Cryptococcosis in Australian Wildlife

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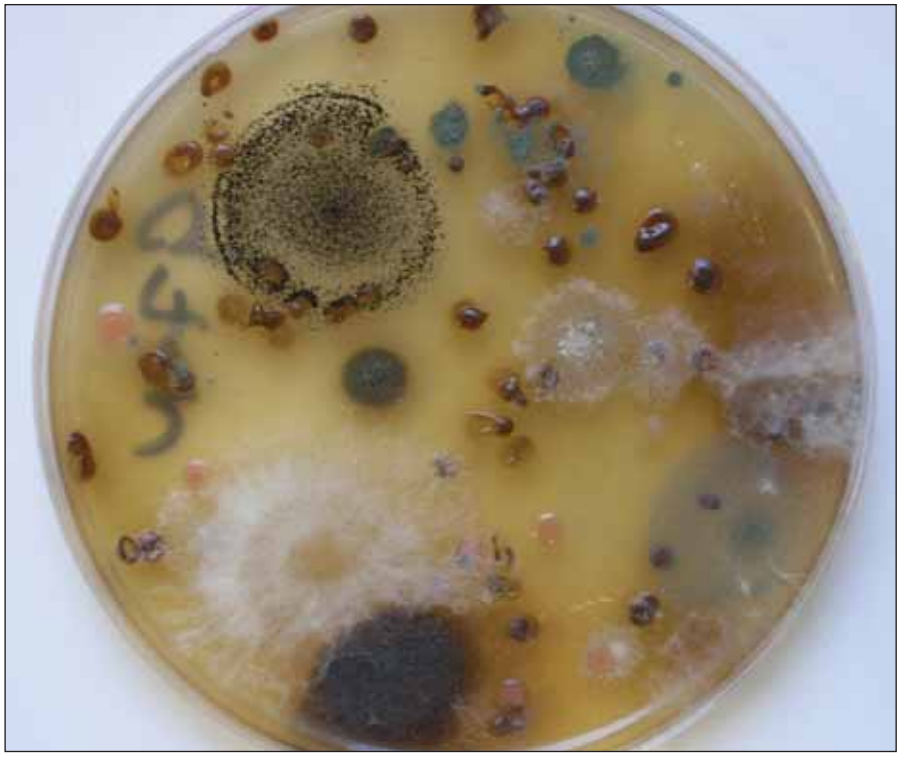
Cryptococcosis is the most important systemic fungal disease to affect mammalian hosts in Australia. Although recent taxonomic developments have somewhat confused the nomenclature of the causative agents, it is now generally accepted that the *Cryptococcus neoformans* complex may be divided into two separate species, namely *C. neoformans* and *C. gattii* (Table 1).

C. gattii is an important cause of cryptococcosis in animals and people in Australia compared with other regions of the world [5,6]. In Australia, the incidence ranges up to 19 cases per 10^6 population (50% caused by *C. gattii)* in the Northern Territory [6]. Cryptococcosis caused by *C. neoformans* var *grubii* in people, tends to be associated with defective cell mediated defences. However, overt immune compromise does not appear to be an important feature of disease caused by *C. gattii* [6] - it is an environmentally acquired disease, usually sporadic in occurrence. While outbreaks from a common environmental source have been recorded in animals and people [7-9], anthropozoonotic or animal-animal transmission has not.

Although *C. n.* var *grubii* is the most common cause of cryptococcosis in cats and dogs in Australia, where it appears to produce disease predominantly in apparently immunocompetent individuals, approximately 30% of cases in cats and 17% of cases in dogs are caused by *C. gattii* [10].

Figure 1. Birdseed agar plate of nasal swab from a koala. Note the mucoid brown colonies consistent with the appearance *C. gattii*. Definitive identification requires further characterisation including assimilation tests and growth on CGB agar



Whilst there are only small numbers of documented cases of cryptococcosis in other domestic animals, *C. gattii* also appears to be a significant cause in ferrets [11], horses [12,13] (A MacLachlan pers comm), goats [14], alpacas [15] and sheep [16].

The cryptococcal story in Australian wildlife is both intriguing and exciting, as *C. gattii* accounts for over 90% of cases as determined by culture and/or immunohistochemistry [17]. Of the 72 cases of cryptococcosis in Australian wildlife brought to our attention, the causative agent was shown to be *C. gattii* in 44 cases (34 koalas, 2 Australian king parrots, 2 corellas, 1 Major Mitchell cockatoo, 1 Gilbert's potoroo, 1 quokka, 1 feather tail glider, 1 sticknest rat, 1 eastern water skink). In only 3 cases (1 gang gang cockatoo, 1 potoroo, 1 sugar glider) has *C. neoformans* var *grubii* been implicated based on immunohistology, while the remaining 24 cases had insufficient material to be classifiable.

Importantly, all koalas with cryptococcosis studied to date have been shown to have *C. gattii* disease based on either culture or immunohistochemistry. Furthermore, in all cases where isolates were submitted for molecular testing, the VGI molecular type was diagnosed. Although this is not surprising, given the association between this species, the molecular type (VGI) and eucalypts (especially *E. camaldulensis* and *E. tereticornis* – for a full list of eucalypt associations, see *Microbiology Australia* 23:29-30), it is interesting that in our work, no *C. neoformans* infections have been recorded, even in suspected immunocompromised koalas living close to the Sydney CBD. Likewise, no VGII infections have been recorded, although this is in accord with the observation that

Figure 2. Histopathology of early nasal mucosal invasion by *C. gattii.* Low power image of nasal mucosa with inset of high power view. It is envisaged that subclinical disease in koalas is represented by this degree of invasion.

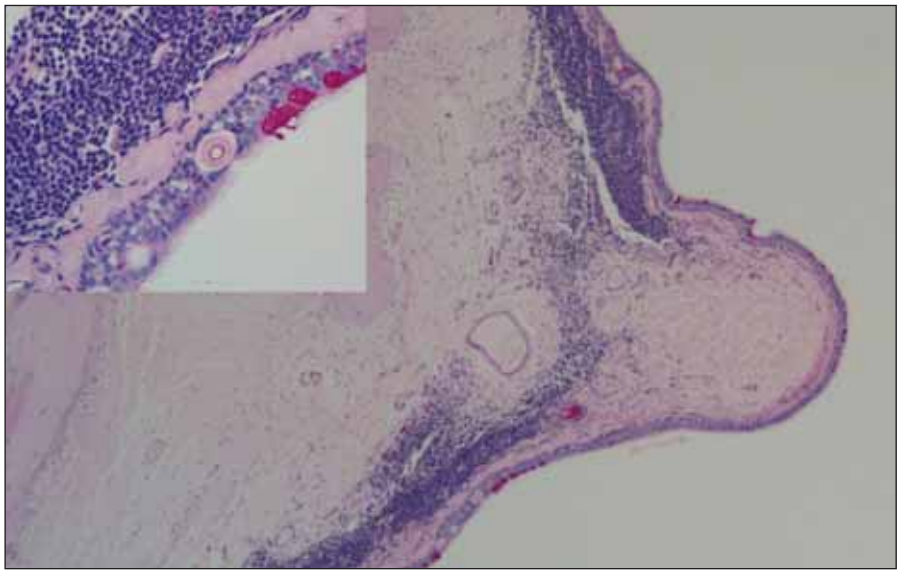


Figure 3. Koala with nasal cryptococcosis. (Photo Courtesy of Robert Johnson.) Note the distortion of the rostral left side of the face.

Table 1. Evolution of nomenclature for *Cryptococcus* spp.

Kwon Chung *et al.* (1982)[1]	**Franzot *et al.* (1999)[2]**	**Boekhut *et al.* (2001)[3]**	**Kwon Chung *et al.* (2002)[4] Currently Accepted**
C. *n*. var. *neoformans* (serotypes A, D, AD)	C. *n*. var. *grubii* (serotype A)	C. *neoformans*s (serotypes A, D, AD)	C. *n*. var. *grubii* (serotype A)
	C. *n*. var. *neoformans* (serotype D)		C. *n*. var. *neoformans* (serotype D)
C. *n*. var. *gattii* (serotypes B, C)	C. *n*. var. *gattii* (serotypes B, C)	C. *bacillisporus* (serotypes B, C)	C. *gattii* (serotypes B, C)

koalas are not native to either Western Australia or the Northern Territory (Arnhem Land), places where VGII is known to be associated with increased prevalence of cryptococcosis in people. Environmental associations for VGII strains have not been well established.

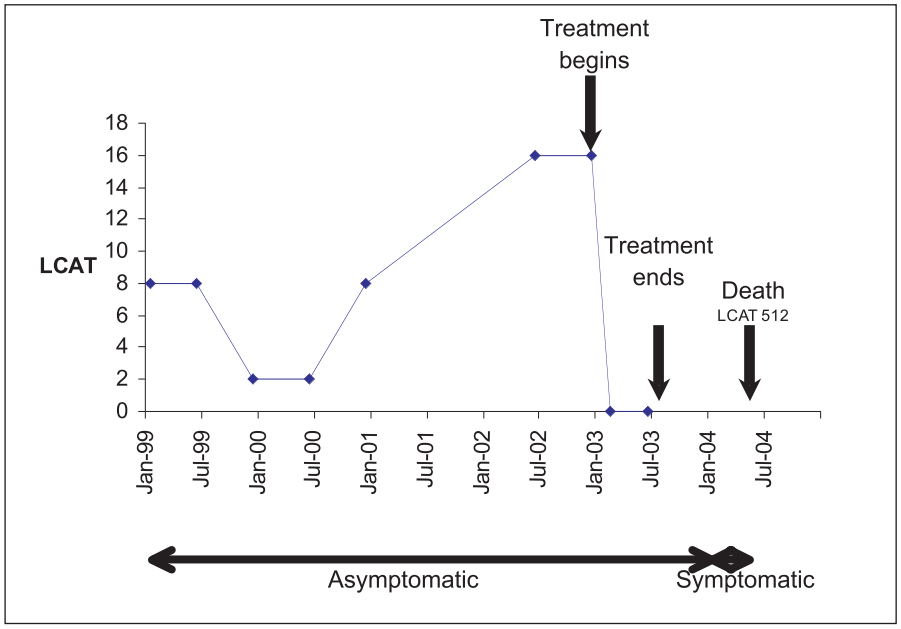
We have been studying *C. gattii* infections in koalas for many years, as this represents a unique 'model system' for charting the progression from nasal colonisation (Figure 1) to subclinical, and sometimes to symptomatic disease. It has become apparent that clinical disease represents only a minor proportion of outcomes of the Host-Pathogen-Environment interaction (HPE). By far the most common result of this dynamic interaction is the establishment of colonisation of the nasal passages or subclinical localised tissue invasion of the sinonasal region (Figure 2). Although it is impossible to be definitive regarding the cut-off between subclinical and clinical disease, with respect to a single serum cryptococcal antigen titre, what the koala story tells us is that subclinical cryptococcosis is likely to be an important entity in all species. This is reinforced circumstantially by the findings concerning exposure of young children to *C. neoformans* in New York City[18]. Of great interest is determination of host, pathogen and environment factors that tip the balance towards symptomatic disease in the case of *C. gattii.*

We continue to monitor certain captive populations in which it appears that koalas somehow amplify the numbers of *C. gattii* in their immediate environment. We are unclear whether the same phenomenon occurs in nature, but the importance of cryptococcosis was recently emphasised by a retrospective study of over 1,100 koala necropsies conducted since 1980, where approximately 4% of animals were diagnosed at necropsy with cryptococcosis[19] Funding to support cryptococcosis research would be much more forthcoming if a similar statistic existed for human patients.

Ongoing studies will help to determine more of the pathogen, environmental and host factors that influence the pathogenesis of cryptococcosis. We are in the process of determining whether therapy with azole antifungals is capable of reducing nasal colonisation – thus far, itraconazole therapy would appear incapable of achieving this end – and whether treating subclinically affected individuals facilitates resolution of inapparent disease. Unfortunately, our present record of treating koalas with cryptococcosis suggests improvement is needed. One recent case with localised invasive mycotic rhinitis eventually succumbed despite many months of treatment with itraconazole and surgical debulking of the major lesion (Figure 3). Another recent case with only subclinical disease was treated pre-emptively in response to a rising cryptococcal antigen titre (Figure 4); despite treatment with itraconazole to the endpoint of a negative serum antigen titre for 6 months, the koala subsequently developed fatal central nervous system disease. Clearly we have a long way to go in developing treatment regimens applicable to captive koalas, and studies of fluconazole for this purpose are planned for later this year.

Naturally-occurring koala cryptococcosis appears to be a perfect model of disease for the investigation of cryptococcosis caused by *C. gattii* in people and animals. We suspect that, as in people, overt host immune compromise is not likely to be an important feature of cryptococcosis caused by *C. gattii* in Australian wildlife. Disease is more likely to be associated with exposure to unusually large numbers of infectious propagules and/or concurrent variables such as nutritional, physiological or environmental factors that impinge on host defences. The role of the pathogen genotype is currently unclear. Determining the critical features of the HPE interaction, including investigation of antibody and cytokine responses, environmental biotic associations and pathogen genotype, is an ongoing process. Hopefully such studies will reveal much about the behaviour of this disease in all species. **We currently have eight staff and graduate students in our koala infectious disease team, and have funding for an additional committed PhD scholar to join us.**

Figure 4. Serial LCAT chart.



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