular polysaccharide by tube agglutination test (Evans and Mehl, 1951; Evans and Kessel, 1951). The fourth serotype D was added by Wilson et al. (1968).

Ikeda et al. (1982) carried out the serotyping by a slide agglutination test with factor sera prepared by adsorption of anti-\textit{C. neoformans} rabbit sera with heterologous heat-killed cells and identified the fifth serotype AD.

The serotype A of \textit{var neoformans} is most common throughout the world and serotype D is uncommon in the USA, but is more frequent in Europe, particularly in Italy (49%) and in France (>20%) according to the recent study by Dromer et al. (1994).
One hundred years after the description, the Curtis's strain appears to be similar to the strain reported by Gatti and Eekels (1970) and named Cryptococcus gattii by Vanbreuseghem and Takashio (1970) or Cryptococcus bacillisporus by Kwon-chung et al. (1978), anamorph of the teleomorph Filobasidiella neoformans var bacillispora. The Curtis’s strain (CBS 1622=ATCC 2344) belongs to serotype B as the strain of C. gattii (CBS 6289=ATCC 32269), while Sanfelice's strain, type of C. neoformans (CBS 132=ATCC 32045) belongs to serotype D and Busse’s strain (CBS 879=ATCC 4189) to serotype A.

The phylogenetic relationship of C. neoformans serotypes studied by Gucho et al. (1993) shows surprisingly, in the phylogenetic tree based on comparisons of LSU r-RNA sequences that, serotype D appears to be closer to serotypes B and C than to serotype A. The phylogenetic distance between serotypes A and D within the var neoformans has not yet been elucidated.

2.7 SEXUALITY

A veritable revolution in the knowledge of C. neoformans was the discovery of its sexual reproduction by Kwon-chung (1975), who proposed the genus Filobasidiella to accommodate this basidiomycete. Shadomy and Utz (1966) and later Shadomy (1970) had reported clamp connections in hypha-forming isolates of C. neoformans. Formation of the F. neoformans teleomorph was observed when crosses were made between two mating types of serotype A or D isolates (Kwon-chung, 1975; Kwon-chung, 1976a), whereas the F. bacillispora teleomorph was observed when similar crosses were made.
among serotype B or C isolates (Kwon-chung, 1976b). Kwon-chung (1976b) concluded that there were two varieties of *F. neoformans*, *F. neoformans* var *neoformans* corresponding to the asexual state, *C. neoformans* var *neoformans* with the serotypes A and D, and *F. neoformans* var *bactilluspora* corresponding to the asexual state, *C. neoformans* var *gattii* with the serotypes B and C.

Although *C. neoformans* is heterothallic and assumes a basidiomycetous state upon mating in the laboratory, isolates of the two compatible mating types from the same clinical or environmental source have rarely been found. This indicates the fungus primarily reproduces asexually and only rarely generates genetically distinct clones within a given population (Kwon-chung and Bennett, 1978).

The existence of self-fertile isolates that produce a complete sexual state in the apparent absence of cross-mating has been also reported (Phaff and Fell, 1970; Erke, 1976; Kwon-chung, 1977).

2.8 ECOLOGY
2.8.1 Distribution of var *neoformans* in nature

*C. neoformans* was isolated in nature first from peach juice (Sanfelice, 1894a), then from milk (Klein, 1901), soil and pigeon excreta (Emmons, 1951 and 1955; Ajello, 1956 and 1958). Although pigeon droppings commonly are colonised with *C. neoformans*, pigeons do not appear to become sick due to cryptococcosis, perhaps because their high body temperature is detrimental to growth of the organism (Abou-gabal and Atia, 1978).
Since Emmon's original reports, *C. neoformans* has been isolated from soil, pigeon excreta and sites contaminated by pigeon excreta in various parts of the world eg. USA (Littman and Schneier, 1959), Australia (Frey *et al.*, 1965), England (Randhawa *et al.*, 1965), Japan (Yamamoto *et al.*, 1957), Sweden (Bergman, 1963), West Germany (Bohm *et al.*, 1967), Czechoslovakia (Halsova and Jesenska, 1973), Nigeria (Gugnani and Nijok-obi, 1973), Poland (Jakubics, 1974), Thailand (Balankura, 1974) and India (Gugnani *et al.*, 1976).

It has been isolated also from other avian species including canaries, chickens, parrots, skylarks, sparrows, starlings and turtle doves (Abou-gabal and Atia, 1978; Bauwens *et al.*, 1986; Pal, 1989a and 1989b). The reason for the high frequency of *C. neoformans* in avian excreta is not clear but may be related to the ability of the fungi to assimilate xanthine, urea, uric acid and creatinine, all of which are abundant in the droppings (Littman and Walter, 1968).

The concentrations of *C. neoformans* in pigeon droppings often exceed $10^6$ viable organisms per gram and most investigators have encountered little difficulty in isolating *C. neoformans* directly from pigeon excreta or samples contaminated by such excreta (Littman and Schneier, 1959; Emmons, 1960; Muchmore *et al.*, 1963; Procknow *et al.*, 1965; Littman and Borok, 1968).

In contrast to the situation with pigeon droppings, a lower percentage of soil samples were positive for *C. neoformans* and the concentration of organisms in soil tend to be less (Ajello, 1958). Probably the soil forms an inhospitable environment for *C. neoformans*. In support of this, anaerobic
conditions, high temperature, decreased humidity, direct sunshine, low pH and the presence of soil amoebae and other microbes have all been shown to be detrimental to the survival of *C. neoformans* in soil (Bunting et al., 1979; Ruiz et al., 1982).

The experimental findings (Bunting et al., 1979) indicate that, *Acanthamoeba polyphaga* in the trophozoite stage, actively ingests and kills the cells of *C. neoformans* and some of the surviving cells of *C. neoformans* developed into colonies containing pseudohyphae. These pseudohyphal forms may be a biological "escape hatch" and that the soil amoebae may be an important biological control mechanism in nature. The studies of Ruiz et al. (1981 and 1982) suggest that many soil organisms like *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Acanthamoeba palestinensis* trophozoites, mites and sow bugs, that occur in pigeon droppings may be a complex of biological factors that influence *C. neoformans* persistence, reproduction, morphology and distribution in nature.

Staib (1963a and 1963b) reported that pigeon droppings provide an excellent culture medium for *C. neoformans*. Walter and Yee (1968) failed to isolate *C. neoformans* from chicken droppings and they suggested that this could be due to their high alkalinity and the presence of low molecular weight, thermostable, growth-suppressing substances in this material. Littman and Borok (1968) reported that *C. neoformans* multiplied rapidly in an extract from sterile pigeon droppings and remained viable in this material for many months.
C. neoformans is occasionally isolated from various non-avian sources, including fruits, vegetables, dairy products and the digestive tract of the cockroach (Pal and Mehrotra, 1984; Swinne et al., 1986). Dead and decaying vegetation could also form a suitable substrata for this fungus (Staib et al., 1972).

The C. neoformans that was isolated from bird excreta or soil (contaminated with bird excreta) throughout the world belong to var neoformans (Muchmore et al., 1980; Mishra et al., 1981; Kwon-chung and Bennett, 1984a; Pal, 1989b; Pal, 1995).

2.8.2 Distribution of var gattii in nature

C. neoformans var gattii has been considerably more difficult to isolate from the environment. Ellis and Pfeiffer (1990a and 1990b) extensively tested environmental samples from sites in rural Australia, an area in which C. neoformans var gattii is endemic. This organism was isolated exclusively from samples collected under the canopies of Eucalyptus camaldulensis trees, during flowering season and later from E. tereticornis, E. blakelyi, E. gomphocephala and E. rudis (Pfeiffer and Ellis, 1992; Pfeiffer and Ellis, 1997). Apart from Australia, the first isolation of var gattii from Eucalyptus trees has been reported also from California (Pfeiffer and Ellis, 1991), Italy (Montagna et al., 1997) and India (Chakrabarti et al., 1997).

Environmental isolations have established that var gattii has a specific ecological association with Eucalyptus trees. The global distribution of
these species of *Eucalyptus* seems to correspond with the epidemiological distribution of cryptococcosis caused by var *gattii* (Pfeiffer and Ellis, 1992). No other environmental source of the var *gattii* has been reported yet.

To date, all environmental isolates of var *gattii* from *Eucalyptus* species were found to be serotype B.

### 2.9 EPIDEMIOLOGY OF CRYPTOCOCCOSIS

#### 2.9.1 Sources of Infection

The only known habitat and source of dissemination of *C. neoformans* is old, dried droppings of pigeon and other avian species, which apparently provides a reservoir of the organisms. Exposure to *C. neoformans* is particularly common in certain occupational groups such as pigeon breeders and laboratory workers. However, there is no evidence of an increased incidence of active cryptococcosis among these groups although, they have a high frequency of cryptococcal serum antibodies than in the general population and an increased incidence of reactivity to skin-test (Walter and Atchinson, 1966; Newberry *et al.*, 1967; Atkinson and Bennett, 1968).

Moreover, the isolates of *C. neoformans* from aerosols generated from soil and pigeon droppings are measuring 0.6 to 3.5 μm in diameter and it is a size ideal for alveolar deposition after inhalation (Powell *et al.*, 1972). Samples of air obtained under flowering *Eucalyptus* trees also have grown *C. neoformans* var *gattii* (Ellis and Pfeiffer, 1990a).
Swinne et al. (1989) have shown that *C. neoformans var neoformans*, is present in domestic dust from the houses of patients with cryptococcosis and have emphasised the importance of the peri-domestic environment and the role of pigeon coops as a source of the yeast for infection in HIV positive and AIDS patients.

2.9.2 Mode of Transmission

Cryptococcosis is not contagious. There is no person-to-person or animal-to-person transmission. However, two unusual cases of person-to-person transmission of cryptococcosis have been reported, in a recipient of a corneal transplant from a donor with cryptococcosis developed cryptococcal endophthalmitis after the transplant (Beyt and Waltman, 1978) and in another case, a health care worker who developed localised cutaneous cryptococcosis after accidentally inoculating himself with blood from a patient cryptococceamia (Glaser and Garden, 1985).

2.9.3 Distribution of Varieties and Serotypes in Patients with Cryptococcosis

The epidemiological studies of the two varieties conducted prior to the AIDS epidemic indicated that infections caused by var *neoformans* are worldwide in distribution. Infections caused by var *gattii* however are prevalent only in tropical and subtropical regions and rarely found in regions with cold climates (Kwon-chung and Bennett, 1984a). In recent years, the overall proportion of infections caused by var *gattii* in tropical and subtropical
regions has been reduced drastically because cryptococcosis in AIDS patients is mostly caused by serotype A of var *neofomans* (Kwon-chung *et al.*, 1990), regardless of the geographical location.

### 2.9.4 Distribution of Mating Types

Several investigators have reported the nature of mating types of *C. neofomans* from clinical sources. Survey revealed that the mating type ‘α’ is predominant among clinical and environmental isolates of *C. neofomans* regardless of the serotype (Kwon-chung and Bennett, 1978; Hironaga *et al.*, 1983; Pal *et al.*, 1991).

### 2.9.5 The Prevalence and Incidence of Cryptococcosis

The prevalence of cryptococcosis in patients with AIDS in Africa and other developing countries appears to be higher than in the developed countries (Kozel, 1995). It is not clear, whether this circumstances is secondary to increased exposure to saprophytic sources of the fungus or the related rarity of other AIDS-related infections in particular those caused by *Pneumocystis carinii* and *Mycobacterium avium* complex, in those parts of the world (Levitz, 1991).

There is no remarkable differences in incidence of cryptococcosis related to age, race or occupation. However, the male preponderance has been reported (Rippon, 1988). There are some reports stating that cryptococcosis in patients with AIDS occurs with increased frequency among blacks, intravenous
drug users and residents of the southern states immediately east of the Mississippi river (Horsburgh and Selik, 1988; Castro et al., 1988).

2.9.6 Epidemiology of Cryptococcosis in India

The first case of cryptococcosis in India was reported by Balakrishna Rao and Lilauwala (1952), subsequently a number of isolated case reports on cryptococcosis have appeared from various parts of the country (Khan et al., 1959; Sinha and Barua, 1960; Padhye and Thirumalachar, 1961; Basu Mallik and Nandi, 1961; Koshi et al., 1964; Subramanian et al., 1965; Aikal et al., 1967). However, some studies with larger series started appearing only after 1985 from different centres (Talwar and Meera, 1986; Banerjee et al., 1995; Chakrabarti et al., 1995; Khanna et al., 1996). But in-depth prevalence study and characterisation of clinical and environmental isolates have not been done.

Some investigators (Pal, 1989a and 1989b; Dhinda et al., 1994) isolated C. neoformans from environmental sources, all of which were var neoformans. Pal et al. (1991) reported that the ‘α’ mating type occurs more frequently in both clinical and environmental isolates. Padhye et al. (1993) reported that of 18 clinical isolates, 15 belonged to var neoformans and 3 belonged to var gattii. It was the first documented record of the var gattii occurring in India. Chakrabarti et al. (1997) reported the first isolation of var gattii from Eucalyptus camaldulensis in India, which were serotype B.
2.10 **HUMAN CRYPTOCOCCOSIS**

*C. neoformans* is not a part of the normal microbial flora of human and it is only transiently isolated from persons with no pathologic features. To be classified as a pathogen, an organism must be able to cause infection under certain conditions. By this definition, *C. neoformans* can certainly be classified as a pathogen. Because the immunodeficiency are more susceptible than the immunocompetent to infection with this yeast-like organism, *C. neoformans* is frequently referred to as an opportunistic pathogen (Table 1). The factors that make *C. neoformans* a pathogen can be divided into two major groups. The first comprises the basic characteristics needed to establish an infection and survive in the human host and the second comprises the virulence factors that affect the degree of pathogenicity (Duperval *et al.*, 1977; Mitchell and Perfect, 1995).

2.10.1 **Pathogenesis**

Generally the organism enters the host by the respiratory route in the form of a dehydrated haploid yeast or basidiospores as an infectious propagule. After sometime in the lungs, the organism spreads haematogenously to extrapulmonary tissues. Since *C. neoformans* has a special predilection for the CNS, most frequently diagnosed form of the disease is cryptococcosis of CNS. The reason for predilection of *Cryptococcus* for the CNS has not been explained, but it is believed that the organism probably encounters less cellular (phagocytic) response there and it has been theorised that selective nutritional
Table 1: Opportunistic infections in AIDS

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<th>Symptom complex</th>
<th>Likely pathogens</th>
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<tr>
<td><strong>Pulmonary</strong></td>
<td><em>Pneumocystis carinii</em></td>
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<td><em>Cytomegalovirus</em></td>
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<td><em>Mycobacteriae</em></td>
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<td></td>
<td><em>Cryptococcus neoformans</em></td>
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<td><strong>Central nervous system</strong></td>
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<td><strong>Chronic meningitis</strong></td>
<td><em>Cryptococcus neoformans</em></td>
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<td><em>Mycobacteriae</em></td>
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<td><em>Toxoplasma gondii</em></td>
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<td><em>Histoplasma capsulatum</em></td>
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<td><strong>Diffuse encephalitis</strong></td>
<td><em>Human immunodeficiency virus</em></td>
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<td><em>Toxoplasma gondii</em></td>
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<td></td>
<td><em>Progressive multifocal leukoencephalopathy</em></td>
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<td></td>
<td><em>Mycobacteriae</em></td>
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<td><strong>Gastrointestinal</strong></td>
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<td><strong>Stomatitis</strong></td>
<td><em>Cytomegalovirus</em></td>
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<td><strong>Esophagitis</strong></td>
<td><em>Herpes simplex</em></td>
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<td><em>Candida albicans</em></td>
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<td><strong>Dysentery-like</strong></td>
<td><em>Cryptosporidium</em></td>
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<td><em>Isospora belli</em></td>
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<td><strong>Colitis</strong></td>
<td><em>Cytomegalovirus</em></td>
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<td><em>Mycobacteriae</em></td>
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<td><em>Clostridium difficile</em></td>
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<td><strong>Fever of unknown origin</strong></td>
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<td><em>Mycobacterium avium-intracellulare</em></td>
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<tr>
<td></td>
<td><em>Other Mycobacteriae</em></td>
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</table>

Source: Bessen et al. (1988)
factors for the yeast are present in the CNS. Entrance of the organism through the skin and naso-pharyngeal mucosa is possible but is also considered extremely rare (Rippon, 1988).

2.10.2 Clinical Manifestations

2.10.2.1 Pulmonary cryptococcosis

Pulmonary cryptococcosis include patchy pneumonitis, single or multiple nodules, lobar consolidation and large-mass lesions mimicking carcinoma. Cavitation and pleural effusions are less common. The course of pulmonary cryptococcosis is also variable. In patients with normal host defenses, it resolves spontaneously and in contrast, pulmonary cryptococcal disease in immunocompromised hosts tends to be progressive and severe. Patients have no symptoms or a minority may experience cough, sputum production, weight loss or low-grade fever (Rippon, 1988; Joseph et al., 1993; Mulanovich et al., 1995).

2.10.2.2 CNS cryptococcosis

Meningitis, usually subacute or chronic in nature, is the most common manifestation of CNS disease. Cryptococcomas are seen in about 5 to 20% of patients, most typically in those with meningitis. Cryptococcomas appear to be less common in patients with AIDS than in other patient groups. Because up to 20% of patients with cryptococcomas have no sign or symptoms of focal neurological disease or increased intracranial pressure. Meningoencephalitis
is an uncommon form, is a fulminant, rapidly developing infection, often leading to coma and death within a short time.

The usual progression of symptoms are fever, headache, stiff neck, and disorientation are accompanied by spinal fluid that is typically clear, increased opening pressure, presence of mononuclear cells, elevated protein and normal or reduced chloride and sugar.

Complications of CNS cryptococcosis include hydrocephalus, visual disturbances including blindness, hearing loss, other cranial nerve palsies, ataxia, seizures and dementia. The mortality rate ranges from 15 to 30% and most deaths occur within the first several weeks of illness (Rippon, 1988; Dismukes, 1993; Powderly, 1993).

2.10.2.3 Miscellaneous forms

The skin, skeletal system and prostate gland follow in frequency of involvement after the lungs and CNS. Cryptococcal infection involves skin is about 10% in patients with AIDS and it may be the first sign of infection (Handa et al., 1996). Cryptococcal skin lesions can be highly variable in appearance and include papules, pustules, nodules, ulcers and draining sinuses. Osteomyelitis is more common than septic arthritis. The prostate gland appears to be an important reservoir of infection, which may serve as a source of relapse after completion of apparently successful primary therapy (Dismukes, 1993).
2.11 **ANIMAL CRYPTOCOCCOSIS**

Naturally acquired cryptococcosis has also been recorded in animals such as cattles, horses, dogs, cats, frogs, ferrets, monkeys, goats, pigs, birds, mice (Carter and Young, 1950; Emmons, 1952; Sacquet et al., 1959) and even in reptiles (McNamara et al., 1994).

2.12 **LABORATORY DIAGNOSIS**

Delay in the diagnosis and failure to institute treatment early in cases of cryptococcal meningitis may result in complications leading to an early death of the patients, as cryptococcal meningitis is a potentially fatal disease. While the diagnosis of this disease in patients without AIDS does not pose much problem, the same may not hold good in cases of cryptococcosis with AIDS (Dismukes, 1988). The laboratory tests of value as diagnostic aids include direct microscopy, isolation of the agent in culture and serodiagnosis.

2.12.1 **Microscopic Examination**

Cerebrospinal fluid (CSF), sputum, aspirates from skin lesions and other appropriate specimens are examined directly in an India ink or nigrosin preparation for the presence of yeast cells with capsules.

2.12.2 **Culture**

*C. neoformans* can be isolated on most laboratory mycological media. The isolated organism is identified depending on a combination of morphological, physiological, nutritional criteria and animal pathogenicity in mice.
In general, isolation of *C. neoformans* from clinical specimens is not difficult, as the fungus grows well on various culture media at 25°C and 37°C. However, for specimens that may be heavily contaminated such as sputum from patients with AIDS, differentiation of *C. neoformans* from other yeasts (eg. *C. albicans*) and bacteria can be time consuming if routine fungal isolation media such as Sabouraud's dextrose agar (SDA) are used.

Staib (1962) reported the discovery of the BCE developed by *C. neoformans* when cultured on the niger seed (*Guizotia abyssinica*). This led to a new diagnostic and epidemiologic implications (Staib, 1963a and 1963b; Staib and Seibold, 1988; Staib and Heissen, 1989).

Generally Staib's agar (*Guizotia abyssinica* creatinine agar, Niger seed agar or Bird seed agar) medium has been widely and successfully used by various investigators and sometimes with minor modifications to isolate *C. neoformans* from clinical specimens. On Staib's agar, *C. neoformans* colonies are recognised by their brown pigment and colonies of other yeast species remaining uncoloured.

Brilliande et al. (1979) reported that, the addition of methyl violet to Staib's agar, improved the isolation of *C. neoformans* from contaminated specimens. Subsequently, several other media such as Buffered agar media containing caffeic acid and glucose (Paliwal and Randhawa, 1981), a medium with esculin and an addition of biphenyl (mold inhibitor) to Staib's agar (Schönheyder and Stenderup, 1982) and use of sunflower seed instead of niger
seed (Pal and Baxter, 1985) have been used for the isolation of *C. neoformans* from clinical or environmental specimens.

Identification of *C. neoformans* by negative staining and culture methods have their own limitations. The demonstration of organisms by negative staining may not always be successful, the cultural isolation and confirmation may take many days to weeks. Therefore serological technique such as the detection of capsular antigens of *C. neoformans* in body fluids is rapid and helps for an early diagnosis.

### 2.12.3 Serologic Tests

The detection of circulating capsular antigen of *C. neoformans* in serum and CSF is a sensitive and specific test for the rapid diagnosis of cryptococcosis (Diamond and Bennett, 1974). The technique described by Bloomfield *et al.* (1963) for detection of the antigen by the latex agglutination (LA) test, has become the most trusted serological method (Kaufman and Blumer, 1973). Koshi *et al.* (1989) developed a Co-agglutination (Co-A) test for the same purpose. Antibodies to *C. neoformans* occurs in the serum of only about 30% of cases, but such tests are of less value than the detection of antigen (Bindschadler and Bennett, 1968).

### 2.12.4 Skin Test

Several investigators have attempted to prepare cryptococcal antigen that would specifically stimulate elements of the delayed-type immune
response (Bennett et al., 1965; Newberry et al., 1967; Atkinson and Bennett, 1968; Graybill and Alford, 1974). A study by Atkinson and Bennett (1968) revealed that cryptococcal antigen utilised for delayed type skin tests elicited positive reactions in up to 91% of patients with cryptococcosis. But the diagnostic and prognostic value of the skin test is still uncertain.

2.13 PROGNOSTIC FACTORS

Cryptococcosis in patients with AIDS is extremely difficult to eradicate and has often been associated with high failure and relapse rate (Holmberg and Meyer, 1986). The acute mortality (during initial therapy) due to AIDS associated cryptococcosis is 10 to 25% and the 12 month survival rate among the patients in 30 to 60% (Chuck and Sande, 1989; Clark et al., 1990).

It is suggested that, a low serum or CSF antigen titre (1:4 or less) at onset of therapy has a favourable prognostic sign (Bennett et al., 1964). Antigen titres of 1:8 or greater in clinical specimens are to be correlated with active disease (Kaufman, 1983). A titer of over 1:400, as is often seen in highly immunocompromised subjects, carry a poor prognosis and management of such cases become very difficult (Roberts and Mackenzie, 1985).

2.14 TREATMENT

Cryptococcal meningitis is generally fatal if untreated and early aggressive treatment with a combination of amphotericin B and 5-flucytosine provides the best opportunity of cure (Bennett et al., 1979; Tjia et al., 1985). Patients treated with amphotericin B alone had a higher mortality (ranging
between 30% and 48%) than those who were treated with the combination therapy. The organism commonly becomes resistant to 5-flucytosine when used alone. The azole group of antifungal drugs has enhanced the therapeutic armamentarium against *C. neoformans* and the newer triazoles, fluconazole and itraconazole are the most effective agents in this class (Dismukes, 1993). Surprisingly, even garlic tablets were used as supplementary treatment with low mortality rate (Anon, 1980; Tjia *et al.*, 1985).

Whether antifungal resistance can develop in *C. neoformans* through the administration of chronic antifungal maintenance therapy is not clear (Brandt *et al.*, 1996). On the other hand, emergence of clinically significant resistance to amphotericin B (Powderly *et al.*, 1993) or fluconazole (Paugam *et al.*, 1994) has been reported in individual cases of recurrent cryptococcosis.

Currie *et al.* (1995) reported that, the chronic use of fluconazole for long term suppressive therapy in AIDS patients may become a factor in the selection of cryptococcal isolates that are more resistant to azole therapy. Resistance to 5-flucytosine in *C. neoformans* was also employed as a useful genetic marker in a previous study (Kwon-chung, 1977), which demonstrated that typical isolates were heterothallic.

### 2.15 MOLECULAR STUDIES IN *C. NEOFORMANS*

In order to discriminate the isolates, that are morphologically and physiologically indistinguishable, a stable and sensitive genetic marker is needed. The different molecular approaches have been used previously in
Cryptococcal epidemiological studies include electrophoretic karyotyping (Kwon-chung et al., 1992b), mitochondrial DNA probes (Varma and Kwon-chung, 1989), genomic DNA probes (Polacheck et al., 1992) and allelic variations at the URA 5 locus (Casadevall et al., 1992). Another method was developed to discriminate between strains of the same organism by the random amplification of polymorphic DNA (RAPD) with the PCR using oligonucleotide primers (Williams et al., 1990). Use of this technique to type C. neoformans, has demonstrated marked heterogenicity among strains (Crampin et al., 1993; Brandt et al., 1995). The molecular studies on the C. neoformans are rather few and remains to be an explorable field.