CRYPTOCOCCOSIS IN ANIMALS

Richard Malik
Centre for Veterinary Education
The University of Sydney
COSMOPOLITAN PATHOGEN WITH CATHOLIC TASTES

SUGAR COATED KILLER IN DESIGNER GENES
You have to understand its environmental niche.
ROADMAP FOR THIS TALK
Amoeba or nematode
Innate immunity

Vertebrate
Central nervous system and adaptive immunity

Human
Latency

Environment

Nature Reviews | Microbiology
“A sugar coated killer with designer genes”
Figure 8. Crypto PS is a lateral flow immunochromatography kit for cryptococcal antigen that is extremely sensitive and thus a useful screening test in the field.
PATHOGEN OF SOIL DWELLING AMOEBA

(SINGLE CELL ORGANISM)
Acanthamoeba: A host for other microbes

Viruses
(Mimivirus, adenovirus)

Bacteria
(Legionella pneumophila)

Yeast
(Cryptococcus neoformans)

Protozoa
(Cryptosporidium parvum)
Manipulate the maturation of macrophages and dendritic cells

A minimal inflammatory response

Higher concentrations of pro-inflammatory cytokines

‘Division of labour’

Neutralisation of ROS

Genome plasticity (microevolution)

Reproduction in clonal and sexual way

Hijack macrophages and dendritic cells

Kill the macrophage (sometimes)

Protean capsule

Titan cells

Vomocytosis

Lateral transfer

Extracellular vesicles

Cross blood:brain barrier in Trojan horses
PATHOGEN OF NEMATODES
Killing of Caenorhabditis elegans by Cryptococcus neoformans as a model of yeast pathogenesis

Elisabeth Mylonakis*, Frederick M. Ausubel†, John R. Perfect‡, Joseph Heitman** and Stephen B. Calderwood†‡

*Division of Infectious Diseases and †Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114, Department of ‡Genetics and ‡Microbiology and Molecular Genetics, Boston Children's Hospital, Boston, MA 02115, and †Department of Pathology
**Department of Molecular Genetics and Microbiology, and ††Howard Hughes Medical Institute, Duke University Medical Center, Durham, NC 27710

Contributed by Frederick M. Ausubel, September 16, 2002

We found that the well-studied nematode Caenorhabditis elegans can use various yeasts, including Cryptococcus neoformans and Cryptococcus gattii, as a food source. Producing similar body sizes compared with growth on its usual laboratory food source Escherichia coli, and dying within three days had the same outcome as Caenorhabditis elegans killed by C. neoformans or C. gattii. However, the yeast forms of Cryptococcus neoformans killed C. elegans, while the C. gattii species forms and the pseudohyphal capsule were much less effective. C. elegans gene expression analysis showed a role in C. elegans killing, similar to that described in C. elegans killing of Fusarium oxysporum. These results support the model that mammalian pathogenesis of C. neoformans may be a consequence of adaptations that have evolved during the interaction of C. neoformans with environmental predators such as free-living nematodes and amoebae and support that C. elegans can be used as a simple model host in which C. neoformans pathogenesis can be readily studied.

Studies of microbial pathogens in nonvertebrate hosts during the past decade have resulted in important insights into the immune evasion mechanisms of fungal and bacterial pathogens and host defense. It is now apparent that many of the same bacterial virulence factors are involved in pathogenesis in coevolutionarily distinct hosts, including plants, nematodes, and mammals (1–5). The nematode Caenorhabditis elegans has proven to be a particularly facile host for studying bacterial pathogenesis. For example, both Gram-negative and Gram-positive human bacterial pathogens kill C. elegans, but bacterial virulence factors important for mammalian pathogenesis are not always required in nematode killing (6). C. elegans can be used as a model for the identification of fungal pathogens. For example, frequent environmental exposure to C. neoformans, immunologically intact individuals spontaneously develop clinical disease. However, the incidence of infection rises dramatically in patients with AIDS and in Nodella T cells (7, 8). Study of the pathogenic mechanisms of C. neoformans has been enhanced substantially by the development of transformation protocols, homologous recombination, and strategies for genetic manipulation, and reproducible experimental models (9–12). The most important C. neoformans virulence factors identified so far include the polysaccharide capsule (36, 35), laccase (an enzyme essential for melanin production) (13), 30 kDa heat shock protein 70 (34, 36, 40), the α/β subunits of the mating type locus (41, 42), and pathogenesis-related (PR) proteins. In this paper, we show that C. elegans can be used to investigate C. neoformans pathogenesis in vivo using a number of virulence genes that are also important in mammalian signaling pathways. C. neoformans is often mediated in the environment (37), and melanization has been associated with protection against environmental conditions such as UV radiation and extremes of temperature (26). Melanin is also a potent free radical scavenger and melanization has been shown to protect C. neoformans from macrophages and has been associated with virulence in murine models (38–40). Melanin is produced when laccase (encoded by C. neoformans) catalyzes the oxidation of dopamine to quinones, which then polymerize to form melanin (36). Two other potential C. neoformans and C. neoformans strains (32–34), respectively, have been extensively studied (43). In C. elegans a strain.
PATHOGEN OF INSECT LARVAE
Figure 1. The larvae acquired from the supplier can be in several grades of conditions. (A) The larvae that arrive are a mixture of light-colored larvae with no markings and larvae with grey markings on the cuticle. Arrows indicated melanization. (B) To inject yeast cells, larvae selected for experiment use are light-colored larvae lacking any gray markings on the cuticle. (C) Larvae are held between the fingers, exposing the larval cuticles. A slight amount of pressure is applied to prevent larvae from moving during the injection. (D) The needle is inserted at the site of the pro-leg for inoculum delivery.

Figure 2. (A) There can be physical and motile changes in larvae post-infection with fungal cells. (A) Larvae infected with C. neoformans (left) remain static in color post-infection. By contrast, larvae infected with C. albicans (right) melanize after being infected. Arrows indicate areas of melanization. (B) A Kaplan-Meier plot of survival after infection with either C. neoformans or C. albicans shows that both pathogens cause a lethal infection. G. mellonello were infected with 104 cfu/larvae with 10 larvae per group. A control group was included in the assay in which G. mellonello were injected with PBS. Larvae infected with C. albicans were found to die faster than larvae infected with C. neoformans. Death of all 16 infected larvae was observed in C. albicans-infected larvae within 48 h but this was delayed to 68 h with C. neoformans infections.
PATHOGEN OF FISH
PATHOGEN OF REPTILES
CRYPTOCOCCOsis IN A COMMON ANAconda (EUNEcTExE MURINUS)

Tracey S. McNamara, D.V.M., Robert A. Cook, V.M.D., John L. Behler, Libero Ajellas, Ph.D., and Aviram A. Padhye, Ph.D.

Abstract: Systemic cryptococcosis was diagnosed in a common anaconda (Eunectes murinus) that died after a 2.5-mo history of progressive neurologic disease. Pathologic examination revealed granulomatous pneumonia and meningoencephalitis associated with yeast organisms morphologically consistent with Cryptococcus. Cryptococcus neofor mans was confirmed as the etiologic agent using a specific fluorescent antibody test for this pathogenic yeast. This is the first reported case of C. neofor mans infection in a poikilothermic host.

Key words: Cryptococcus neofor mans, common anaconda, Eunectes murinus, systemic cryptococcosis.

INTRODUCTION
Numerous mycotic infections have been reported in reptiles.1-13,28,31,36,37 Infestations of the skin, alimentary tract, and pulmonary system are most common. Reports differ as to the relative frequency of infections by various species of mycotic agents; however, aspergillosis, candidiasis, and zygomycosis are cited as common.1 We report a case of cryptococcosis in a common anaconda (Eunectes murinus). This is the first reported case of Cryptococcus neofor mans infection in a poikilothermic host.

CASE REPORT
History
A 10-yr old 30-kg captive-raised male common anaconda (NYZS 780059) was presented in October 1988 with a 3-mo history of anorexia. No previous abnormal behavior or significance had been noted. The animal was kept in the New York Zoological Park's Reptile House in its entire life; management protocols have been addressed elsewhere.36 The snake was transferred during growing to a series of enclosures of increasing size with a substrate of fiberglass, concrete, or a combination of the two. The snake was initially housed with several siblings but was separated at 2 mo of age and maintained in isolation until 22 mo of age when it was paired with a female of compatible size. Thereafter, it shared its facility with other individual females. Courting was first noted at 25 mo of age and regularly in following years. This specimen was observed copulating with a cage mate one time in April 1987. The snake was fed a diet of rats, chickens, adult chickens, rabbits, or guinea pigs. All items offered were fresh after being euthanized with CO2.

Medical treatment
On physical examination, the snake had difficulty righting itself and tremors were noted in the head and neck regions. The snake was thin and lethargic. A blood sample was obtained from the caudal vein using standard techniques,37 and a complete blood count, biochemical profile, and aerobic and anaerobic blood cultures were carried out. A 10-day course of chloromphenicol sodium succinate (Chloromycetin Sodium Succinate, Parke-Davis, Morris Plains, New Jersey 07950, USA) was initiated i.m. once daily, at a dose of 50 mg/kg.
Five days later, the snake was more active with no signs of righting problems. However, anisocoria with a decreased pupillary
SO FAR, ALL SPECIES INFECTED HAVE INNATE IMMUNITY BUT NOT ADAPTIVE IMMUNITY
Birds have adaptive immunity – B cells, T cells etc.
Avian cryptococcosis


*University Veterinary Centre and Diagnostic Services Laboratory, Faculty of Veterinary Science, The University of Sydney, NSW 2006, Australia; †West Toowoomba Veterinary Surgery, Toowoomba, Qld 4350, Australia; §Springvale Veterinary Clinic, Springvale South, Vic 3172, Australia; §§RMB 2235, Moama, NSW 2731, Australia; ‡Highbury Veterinary Clinic, Bunwood, Vic 3125, Australia; **Bird Vets of Australia, Pagewood, NSW 2035, Australia; ***Veterinary & Quarantine Centre, Taronga Zoo, Mosman, NSW 2088, Australia; ‡‡Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand; ††Little Grave, WA 6330, Australia; §§§Animal Health Laboratories, Albany, WA, Australia

Fig. 5  A king parrot with localized nasal cryptococcosis. Note the distortion of the cere.

Fig. 7  Localized cutaneous cryptococcosis in a stud racing pigeon also viewed in profile.
Fig. 2. An African grey parrot with nasal cryptococcosis. Note the fleshy lesions on either side of the beak. The nasopharynx, choana and infraorbital sinus were involved also.

Fig. 3. An African grey parrot with localized cryptococcosis involving the nasal cavity and nearby structures.

Fig. 4. Major histopathological and histological features of the nasal tissues with nasal cryptococcosis. (A) Hematoxylin and eosin (H&E) stained sections of the nasal mucosa showing Cryptococcus neoformans budding yeast cells. (B) Immunohistochemistry showing Cryptococcus neoformans intracellularly in the vertical septum of the mucosa.

Fig. 5. Major histopathological and histological features of the nasal tissues with nasal cryptococcosis. (A) Hematoxylin and eosin (H&E) stained sections of the nasal mucosa showing Cryptococcus neoformans budding yeast cells. (B) Immunohistochemistry showing Cryptococcus neoformans intracellularly in the vertical septum of the mucosa.

Fig. 6. Eclectus parrot with severe localized nasal cryptococcosis.

Fig. 7. Same patient as Fig. 6, but viewed in profile.

Fig. 8. Same patient as Fig. 7, but viewed in profile.
Multicentric Cryptococcosis in a Congo African Grey Parrot (Psittacus erithacus erithacus)

Raina S. K. Schunk, DVM, Nicholas E. Sitzman, VMD, DipI ABVP (Avian), Katherine E. Queenenberry, DVM, MPH, DipI ABVP (Avian), and Jessica L. Grodin, DVM, PhD

A 39-year-old female Congo African grey parrot (Psittacus erithacus erithacus) developed progressive, bilateral esophalangitis and buphthalmia. Survey radiographs revealed a large, coccidial, wall tissue mass, which was confirmed on computed tomography scan. Arteriograms of both the contents of the buphthalmic globe and coccidial mass were consistent with Cryptococcus sp. Initial results were later confirmed with serum antigen latex agglutination and polyclonal DNA hybridization tests, and the results were then identified as Cryptococcus neoformans by DNA sequencing. During the course of 1 year, the bird was treated with combination of oral itraconazole, mycostatin, and Theracon, as well as intramuscular anti-infectives. The coccidial mass dramatically decreased in size during the course of treatment, but the globe remained inactive. The bird died of aspergillosis and septicemia approximately 11 months after initial diagnosis. Necropsy confirmed colonization of the urethra and intestines with Cryptococcus neoformans. Cryptococcus neoformans is a rare fungal disease of birds that is often refractory to treatment.

Key words: Cryptococcus species, fungal disease, coccidial, parrot, bird,它头, African grey parrot, Psittacus erithacus

Figure 1. Ventrodorsal (A) and right lateral (B) radiographs of the African grey parrot described in Fig. 1. A large mass is visible in the left coelom (arrow).

Figure 2. Computed tomography images of the African grey parrot described in Fig 1 showing esophalangitis and buphthalmia of the left eye and loss of normal intracoelomic architecture (arrow).
Cryptococcus neoformans
Thermotolerance to Avian Body Temperature Is Sufficient For Extracellular Growth But Not Intracellular Survival In Macrophages

Simon A Johnston¹,², Kerstin Voelz³ & Robin C May³,⁴

Cryptococcus neoformans is a fatal fungal pathogen of humans that efficiently parasitises macrophages. Birds can be colonised by cryptococci and can transmit cryptoccocosis to humans via inhalation of inoculated bird excreta. However, colonisation of birds appears to occur in the absence of symptomatic infection. Here, using a pure population of primary bird macrophages, we demonstrate a mechanism for this relationship. We find that bird macrophages are able to suppress the growth of cryptococci seen in mammalian cells despite C. neoformans being able to grow at bird body temperature, and are able to escape from bird macrophages by vomocyctosis. A small subset of cryptococci are able to adapt to the inhibitory intracellular environment of bird macrophages, exhibiting a large cell phenotype that rescues growth suppression. Thus, restriction of intracellular growth combined with survival at bird body temperature explains the ability of birds to efficiently spread C. neoformans in the environment whilst avoiding systemic disease.
Screening of antigenemia and isolation of Cryptococcus neoformans and C. gattii from cloaca and crop of birds in the state of Paraná, Brazil

Camile Lugarini²*, Larissa A.Z. Condás³, Grazielle C.G. Soresini⁴, Renata C.F. Santos³, Marisol D. Muro⁵, Margaret Ono⁵, Marconi R. Fanas⁴ and Fabiano Montianni-Ferreira³

ABSTRACT: Lugarini C., Condas L.A.Z., Soresini G.C., Santos R.C.F., Muro M.D., Ono M., Farias M.R. & Montianni-Ferreira F. 2008. Screening of antigenemia and isolation of Cryptococcus neoformans and C. gattii from cloaca and crop of birds in the state of Paraná, Brazil. Pesquisa Veterinária Brasileira 28(7):341-344. Departamento de Medicina Veterinária, Rua dos Funcionários 1540, Juvevé, Curitiba PR 80035-050, Brazil. E-mail: camilelug@gmail.com

Cryptococcus neoformans and C. gattii are associated with dry bird excreta but rarely recovered from birds’ digestive tract. The objective of the present study was (1) to verify the existence of C. neoformans and C. gattii in crop and cloaca of wildlife and captivity birds hypothesizing about a possible primary source of this yeast in the excreta, and (2) to determine the fungi’s invasive capability in avian species through latex agglutination. For that purpose, 172 cloacal and 77 crop samples of domestic pigeon, Passerine, and Psittacine birds were collected. None of these samples was positive, suggesting that the yeast is not saprobic in the digestive tract of these birds. Only one out of 62 serum samples collected from pigeons and Psittacine birds was positive (titre 1:2) showing that Cryptococcus sp. probably has a low invasive capability in birds, and is thus considered only a dry excreta colonizer.

INDEX TERMS: Ecology, birds, yeast, Cryptococcus neoformans, Cryptococcus gattii.

Brief Report

Multi-locus sequence typing as a tool to investigate environmental sources of infection for cryptococcosis in captive birds

Laura J. Schmertmann¹,²,³, Kate Bodley¹, Wieland Meyer²,³,⁵, Richard Malik⁶ and Mark B. Krockenberger¹,⁵,⁶,⁷

¹Sydney School of Veterinary Science, The University of Sydney, Sydney, New South Wales, Australia, ²Molecular Mycology Research Laboratory, Centre for Infectious Diseases and Microbiology, The University of Sydney – Westmead Clinical School, Faculty of Medicine and Health, Sydney, New South Wales, Australia, ³The Westmead Institute for Medical Research, Westmead, New South Wales, Australia, ⁴Zoo Victoria, Parkville, Victoria, Australia, ⁵Marie Bashir institute for Infectious Diseases and Biosecurity, The University of Sydney, Sydney, New South Wales, Australia, ⁶Centre for Veterinary Education, The University of Sydney, Sydney, New South Wales, Australia and ⁷Veterinary Pathology Diagnostic Services, B14, The University of Sydney, Sydney, New South Wales, Australia 2006
Identification of the likely point source of infection in a case of avian cryptococcosis

Laura Schmertmann1, Kate Bodley2, Mark Kronckenberger2, Richard Malik3 & Wieland Meyer3

Contact: Laura Schmertmann@sydney.edu.au

1 Molecular Microbiology Research Laboratory, Centre for Infectious Diseases and Microbiology, Sydney Medical School—Newman Hospital, Marie Bashir Institute for Infectious Diseases and Biotechnology, The University of Sydney, Sydney, NSW, Australia; Faculty of Veterinary Science, The University of Sydney, Sydney, NSW, Australia.
2 Centre for Veterinary Education, The University of Sydney, Sydney, NSW, Australia.

Introduction
Cockatoos are invasive fungal disease caused by members of the Cryptococcus neoformans complex. Infections can occur in captive and free-ranging red cockatoos (Calyptorhynchus banksii), the species most commonly affected in Sydney. Cryptococcus neoformans complex infections can cause disease in various animals, with some examples of human, canine, feline, avian, and equine infections. An outbreak of cryptococcosis was observed in 2009 at a bird aviary in Melbourne, Australia due to an outbreak in a captive red cockatoo. An investigation was conducted to determine the point source of infection.

Methods
Environmental samples were obtained from the aviary and the cage of the affected bird. Cryptococcus neoformans complex organisms were isolated and identified by standard methods. For the molecular analysis, DNA was extracted from the environmental samples using high-volatility extraction kits. DNA sequencing was performed using the Illumina MiSeq platform for taxonomic identification. MLST analysis was conducted for population genetic characterization of the outbreak isolate.

Results
Cryptococcus neoformans isolates were identified in 4/159 environmental samples, all from a single tree hollow in a dead eucalyptus tree. Cryptococcus neoformans complex isolates (n=4) were identified as C. gattii molecular type VDI (n=3) and C. neoformans molecular type VNI (n=1). The disease isolate was identified as C. gattii molecular type VDI.

Conclusions
The discovery of C. gattii in the environmental samples highlights the risk of infection in higher bird populations. Public health implications must be considered for environmental samples, and molecular analysis is critical in determining the source of infection.

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CANINE & FELINE CRYPTOCCOCCOSIS

SIX TIMES AS COMMON IN CATS THAN DOGS
Retrospective study of feline and canine cryptococcosis in Australia from 1981 to 2001: 195 cases


A retrospective study of 155 cats and 40 dogs diagnosed with cryptococcosis between 1981 and 2001 was undertaken. Age, sex, breed, clinical findings, feline immunodeficiency virus and feline leukaemia virus status (in cats), species of Cryptococcus causing disease and region of domicile were recorded. Associations between variables were tested. Male and female cats were affected equally. Age ranged from 1 to 16 years, with a preponderance of cats aged between 2 and 3 years. Siamese, Himalayan and Ragdoll breeds were over-represented. Rural cats were more frequently infected with Cryptococcus gattii. Retroviral infection was not identified as a predisposing condition and was not correlated with either species of Cryptococcus or physical findings. Most cats had signs of nasal cavity infection, which was typically localised for a substantial period before invasion of adjacent structures or dissemination. Male and female dogs were affected equally. A marked preponderance of young, large breed dogs was noted. Border Collies, Boxers, Dalmatians, Doberman Pinschers, Great Danes and German Shepherds were over-represented. Cryptococcus species involved was not affected by place of domicile. Although nasal cavity involvement was important, the canine cohort had a greater propensity to develop secondary central nervous system involvement and disseminated disease than feline cases. There were no clinical findings in either cats or dogs which could be reliably used to distinguish disease caused by Cryptococcus neoformans variety grubii from disease caused by Cryptococcus gattii. Both Cryptococcus species appear to be primary pathogens of cats and dogs, with the upper respiratory tract presumed to be the predominant primary site of inoculation in most but not all cases.

Keywords: cat, Cryptococcus gattii, Cryptococcus neoformans, dog
FELINE CRYPTOCOCCOSIS
Impact of current research on clinical management

Disease summary: Cryptococcosis, principally caused by Cryptococcus neoformans and Cryptococcus gattii, is the most common systemic mycosis of cats worldwide. Cats may be infected following inhalation of spores from the environment, with the nasal cavity suspected as being the initial site of colonization and subsequent infection. Other sites of infection in cats are the skin, lungs, lymph nodes, central nervous system (CNS), eyes, and, occasionally, extracranial connective tissue. Cryptococcosis can be diagnosed using serology (antigen testing), coccidiotypic examination of smears, histopathology, or culture. Treatment of localized disease is generally successful using antifungal drugs; however, cats with CNS involvement or disseminated disease require additional treatment with amphotericin B, with or without flucytosine. The prognosis is variable, depending on host and pathogen factors. Some cats require long-term (1-3 year) treatment or lifelong therapy.

Patient group: Cats of any breed, gender, and age may be affected. Vaccination status does not appear to be a risk factor for developing cryptococcosis and inoculated cats are not protected from disease.

Global importance: Feline cryptococcosis occurs worldwide, but is most frequently reported in Australia, western Canada, and the western United States. Species and molecular type vary in different geographical regions and may affect clinical presentation and antifungal susceptibility patterns.

Clinical challenges: Serologic tests that detect cryptococcal antigens in serum are sensitive and specific, but false negatives can occur in cats with localized disease. Long-term drug therapy can be expensive and has the potential for toxicity. The extent to which the pathogenicity and antifungal susceptibility is affected by molecular type is currently under study.

Evidence-based: This review draws on recent literature relating to epidemiology, CNS involvement, and advanced diagnostic imaging to update clinicians regarding research findings relevant to clinical practice.

Which are the relevant species and molecular types?

Cryptococcus is the most common systemic mycosis of domestic cats and has been reported in other felines, especially cheetahs. The disease is caused by an encapsulated yeast species belonging to the genus Cryptococcus, a dimorphic, basidiomycete fungus. Infection most commonly results in upper respiratory, cutaneous, and central nervous system (CNS) abscesses. The two most common species infecting cats are Cryptococcus neoformans and Cryptococcus gattii (formerly C. neoformans var. gattii). Other species, including Cryptococcus laurentii and Cryptococcus albidus, can cause disease when associated with immune compromise. Cryptococcosis has been described as the cause of otitis externa in a cat because cats are more susceptible than humans to cryptococcosis, they may represent a sentinel for human exposure.

Classically, five serotypes of Cryptococcus (A, B, C, D, and AD) have been recognized. C. neoformans var. grubii and C. neoformans var. neoformans cause most of the cases of cryptococcosis. Serotypes B and C are notable. Serotypes A and D are responsible for the majority of the two varieties. Serotypes B and C belong to C. gattii. Using molecular typing methods, including pulsed-field gel electrophoresis and multilocus sequence typing, epidemiological analysis is obtained from multi-
LOCALISED DISEASE IN AN IMMUNE COMPETENT HOST

- Localised cutaneous disease

- Localised nasal cavity disease
WIDELY DISSEMINATED DISEASE
FELINE CRYPTOCOCCAL RHINITIS WITH REGIONAL LYMPH NODE INVOLVEMENT
NASOPHARYNGEAL CRYPTOCOCCOSIS

Canada crypto
FELINE CRYPTOCOCCAL OPTIC NEURITIS AND MENINGOENCEPHALITIS
BEFORE AND AFTER THERAPY
BEFORE AND AFTER THERAPY
A RECENT CASE......
A RECENT CASE......

The “glassy stare” of crypto optic neuritis
AFTER SUBCUTANEOUS AMPHOTERICIN B AND FLUCONAZOLE

“King George”
AFTER SUBCUTANEOUS AMPHOTERICIN B AND FLUCONAZOLE

Where is my dinner?
Cryptococcosis in dogs: a retrospective study of 20 consecutive cases


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Abstract

The clinical and mycological findings in 20 consecutive cases of cryptococcosis evaluated between 1981 and 1995 were analysed retrospectively. Typically, young adult dogs (median age 2 years) of either sex were affected. Dobermann Pinschers and Great Danes were significantly over-represented in relation to other breeds and crossbred dogs, and there was no trend for cryptococcosis to be acquired at a particular time of year. Cryptococcus neoformans was cultured from 18 dogs, with 16 isolates further characterized. Of these, C. neoformans var. neoformans was isolated from 12 cases, while the remaining four strains were C. neoformans var. gattii. Dogs with C. neoformans var. gattii infections resided in rural (two cases) or suburban (two cases) environments. Ten dogs were presented as a result of infection of structures inside, adjacent to, or contiguous with the nasal cavity. Seven dogs were presented primarily for signs of central nervous system disease, of which at least three also had cryptococcal rhinosinusitis. One dog had cryptococcal pneumonia and also possible mycotic rhinitis, another had disseminated disease with lymph node and skin involvement, while the last dog was presented for vomiting referable to cryptococcal mesenteric lymphadenitis. Treatment consisting of surgery and/or antifungal drug therapy was successful in the majority of animals in which it was attempted, including two of three cases with meningoencephalitis.
Abdominal Cryptococcosis in Australia: A retrospective evaluation of 25 cases

Anna Tebb, Alan Kessell, Sonia McGill, Terry King & Richard Malik
CAPRINE AND OVINE CRYPTOCOCCOSIS
Cryptococcal meningitis in a goat – a case report

George Silvelt and Hugo Flicone

Abstract

Background: Cryptococcus neoformans is a saprophytic and opportunistic fungal pathogen that can be found in soil, compost, and bird droppings. In immunocompromised human and veterinary patients, Cryptococcus neoformans infection may result in meningitis, meningoencephalitis, and disseminated disease. The presentation of signs and symptoms can vary widely and may include fever, headache, and altered consciousness.

Case presentation: A 3-year-old boer goat was admitted to the clinic with a one-week history of fever, weight loss, anorexia, and depression. Physical examination revealed a temperature of 40.5°C, tachycardia, and a reduced level of consciousness. The goat had a pronounced meningeal sign (Kernig's sign) and a positive Babinski's reflex. Serological testing was negative for Neospora caninum and Toxocara canis.

Cerebrospinal fluid analysis revealed a Pleocytosis with a high percentage of lymphocytes, increased protein, and decreased glucose levels. Cryptococcal antigen was detected in the cerebrospinal fluid by latex agglutination test. Cultures on slopes of Sabouraud dextrose agar and potato dextrose agar with chloramphenicol were positive for Cryptococcus neoformans.

Conclusions: Cryptococcal meningitis should be considered in the differential diagnosis of central nervous system diseases. Early recognition and institution of appropriate antifungal therapy are crucial for achieving a good outcome in affected animals.

Keywords: Cryptococcosis, Meningitis, Goat, Northland, New Zealand, Coccidioides immitis

References


Short contributions

Cryptococcus neoformans infection in goats

HM CHAPMAN, WJ ROBINSON, JF BOLTON, JP ROBERTSON

Reports of cryptococcosis in small ruminants are uncommon. In bovines, the disease is typically seen in neonates and is caused by Cryptococcus neoformans var. gattii. In other species, it manifests as a systemic disease or as localized lesions such as meningitis, meningoencephalitis, or peritonitis. The classic presentation includes fever, lethargy, anorexia, and sometimes central nervous system signs. The diagnosis is often made by isolation of the fungus from clinical samples or detection of Cryptococcus antigen in the cerebrospinal fluid.

In this case, a 4-year-old female Boer goat was presented with a 1-week history of fever, weight loss, anorexia, and depression. The goat had a pronounced meningeal sign (Kernig's sign) and a positive Babinski's reflex. Serological testing for Neospora caninum and Toxocara canis was negative. Cerebrospinal fluid analysis revealed a pleocytosis with a high percentage of lymphocytes, increased protein, and decreased glucose levels. Cryptococcal antigen was detected in the cerebrospinal fluid by latex agglutination test. Cultures on slopes of Sabouraud dextrose agar and potato dextrose agar with chloramphenicol were positive for Cryptococcus neoformans.

The goat was treated with oral fluconazole (10 mg/kg BID) for 14 days, followed by a maintenance dose of 5 mg/kg BID. The goat made a full recovery and has remained asymptomatic for 6 months post-treatment.

In conclusion, Cryptococcus neoformans infection must be considered in the differential diagnosis of central nervous system diseases in small ruminants. Early recognition and treatment are crucial for achieving a good outcome. Further studies are needed to better understand the disease epidemiology and to improve diagnostic tools and treatment regimens.

Figure 1. Nodular mass protruding from the opening of the left nostril.

Figure 2. Cross section of the cranial nasal cavity showing thickening and distention of the middle turbinate. There is deviation of the nasal septum.
Short Communication

Cryptococcal Mastitis in Dairy Animals

Cryptococcen-Mastitis bei Milchvieh

M. Pal and B. S. Mehta*

Valabhbhai Patel Chest Institute, University of Delhi, Delhi/India
*Dean, Faculty of Science, Kumaun University, Nainital/India

Keywords: Cryptococcus neoformans - mycotic mastitis

Schlüsselwörter: Cryptococcus neoformans - mykotische Mastitis

Summary: The occurrence and etiological significance of Cryptococcus neoformans in mycotic mastitis has been investigated in 79 milk animals during April 1978 to July 1980. The pathogen could be isolated from mastitic milk of 2 buffaloes and 1 Holstein-Friesian cow. The clinical, mycological and epidemiological findings have been discussed.
First Identification of Autochthonous Cryptococcus neoformans var. gattii Isolated from Goats with Predominantly Severe Pulmonary Disease in Spain

TERESA BARÓ,1 JOSEP M. TORRES-RODRIGUEZ,1,2 MIGUEL HERMOSO DE MENDOZA,2 YOLANDA MORERA,1 and CONCEPCIÓN ALÍA1

Clinical and Experimental Mycology Research Group, Institut Municipal d’Investigacions Mèdiques, Autonomous University of Barcelona, Barcelona,1 and Department of Infectious Diseases, Faculty of Veterinary Medicine, University of Extremadura, Cáceres,2 Spain

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Cryptococcus neoformans var. gattii is associated with Eucalyptus trees growing in various tropical and subtropical regions of the world. The identification of 13 autochthonous strains of C. neoformans var. gattii in Spain is reported. These strains were isolated from lung (10 samples), liver (1 sample), and brain (2 samples) tissue specimens from six goats suffering from predominantly severe pulmonary disease that were autopsied. The animals were members of five different herds of goats grazing in rural areas of the province of Cáceres (Extremadura, Spain). Between 1990 and 1994, there were five outbreaks, in which between 25 and 12% of the goats were affected. Although respiratory symptoms (pneumonia) associated with cachexia were the predominant clinical picture in all outbreaks, brain and liver involvement was also documented in three of the five outbreaks. Biotyping was performed by culturing the isolates on t-tryptophan-glycine-brucemohy blue medium and testing them for the assimilation of n-proline and n-tryptophan. Serotyping by agglutination tests confirmed the characterization of all strains as C. neoformans var. gattii serotype B. This is the first confirmation of the presence of this variety in Spain, with a peculiar ability to produce severe pulmonary and systemic disease in normal goats, particularly in the form of outbreaks of pneumonia in association with cachexia.

FIG. 2. Map of Extremadura, Spain, showing the locations of the five outbreaks of cryptococcal infections in goats and forested areas with Eucalyptus spp.
Nasal cryptococcosis in a sheep in Brazilian Semi-Arid

Saulo T. Gusmão da Silva, José C. de Almeida Souza, Carla L. de Mendonça, Marisa A. Izael, Antônio F. Dantas, Roscana Portela, Franklin Riet-Correia, José A. Bastos Afonso

Abstract
A case of nasal cryptococcosis is reported in a two years old male hair sheep in the Brazilian semi-arid. Severe respiratory signs and a mass occupied a large portion of the right nasal cavity were observed. Cryptococcosis was diagnosed by the typical histologic appearance of the fungus.

Key words: Cryptococcus; nasal cavity; Brazilian Semi-Arid

The examination of the nasal cavity revealed a pendulous mass with a rough appearance that occupied a large portion of the right nasal cavity, comprising the septum and causing partial occlusion of the left nasal cavity. The animal remained at the clinic for 31 days, during which time it had a reduced appetite, a variable degree of dehydration, an area of alopecia in the region of the nasal sinus, and swelling of the nasal bone structure. At that time it also exhibited an abdominal breathing pattern with respiratory noises, severe inspiratory dyspnea, sporadic cough, movements of the neck, and febrile non-purulent nasal secretion.

The animal was submitted to penicillin-based antibiotic treatment (Pencinet Pen PPU/Intratet) at 24-hour intervals and also received three applications of dexamethasone (AstraSering-Plemed). Daily cleaning of the nostril was performed with a boric acid nasal mucosa and infiltrated the adjacent structures, extending throughout the entire nasal cavity from the nasal vestibule to the ethmoid bone, causing facial deformity and obstructed airflow. Resorption of the right nasal bone and nasal turbinates bones (dental and nasal) was also noted. The remaining organs did not exhibit any significant lesion.

Fragments from different regions of the mass were collected, fixed in 10% buffered formalin, embedded in paraffin, sliced to 5-6 μm and stained with hematoxylin-eosin (H&E), periodic acid-Schiff (PAS) and Alcian blue. Microscopically, the lesion was a locally extensive, necrotizing granulomatous rhinitis, with infiltration by macrophages, lymphocytes and plasma cells. Extensive areas of necrosis were also observed. Myriads of round or oval leucocytes structures, with a thin basophilic wall, measuring approximately 5.0 to 20 μm in diameter were present within the cytoplasm. Surrounding the yeast, there was a light halo not stained by H&E, giving a soap bubble appearance, which was stained by Alcian blue and PAS (Figure 2). These characteristics are typical of Cryptococcus spp. There was also moderate proliferation of the fibrous tissue, areas of hemorrhage, and infiltration neutrophils in the periphery of the lesion.

Figure 1: Nasal granulation tissue in sheep.

Figure 2: Sheep with nasal cryptococcosis. The histologic section of the nose shows numerous yeast of Cryptococcus neoformans and few inflammatory cells. PAS: X40.
KOALA CRYPTOCOCOSIS
Cryptococcus neoformans var. gattii in the koala (Phascolarctos cinereus): a review of 43 cases of cryptococcosis

M.H. Krockenberger, P.J. Canfield & R. Malik
Faculty of Veterinary Science, University of Sydney, Sydney, New South Wales, Australia

Details of 11 previously reported cases and 32 new cases of cryptococcosis in captive and wild koalas were analysed. Cryptococcus neoformans var. gattii accounted for all 32 cases in which varietal status was determined. No age or sex predisposition was observed. The respiratory tract was the primary focus of disease in 77% of cases. Although the lower respiratory tract was affected most commonly (60% of cases), 30% of cases had upper respiratory tract lesions and 14% had both. Dissemination was common, especially to the central nervous system (37% cases). Local extension to surrounding tissues was a feature of upper respiratory tract disease. Other tissues showing cryptococcal invasion included lymph nodes (19%), alimentary tract (12%), kidneys (12%), spleen (9%) and skin (7%). Only three cases (7%) had no respiratory tract or central nervous system involvement, two of primary skin inoculation and one case of primary lymphadenopathy. Late presentation was a likely factor in the high proportion of cases with disseminated disease (40%). The proportion of koala cases with involvement of the central nervous system, lower respiratory tract and skin, parallels what has been reported for immunocompetent people. Cryptococcosis in the koala appears to be an excellent naturally occurring model for examination of the cryptococcal host-parasite relationship in all species.

Keywords: cryptococcosis, Cryptococcus neoformans, gattii, koala
BEFORE AND AFTER THERAPY
JET-SETTING KOALAS

Australia

Canada crypto
Clonality and α-a Recombination in the Australian Cryptococcus gattii VGII Population - An Emerging Outbreak in Australia

Fabian Carriconde¹, Félix Gilgado¹, Ian Arthur², David Ellis³, Richard Malik⁴, Nathalie van de Wiele⁴, Vincent Robert⁵, Bart J. Currie⁶, Wieland Meyer⁷

1. Melbourne Molecular Research Institute, School of Medical Sciences, University of Melbourne, Parkville, Victoria, Australia
2. Northern Australia Primary Health Care Research Unit, Department of Primary Health Care, University of Queensland, Brisbane, Queensland, Australia
3. Institute for Mycology, University of Zürich, Zürich, Switzerland
4. Department of Microbiology, University of British Columbia, Vancouver, British Columbia, Canada
5. Department of Microbiology and Immunology, University of Western Ontario, London, Ontario, Canada
6. Department of Epidemiology and Biostatistics, Doherty Institute for Infectious Diseases, University of Melbourne, Parkville, Victoria, Australia
7. European Reference Laboratory for Cryptococcus, Robert Koch-Institut, Berlin, Germany

Figure 4. Spatial distribution of the different sequence types delineated in the Australian C. gattii VGII population. Pie charts are proportional to the number of samples. The symbols n and nST corresponds to the number of samples and the number of sequence types observed, respectively.

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WHY DON’T PEOPLE GET NASAL CRYPTOCOCCOSIS
Humans have poorly developed nasal cavity (reductive evolution)
CRYPTOCOCCUS GATTII VGI IN A SPINNER DOLPHIN (STENELLA LONGIROSTRIS) FROM HAWAII


Abstract: A spinner dolphin (Stenella longirostris) was found stranded in Hawaii with cutaneous nodules and enlarged lymph nodes. Numerous Cryptococcus gattii VGI yeast were observed in multiple organs with minimal inflammation. This case represents the first reported infection of C. gattii in a dolphin from Hawaii.

Key words: cryptococcosis, Cryptococcus gattii VGI, granulomatous, Hawaii, Stenella longirostris.

Figure 1. Spinner dolphin with numerous skin lesions, including a focal 12-cm, raised volcanic ulceration.

Figure 2. Fungal yeast with an expansive clear capsule forming large aggregates within the lung. Hematoxylin and eosin, x400.
Cryptococcosis in a Bottlenose Dolphin (Tursiops truncatus) Caused by Cryptococcus neoformans var. gattii

W. George Miller,1 Arvind A. Padhye,2* William van Bonn,1 Eric Jensen,1
Mary E. Brandt,2* and Sam H. Ridgway1

Space and Naval Warfare Systems Center, San Diego, California 92152,* and Mycotic Diseases Branch,
Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases,
Centers for Disease Control and Prevention, Atlanta, Georgia 30333

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We describe the first case of cryptococcosis caused by Cryptococcus neoformans var. gattii in a male Atlantic
bottlenose dolphin (Tursiops truncatus). The dolphin showed clinical signs of tachypnea, transient dyspnea, and
mild tachycardia and developed multiple hyperemic nodules, parenchymal consolidation, and thickening of
pleura. A diagnosis of bronchopneumonia with pleuritis was made. Intravenous therapy was initiated for
120 days, and trough levels in serum were within or above the suggested therapeutic range. Levels of cryp-
tococcal antigen in serum increased eightfold during therapy, and the case had a fatal outcome. Necropsy
examination findings included enlarged pulmonary lymph nodes and extensive constricting granulomatous
lesions throughout both lungs. Histologic examination revealed numerous, spherical to ellipsoidal, mucicar-
nine-positive, 5- to 14-μm, encapsulated, budding cells consistent with C. neoformans. Culture of the lung
tissue yielded colonies of C. neoformans. The isolate was urease positive and nitrate negative and exhibited
phenoloxidase activity. It was positive on cornstearine-glucomethyl blue agar. When tested by the
hornon serodiagnostic reagent kit (Barron Laboratories, Inc.), it was shown to belong to serotype B.

Schematic illustration of a dolphin’s head anatomy

Sound generator: The Monkey Lips/Dorsal Burssae Complex (MLDB)

Modified and adapted from Crawford et al. 1996
Cryptococcus neoformans is a ubiquitous saprophytic environmental fungus that causes localized or disseminated mycoses in humans and animals. Disease associated with this organism is usually rare or sporadic. An unusual cluster of human and animal cryptococcosis was identified in British Columbia (BC) in the summer of 2001. The majority of animal cases were initially identified through a single private veterinary laboratory that serves BC and Alberta. Historically, this laboratory diagnoses 4 to 6 animal cases of cryptococcosis per year, but by August 2001, it had diagnosed 12 cases on Vancouver Island alone. The University of BC Centre for Disease Control became aware of a consistent increase in human cases in the same geographic location. This report focuses on the veterinary aspects of the outbreak.

By the end of March 2002, a total of 45 laboratory-confirmed animal cases and 50 human cases had been identified. Only cases that were substantiated by cytological, histopathological, or culture methods were counted. Additional animal cases were found but could not be confirmed due to lost records or because they had been diagnosed on clinical signs alone. Table 1 summarizes the species involved, plus their predominant clinical or pathological presentation.

The 1st animal case was diagnosed in February 2000. No seasonal pattern of disease has been identified. Given the lack of information on the incubation period of cryptococcosis, the dates of infection remain speculative. The delay between the date of diagnosis and the date of the reported onset of clinically compatible symptoms ranges from 2 d to over 2 mo. All but 7 cases have been found along the east coast of Vancouver Island from Courtenay to Victoria, as well as offshore waters (Figure 1). Three cases occurred in the Fraser Valley on the mainland of BC and 1 occurred in Prince Rupert on the north coast of the province. Four puppies were found dead and stranded along the east coast of Vancouver Island, a solitary animal was recovered near West Vancouver, and 1 of 9 puppies from recent strandings in the San Juan Islands, Washington, USA

Table 1. Species and predominant clinical or postmortem presentation of animals involved in an outbreak of cryptococcosis in British Columbia (2000–2001)

<table>
<thead>
<tr>
<th>Species</th>
<th>Upper respiratory tract</th>
<th>Subcutaneous</th>
<th>Meningitis</th>
<th>Central nervous system</th>
<th>Oral</th>
<th>Ocular</th>
<th>Lymphadenopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat (n = 18)</td>
<td>7</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Dog (n = 17)</td>
<td>11</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Dali’s porpoise</td>
<td>11</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Haribo porpoise</td>
<td>11</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Ferret (n = 2)</td>
<td>1</td>
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<td></td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Llana (n = 7)</td>
<td>1</td>
<td>12</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total (n = 43)</td>
<td>18</td>
<td>42</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

1 Case also presented with lymphadenopathy as a primary sign
2 Dog also had concurrent meningitis without another dog had concurrent central nervous system signs. In each case, respiratory signs occurred first
3 Dog had concurrent central nervous system signs
4 All 5 porpoises had multifocal pulmonary cryptococcosis with generalized lymphadenopathy
5 2 llanas had pulmonary and central nervous system lesions

Figure 1. Map of cases of animal cryptococcosis in British Columbia 2000–2001. Locations for domestic species indicate place of residence and for porpoises indicate location of recovery on the beach.
VIRULENCE STUDIES
VIRULENCE STUDIES
1. First step is **COLONISATION**

- This can be demonstrated by collecting deep nasal washings (and sometimes even superficial swabs) and plating on Staib’s bird seed agar – the only colonies that develop ‘brown colour effect’ are *Cryptococcus* spp

- *C. gattii* VGI tends to have highly mucoid colonies

- Note that there are many fungi in the normal mycobiome of cats and dogs
1. First step is **Colonisation**

2. Something facilitates **Invasion** of the epithelium

   – FHV-1?
INFECTION

1. First step is **COLONISATION**

2. Something then facilitates **INVASION** of the epithelium – **FHV-1**?

3. This gives rise to **LOCALISED INFECTION**

Nasal turbinates (mouse)
ONE MORE TIME!
COLONISATION
Early **INVASION** of the epithelium
LOCALISED INFECTION
The HOST responds by non-specific cellular response (neutrophils, macrophages) which is LATER organized by SPECIFIC CMI (cell mediated immunity)
Use antigen testing (IMMY lateral flow or latex cryptococcal agglutination [LCAT]) to demonstrate infection (subclinical or clinical)

Clinical disease gives rise to signs and symptoms
LCAT/IMMY

Colonization

Subclinical Disease

Clinical disease

Negative

Positive (low)

Positive (higher)
THANK YOU FOR YOUR ATTENTION