

Medical Microbiology and Infectious Diseases:-

A STUDY

OF

CRYPTOCOCCUS NEOFORMANS

VARIETIES

GATTII AND NEOFORMANS

IAN F. LAURENSEN

Submitted for the degree of Doctor of Medicine

University of Edinburgh

1997-8

ABSTRACT

Cryptococcus neoformans is a fungus that can be biotyped as variety (var.) *gattii* or var. *neoformans*. Four primary serotypes, A,B,C, and D have been described. *Cryptococcus neoformans* var. *gattii* is predominant in the tropics and subtropics.

Environmental investigations have established the association of *Cryptococcus neoformans* var. *gattii* with *Eucalyptus camaldulensis*, *E. tereticornis*, *E. gomphcephala*, *E. rudis* and most recently *E. blaykeli*. Worldwide, *C. neoformans* var. *neoformans* has been associated with avian sources. This thesis examines *C. neoformans* and the meningitis it causes, based predominantly in Papua New Guinea (PNG).

Investigations of possible plant, mammal and avian associations in PNG, revealed that few *E. camaldulensis* survive experimental planting, while *E. tereticornis* is endemic. In Port Moresby, *E. confertiflora*, *E. papuana* and *E. alba* are common. Examination of 1130 specimens from plant, bird and animal sources, failed to identify the ecological niche of *C. neoformans* in PNG.

Epidemiological studies of 96 patients presenting with cryptococcal meningitis to Port Moresby General Hospital (PMGH) from 1972-1993 showed an annual incidence of 33 cases per million population of Central Province and the National Capital District. Twenty one of these are infected with *C. neoformans* var. *gattii* and 12 with *C. neoformans* var. *neoformans*. On average 11 cases present annually

to PMGH. Geographical clustering occurred amongst those from some parts of Gulf and Central Provinces, with a male predominance. Possible seasonal variation was found, with increased presentation rates in May/June and September/October. This could reflect seasonal exposure to *Cryptococcus neoformans* var. *gattii*. The pattern of childhood infection may result from varying exposure or susceptibility.

Eleven sequential patients with cryptococcal meningitis were diagnosed and isolates biotyped. Seven were var. *gattii* (one patient with diabetes mellitus) and four were var. *neoformans*. The latter came from adult patients with HIV 1 infection, tuberculosis or *Plasmodium vivax* malaria. Significant clinical findings were headache, fever, meningism, vomiting, photophobia, papilloedema and cranial nerve lesions. Five patients (45.5%) died; the 2 var. *neoformans* HIV 1 infected men and 3 adult var. *gattii* patients. In PNG where var. *gattii* has been predominant in the immunocompetent, var. *neoformans* is emerging as the predominant biotype amongst immunosuppressed patients, notably those with HIV1 infection.

Laboratory comparison in Edinburgh confirmed that var. *gattii* were usually more mucoid than var. *neoformans* colonies and that this was correlated with capsule size.

A neutrophil-monolayer assay was unable to demonstrate a clear difference in varietal binding.

Cerebrospinal fluid examination by microscopy and cryptococcal latex antigen failed to detect an apparently acapsulate cryptococcal isolate in an HIV infected

patient in Edinburgh. The isolate was detected by culture on malt extract agar. The human neutrophil-cryptococcal assay subsequently confirmed the presence of capsule.

These studies confirm the high prevalence in PNG of meningitis caused by *C. neoformans* variety *gattii* in immunocompetent individuals, and the presence of potential mammal and plant sources similar to those found in Australia. Although cases are clustered geographically, the source(s) of *C. neoformans* remain elusive. Alone, the small series of prospectively studied patients cannot indicate whether the course of meningitis caused by the two varieties of *C. neoformans* differs in the immunocompetent. Laboratory studies investigated the intriguing differences in pathogenesis between the two varieties and emphasise the importance of culture in clinical diagnosis.

The work described here has formed a foundation for further clinical, epidemiological and laboratory studies of *C. neoformans* meningitis in PNG and London, England.