

Cryptococcosis in koalas

Fact sheet

Introductory statement

Cryptococcosis is an important systemic mycosis of animals and people. It is the second most common infectious disease of koalas overall, after chlamyphilosis, but appears more frequently than chlamyphilosis in captive populations.

Aetiology

Cryptococcosis is caused by two species of encapsulated, saprophytic fungus: *Cryptococcus neoformans* and *Cryptococcus gattii*. Historically these two species were regarded as varieties of the same species named *Cryptococcus neoformans* var. *neoformans* and *Cryptococcus neoformans* var. *gattii*. Almost all koala cryptococcosis cases studied to date have been caused by *C. gattii* (Krockenberger 2012, Krockenberger et al 2005).

Natural hosts

Cryptococcus spp. have a very broad host range. Disease has been reported in a large number of mammalian species and rarely in reptiles. While cryptococcosis does not occur in birds *C. neoformans* is frequently found in pigeon droppings (Pare and Jacobson 2007, Burek 2001).

World distribution

Cryptococcus spp. have a worldwide distribution.

Occurrences in Australia

Cryptococcus spp. are found throughout the sub-tropic and temperate regions. *C. gattii* is particularly prevalent in the Murray Darling river system and temperate regions of south-east Australia (Krockenberger 2012).

Epidemiology

Infections are acquired from the environment and the disease is not considered to be contagious. Transmission is by inhalation of airborne cryptococcal organisms. Clinical disease is usually associated with high environmental loads.

The fungus is dimorphic and exists as either a yeast form or a hyphal form. The yeast form is large (5-50 µm in diameter) and has a thick extracellular mucopolysaccharide capsule. It is not easily aerosolised and is generally too large to evade normal innate respiratory defences. Therefore, it is not highly infective but is the predominant form in diseased tissue.

The hyphal form produces small basidiospores, 1-2 µm in diameter that can evade host defences and penetrate to the alveoli causing disease.

In many infections in koalas the fungus can be cultured from the mucosal surface of the nasal cavity or the skin without any evidence of tissue invasion or disease. Some cases progress to tissue invasion but still with no evidence of disease. Fewer still develop clinical disease mostly involving the respiratory tract or central nervous system.

C. neoformans is widespread in the environment while *C. gattii* is commonly associated with a number of *Eucalyptus* spp. trees, particularly the river red gum (*E. camaldulensis*), but it has also been isolated from *E. teretecornis*, *E. gomphocephala*, *E. blakelyi*, *E. rudis*, *E. microcorys*, *E. grandis*, *Angophora costata* and *Syncarpia glomulifera* (Krockenberger 2012, Blanshard and Bodley 2008).

Cryptococcus spp. survive well in the environment, more than 24 weeks in a soil and water mixture held at 20°C and 30°C (Theraud et al 2004). *C. neoformans* can remain viable for over two years in pigeon droppings. It is killed by moist heat of 121°C for a minimum of 20 minutes or dry heat of 165-170°C for two hours (Spickler 2013).

Clinical signs

Clinical signs include depression, anorexia and weight loss. When the nasal cavity is affected epistaxis, a mucopurulent nasal discharge and facial distortion may occur. Involvement of the caudal nasal cavity and lungs results in stertor and dyspnoea.

Neurological signs include blindness, nystagmus, limb paresis, opisthotonus and seizures (Krockenberger 2012, Ladds 2009, Blanshard and Bodley 2008).

Diagnosis

Diagnosis is based on clinical signs presenting in conjunction with laboratory test results. Normal koalas will display nasal colonisation by *C. gattii* without tissue invasion or clinical signs of disease. Therefore, culture alone is insufficient to diagnose cryptococcosis. Evidence of tissue invasion and/or host response must be documented either microscopically or serologically (Krockenberger 2012).

Pathology

Grossly, cryptococcosis may form circumscribed to diffuse, focal or multifocal nasal cavity masses that extend into the surrounding tissues including the maxillary sinuses, the subcutaneous tissues of the face and lips, the oral cavity and pharynx, the cribriform ethmoidal turbinates and the cranial cavity. Occasionally nodules may also be found on the ears, hind limbs and feet. Masses are generally gelatinous but may also be firm. Lung lesions include oedema, erythema, emphysema and firm pale nodules. Nervous system lesions include hyperaemia, oedema and swelling of the brain with dilation of the lateral ventricles.

Histologically there are abundant, usually budding, encapsulated spherical organisms. The organisms are 5-25 µm in diameter (10-50 µm in diameter including the capsule). The capsule does not stain with haematoxylin and eosin and stains poorly with PAS. The inflammatory response is variable and tends towards granulomatous with predominantly macrophages and multinucleated giant cells. Often there are large numbers of organisms with very little host response. The response tends to become more marked after therapeutic intervention (Krockenberger 2012, Ladds 2009).

Differential diagnoses

Differential diagnoses include other causes of nasal discharge such as *Bordetella bronchiseptica*, and other causes of facial deformities such as cranio-facial tumours.

Laboratory diagnostic specimens

A complete necropsy should be performed on all dead koalas. Collect a range of tissues including brain, nasal passages and any obvious lesions, and submit them in formalin for histopathology.

Masses from live koalas should be aspirated or biopsied.

Nasal swabs may be submitted in transport medium for culture.

Serum, urine or cerebrospinal fluid can be collected and submitted for examination with the latex-cryptococcal antigen test (LCAT).

Laboratory procedures

Aspirated or biopsied masses should be stained with Diff Quik, new methylene blue, Gram or India ink and examined microscopically for the presence of budding yeast-like organisms surrounded by a clear capsule.

Immunohistochemistry or PCR can be used to differentiate *C. neoformans* from *C. gattii* in formalin fixed tissue.

False positives are uncommon with the LCAT but may occur with *Klebsiella* spp. or *Trichosporon* spp. infections. False negatives can occur with localised lesions. Generally, titres ≥ 128 indicate clinical disease while titres ≤ 64 are likely subclinical. Any titre ≥ 2 is considered evidence of tissue invasion. Koalas with positive antigen titres should be retested monthly. Sustained or rising titres justify further investigation or the initiation of treatment (Krockenberger 2012, Blanshard and Bodley 2008).

Treatment

Treatment involves the administration of amphotericin B (0.5 mg/kg in 300 ml 2.5% dextrose and 0.45% NaCl SC twice weekly) in conjunction with either itraconazole (100 mg/day PO) or fluconazole (50-100 mg BID PO). Therapy time ranges from two to 18 months so nutrition needs to be optimal and other stresses minimised. Nephrotoxicity associated with amphotericin B treatment has not been seen in koalas. CNS involvement is associated with a poor prognosis (Blanshard and Bodley 2008).

Prevention and control

To maintain a cryptococcus free captive koala population all new koalas must be isolated and held in quarantine for a minimum of 45 days. They should be chemically restrained, examined and tested twice with the LCAT at least 21 days apart. See the Australian Government “Conditions For The Overseas Transfer Of Koalas” document (2009) which can be found at:

<https://www.environment.gov.au/system/files/resources/e54cd87f-22b5-46e9-8a21-5e16f7c645cb/files/koala-export-conditions.rtf>

Fungal environmental load should be reduced. Enclosures that have housed *Cryptococcus* spp. positive koalas should have their branches removed and replaced. If this is not possible they should be disinfected with 0.5% chlorhexidine or 70% ethanol (Krockenberger 2012, Rutala et al 2008, Theraud et al 2004).

Surveillance and management

There is no targeted surveillance program or AUSVETPLAN for koala cryptococcosis.

Statistics

Wildlife disease surveillance in Australia is coordinated by Wildlife Health Australia. The National Wildlife Health Information System (eWHIS) captures information from a variety of sources including Australian government agencies, zoo and wildlife parks, wildlife carers, universities and members of the public. Coordinators in each of Australia's States and Territories report monthly on significant wildlife cases identified in their jurisdictions. NOTE: access to information contained within the National Wildlife Health Information System dataset is by application. Please contact admin@wildlifehealthaustralia.com.au.

There are currently 11 reports of koala cryptococcosis in the National Wildlife Health Surveillance Database. Of these, eight are from Queensland and three are from NSW. Wildlife Health Australia is interested in receiving reports of this condition in koalas (contact admin@wildlifehealthaustralia.com.au).

One study found approximately 4% of over 1100 koalas necropsied since 1980 to be diagnosed with cryptococcosis (Krockenberger et al 2005).

Another study investigated koalas from four sites around NSW (Coffs Harbour Zoo, Taronga Zoo, Port Macquarie and western Sydney wildlife parks) and found evidence of cryptococcal nasal colonisation in 97 of the 237 koalas sampled. The same study found no evidence of cryptococcal colonisation in the 58 koalas sampled from Phillip Island, Victoria (Krockenberger et al 2002).

Research

As most infected koalas do not go on to develop clinical disease research is required to determine which host, pathogen and environmental factors tip the balance towards symptomatic disease. These include pathogen genotype, environmental load, nutritional and other stressors, antibody and cytokine responses, retrovirus infection and environmental biotic associations.

It is also not clear if treating subclinically affected individuals facilitates the resolution of inapparent disease and prevents the infection from becoming clinically apparent. Treatment success of clinical cases is not high and the effectiveness of newer antifungal agents, such as voriconazole, needs to be assessed.

Human health implications

Cryptococcus spp. are generally opportunistic mostly affecting people with depressed immune systems. Humans can be affected by both *C. neoformans* and *C. gattii*. Infections are usually acquired from the environment rather than infected hosts. *C. neoformans* infections are occasionally associated with exposure to pigeon droppings. Most *Cryptococcus* spp. infections are asymptomatic. Clinical signs are usually referable to the respiratory tract. Disseminated spread occurs more commonly in immunosuppressed patients and usually involves the CNS, eyes and skin (Spickler 2013).

Conclusions

While cryptococcosis is an important disease of koalas it appears to be a more significant problem in Queensland and NSW than other parts of Australia. Many apparently healthy koalas are colonised by the fungus and appear to live with it, showing no deleterious effects. Given the fungus's association with trees, particularly *Eucalyptus* spp. it seems likely that the koala has developed an almost symbiotic relationship with it that only allows clinical disease to occur when this balance is upset. Much remains to be discovered about the true nature of this relationship.

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To provide feedback on this fact sheet

We are interested in hearing from anyone with information on this condition in Australia, including laboratory reports, historical datasets or survey results that could be added to the National Wildlife Health Information System. If you can help, please contact us at admin@wildlifehealthaustralia.com.au.

Wildlife Health Australia would be very grateful for any feedback on this fact sheet. Please provide detailed comments or suggestions to admin@wildlifehealthaustralia.com.au. We would also like to hear from you if you have a particular area of expertise and would like to produce a fact sheet (or sheets) for the network (or update current sheets). A small amount of funding is available to facilitate this.

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