Australian
VETERINARY
JOURNAL

CLINICAL SECTION

CONTRIBUTIONS INVITED

Practising veterinarians and others are invited to contribute clinical articles to the Australian Veterinary Journal. We will consider material in a variety of formats, including clinically orientated reviews, reports of case series, individual case studies, diagnostic exercises, and letters containing comments or queries. Practitioners are also invited to contribute to the case notes feature, where accepted articles are not peer reviewed but are edited for publication. Contributors should consult instructions to authors and recent issues of the journal for guidelines as to formatting. Over referencing should be avoided: authors should preferably quote only those articles they feel are most likely to be of interest and benefit to readers.

Send all contributions to:
Editor, AVJ Clinical Section
AVA House, 272 Brunswick Road,
Brunswick, Vic. 3056,
phone: (03) 9387 2982
fax: (03) 9388 0112

Pulmonary cryptococcosis and *Capillaria aerophila* infection in an FIV-positive cat

VR BARRS[a], P MARTIN[b], RG NICOLL[a], JA BEATTY[a] and R MALIK[a]
Faculty of Veterinary Science, The University of Sydney, New South Wales 2006

A 12-year-old, FIV-positive, domestic longhair cat was presented with a history of sneezing and coughing during the previous seven months. On thoracic radiographs, a prominent bronchial pattern and three focal, opacified nodules were seen. Cytology of bronchoalveolar lavage fluid demonstrated spherical, capsulate, narrow-necked, budding yeasts within macrophages. Culture of the fluid yielded a heavy growth of *Cryptococcus neoformans* var *neoformans*. The serum latex cryptococcal antigen agglutination test titre was 158. The cat was treated with itraconazole and the cough resolved over a 5-month period but then recurred. Repeat thoracic radiographs showed resolution of the pulmonary nodules but a persistent bronchial pattern. Adult nematodes and ova with morphology characteristic of *Capillaria aerophila* were seen in bronchoalveolar lavage fluid and no yeasts were cultured from the fluid. The cryptococcal titre was zero. The lungworm infection was treated successfully with abamectin and the cough resolved. Immunosuppression related to FIV infection may have predisposed this cat to sequential respiratory tract infections.

Aust Vet J 2000;78:154-158
Key words: *Cryptococcus neoformans*, *Capillaria aerophila*, FIV, cat, ivermectin.

AIDS	Acquired immune deficiency syndrome
ALP	Alkaline phosphatase
ALT	Alanine transaminase
BAL	Bronchoalveolar lavage
FIV	Feline immunodeficiency virus
LCAT	Latex cryptococcal antigen agglutination test
PO	Orally

Case Report

A 12-year-old, castrated domestic longhair cat (bodyweight 5.3 kg) presented to a veterinarian with a 3-month history of sneezing and coughing. The cat had unlimited outdoor access. It was treated with oral prednisolone and bromhexine hydrochloride (doses unrecorded) for 10 days for suspected allergic bronchitis and rhinitis. The cat improved initially but then began coughing again. A short time later the cat was examined by another veterinarian and found to have tracheal hypersensitivity and increased breath sounds on thoracic auscultation. Referral was recommended to further evaluate the cat's respiratory disease. Four months later the cat was presented to the University Veterinary Centre, Sydney. Coughing was now the major presenting complaint as there had been no recent history of sneezing.

[a]Department of Veterinary Clinical Sciences
[b]Department of Veterinary Anatomy and Pathology

On physical examination the cat was tachypnoeic (respiratory rate 54/min) and mildly increased breath sounds were auscultated during inspiration and expiration. Heart rate (160/min) and rectal temperature (38.1°C) were normal. No murmur was audible and no nasal discharge was detected. Lower respiratory tract disease was suspected and ventrodorsal and bilateral thoracic radiographs were taken. In the left lateral projection there were two poorly defined soft tissue opacities overlying the cardiac silhouette in the region of the right middle lung lobe (Figure 1). In the right lateral projection a well-defined nodule with soft tissue density was present overlying the cardiac silhouette in the region of the caudal part of the left cranial lung lobe (Figure 2). In addition, there was a diffuse bronchial pattern throughout the remainder of the lung fields.

An unguided BAL was performed under general anaesthesia induced with ketamine, midazolam and atropine and

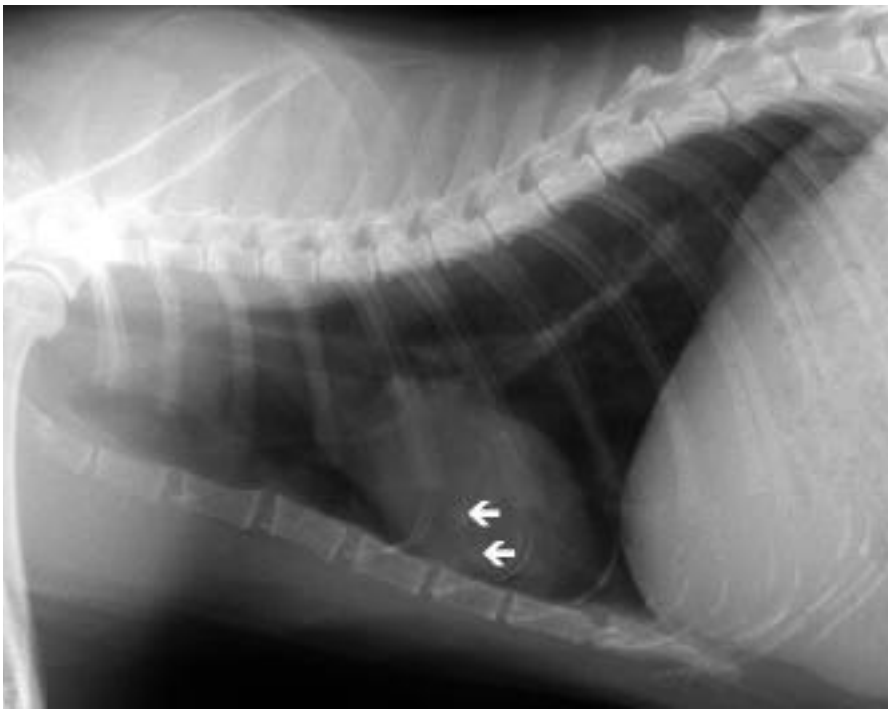

Figure 1. Left lateral thoracic radiograph, showing two poorly defined nodules of soft tissue opacity (arrows) overlying the cardiac silhouette in the region of the right middle lung lobe.

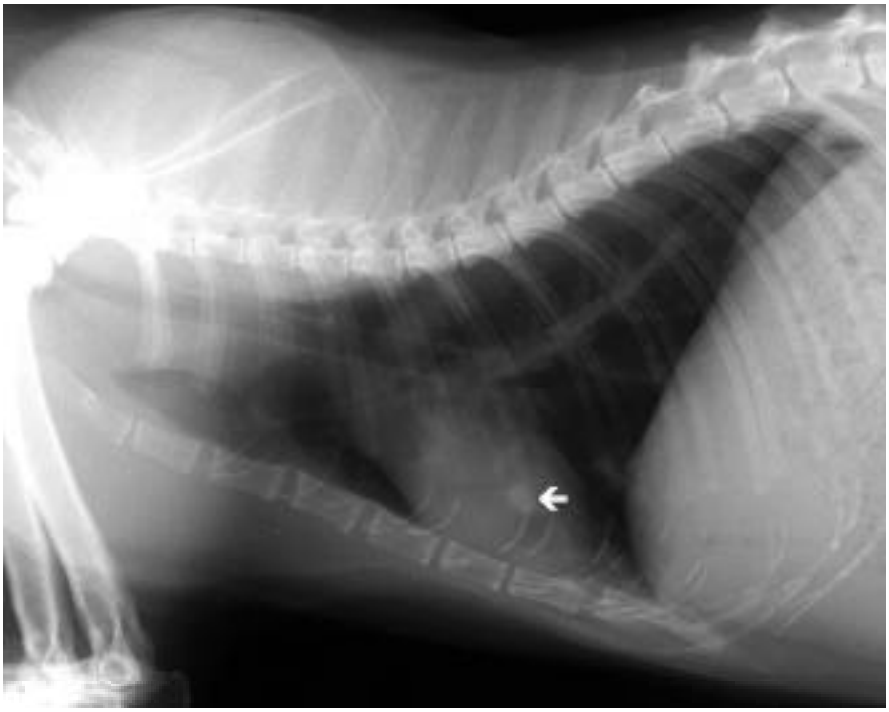

Figure 2. Right lateral thoracic radiograph, showing a well-defined nodule of soft tissue opacity (arrow) overlying the cardiac silhouette in the region of the caudal part of the left cranial lung lobe.

maintained using halothane in oxygen. The cat was intubated with a sterile, cuffed endotracheal tube (internal diameter 4 mm). The plane of anaesthesia was lightened until a cough reflex could be elicited. A sterile urinary catheter (size 8 French) was gently advanced through the endotracheal tube until it became wedged in a bronchus. Two 10 mL aliquots of sterile 0.9% saline were flushed through the urinary catheter and retrieved via negative syringe-pressure as the cat coughed. Many clumps of flocculent material were collected for cytological examination and culture.

Diff-Quik stained smears of the BAL fluid demonstrated much mucus, some inspissated Curschmann's spirals and scattered ciliated, columnar epithelial cells. The sample was highly cellular, consisting of 80% macrophages, 15% neutrophils, 5% eosinophils and scattered lymphocytes. Many macrophages contained multiple intracellular, spherical, capsulate, narrow-necked, budding yeasts. The cytological diagnosis was cryptococcal bronchopneumonia. Culture of the fluid on birdseed agar yielded a heavy growth of non-mucoid colonies of *C neoformans* demonstrating the brown-colour-effect. The organism was typed as *C neoformans* var *neoformans* at a reference laboratory. The serum LCAT titre was 128. An FIV antibody test (Witness, Rhone Merieux) was positive and a feline leukaemia virus antigen test (ViraCHEK, Synbiotics) was negative.

Treatment was commenced with itraconazole (100 mg PO once daily). Six weeks later the cat became inappetent. Serum ALP activity was mildly elevated (52 U/L, reference range < 50), while ALT activity was moderately elevated (239 U/L, reference range < 60). Thoracic radiographs showed some resolution of the nodules, but the bronchial pattern and a single focal pulmonary opacity in the right middle lung lobe persisted. The serum LCAT titre was unchanged at 128. Itraconazole was continued at 50 mg PO once daily then increased gradually to 100mg PO once daily with no adverse effects. The owner declined adjunctive therapy with amphotericin and/or 5-flucytosine.

Itraconazole therapy was continued for a further 5 months and during this time the cat's cough resolved. The cat then began to cough again. The owner (a pharmacist) did not seek veterinary attention for a further 3 months but continued to administer itraconazole. Two weeks prior to the third presentation, the cat began sneezing and developed an ocular discharge. On physical examination the cat had a moist cough, marked tracheal hypersensitivity and increased breath sounds on auscultation of the lung fields. Purulent ocular and

nasal discharges were present. Repeat thoracic radiographs showed resolution of the focal pulmonary nodules. However, a bronchial pattern was evident and more prominent than previously. An unguided BAL was repeated and yielded abundant thick, mucoid material. Diff-Quik stained smears of the BAL fluid demonstrated moderate numbers of inflammatory cells comprising 67% macrophages, 20% eosinophils, 11% neutrophils and 2% lymphocytes. No yeasts were seen. Low-power, light microscopic examination of wet preparations of the lavage fluid revealed three female nematodes, containing and surrounded by bipolar operculate ova, with morphological features characteristic of *Capillaria aerophila* (Figures 3 & 4). Culture of the BAL fluid on birdseed agar, Sabaroud's dextrose agar and sheep blood agar yielded no growth. The serum LCAT titre was zero. No budding yeasts or *C aerophila* ova were seen in cytological preparations of nasal swabs. On a routine biochemical and haematological profile mildly increased ALT activity (81 U/L), a mature neutrophilia (15.6 x 10^9/L) and lymphocytopaenia (0.8 x 10^9/L) were detected. Circulating numbers of eosinophils were within the reference range (0.34 x 10^9/L).

Itraconazole was discontinued and the cat was treated with abamectin (300 µg/kg subcutaneously), terbutaline (0.6 mg PO twice daily) and doxycycline was prescribed for the upper-respiratory tract infection (25 mg PO twice daily). At recheck examination 2 weeks later the oculonasal discharge and cough had resolved and a faecal flotation test was negative for parasitic ova; a second dose of abamectin was administered (300 µg/kg subcutaneously).

Discussion

Solitary lung nodules are rare in the cat and are most commonly associated with mycotic pneumonia, primary or metastatic neoplasia and parasitic granulomas. Less common causes are toxoplasmosis, focal pneumonia including infections caused by *Mycobacterium* spp, *Rhodococcus* spp and *Mycoplasma* spp, localised haemorrhage, cysts, infarcts, feline allergic bronchitis and feline infectious peritonitis.[1]

Although the initial physical and radiological findings in this case were indicative of lower respiratory tract disease, the prior history of sneezing suggested the possibility of an infectious disease process starting in the nasal cavity with subsequent extension into the lower airways. Methods for obtaining a sample from the lower respiratory tract for cytological examination and culture include deep bronchial washing, unguided BAL, bronchoscopically-guided brushing and ultrasound-guided fine-needle aspiration of a pulmonary nodule. Unguided BAL was chosen in this case because the diffuse bronchial pattern suggested the disease process involved airways in addition to localised regions of pulmonary parenchyma. Given that the pulmonary nodules and the cough resolved following itraconazole therapy, it would seem most likely that these nodules were cryptococcal granulomas. However, the possibility exists that they were pneumonic foci surrounding *C aerophila* adults or aspirated eggs.[2]

Infection of cats by *C neoformans* is most commonly restricted to the nasal cavity and adjacent or contiguous structures, including the nasopharynx. Two variants (*neoformans* and *gattii*) occur, of which *C neoformans* var *neoformans* more commonly causes clinical disease in cats in Sydney.[3-4] Infectious propagules presumably establish infection following deposition in the upper respiratory tract. In some cats (< 7%) subclinical colonisation of the nasal passages may occur.[5] In contrast, in humans, the lower respiratory tract is thought to be the primary site of cryptococcal deposition and replication following inhalation. In horses and koalas, the upper and lower respiratory tract can both be primary sites of infection. In this cat it was not possible to determine if cryptococcal rhinitis preceded pneumonia, or vice versa, although the history of sneezing suggests the former scenario.

In cats, cryptococcal infection may spread from the nasal cavity by direct extension through the cribriform plate

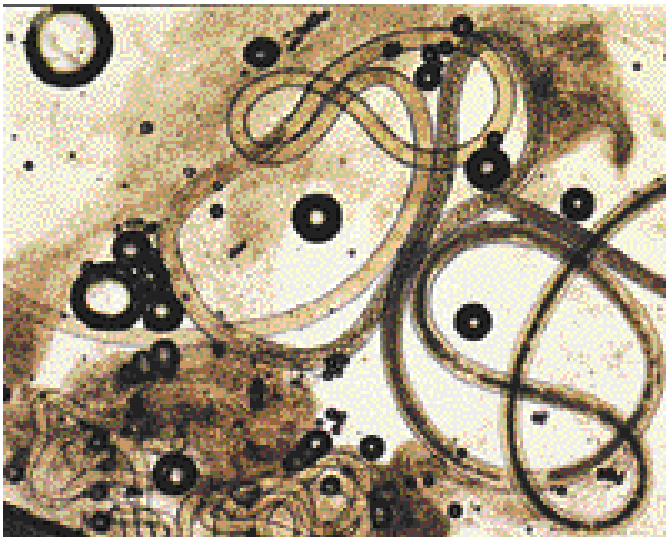

Figure 3. Wet preparation of bronchoalveolar lavage fluid. Adult *Capillaria aerophila* contain and are surrounded by ova. The nematodes were thin, had a uterine opening at the oesophageal intestinal junction and a stichosome (13.2 x).

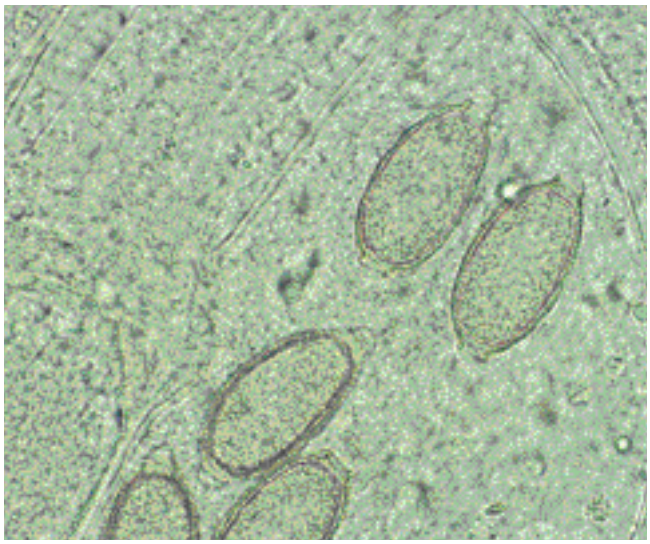

Figure 4. Wet preparation of bronchoalveolar lavage fluid. Unembryonated and double-operculated *Capillaria aerophila* ova with characteristically asymmetric bipolar plugs (132 x).

or haematogenously to cause meningoencephalitis.[4,6] The disease may also disseminate via blood or lymphatics to other tissues including lymph nodes, skin, lung and kidney.[6-7] Concurrent upper and lower respiratory tract disease without systemic dissemination, as seen in this case, has rarely been described.[8]

Cryptococcal infections in cats may be treated with oral triazole drugs including fluconazole and itraconazole, either alone or in combination with amphotericin B and flucytosine.[4,5] Treatment using azole drugs alone takes months to years to effect a cure and concurrent FIV infection may necessitate longer courses of therapy.[9] Itraconazole may cause reversible, dose-related, hepatotoxicity in cats[10] as in the present case, although a 50% reduction in the dosage usually permits therapy to be continued. Serology is a useful noninvasive method of monitoring efficacy of therapy. Ideally, antifungal therapy should be continued until the LCAT titre declines to less than 1.[10,11]

It is well established that immune dysfunction, usually resulting from HIV infection, is a major predisposing factor for the development of cryptococcosis in humans .[12] Whether the same is true in cats remains the subject of debate. The prevalence of FIV infection in cats with cryptococcosis in Australia has been reported by Malik et al to be 28%.[9] Since this prevalence approximated that reported among healthy cats in Australia[13] it was interpreted as indicating that FIV-positivity is not a significant risk factor for the development of cryptococcosis. However, subsequent seroprevalence surveys from the same region as cats studied by Malik et al indicated that only 8% of healthy cats are FIV-positive.[14] Although FIV-infection does not impart an unfavourable prognosis, affected cats tend to have advanced and/or disseminated disease.[9,15] It is possible that FIV infection in this cat was associated with immune dysfunction manifested clinically as sequential infections including cryptococcosis, capillariasis and probable viral upper respiratory tract disease. Quantification of lymphocyte subsets or viral load may have clarified whether the cat had an AIDS-like status associated with long-standing FIV infection. However, lymphocyte subset numbers may not be useful as a marker of immune-dysfunction in FIV-infected cats since impaired lymphocyte responsiveness precedes the decline in CD4 cell counts and some chronically infected cats have very low CD4 counts without obvious clinical immunodeficiency.[16-17] In this cat, impaired T-cell immunity due to FIV infection may have been associated with an increased propensity to heavy parasitism by *C aerophila* and subsequent development of bronchitis. The role of T-cell mediated immunity in nematode infections is well documented and involves a strong T-helper2 cell-mediated response.[18]

Capillaria aerophila and *Aelurostrongylus abstrusus* are nematode lungworms that may infect cats. A similar prevalence (3-5%) has been reported for both in surveys of Australian cats.[19-21] *C aerophila*, a parasite of foxes, dogs and cats, most commonly causes subclinical infections. Clinical disease was common in foxes farmed for fur where it was associated with poor husbandry practices.[22] In cats, clinical infections are reportedly rare but, like *A abstrusus*, heavy worm burdens may result in severe bronchitis sometimes with secondary bronchopneumonia.[23-27]

C aerophila belongs to the family Trichuridae. The double-operculated ova of *C aerophila* may be mistaken for *Trichuris* spp when found in faecal preparations from cats, although they are smaller (usually less than 70 µm long), have radial striations and their asymmetric bipolar plugs are less protruberant.[23] The life cycle of *C aerophila* is direct, although earthworms and rodents may act as paratenic hosts. Adult worms reside beneath the epithelium of trachea, bronchi and bronchioles. In dogs, *C aerophila* has been reported to occur also in the frontal sinuses and nasal cavity, although, in retrospect these nematodes were likely mistaken for *C böhmi*, a parasite of the frontal sinus mucosae of the fox, that can be distinguished from *C aerophila* by their ova which have pitted rather than striated surfaces.[22,28-30]

C aerophila ova are shed into the airways, coughed up, swallowed and passed in faeces. Infective larvae develop within the egg and may survive harsh environments for up to 1 year. Ingested ova hatch in the intestine and larvae migrate haematogenously to the lungs within a week. The prepatent period is 6 weeks and infections remain patent for 8 to 11 months.[23] Specific treatment protocols have not been evaluated but fenbendazole, levamisole and ivermectin have been used successfully. Avermectins are effective for treatment of *A abstrusus* infections and appear to be effective in the treatment of *C aerophila* infections in cats at a dose of 300 µg/kg.[30]

Allergic bronchitis is the most common cause of chronic coughing in cats.[31] This case shows that therapeutic trials with corticosteroids for this condition should be undertaken with caution since infectious agents or parasites may occasionally cause similar signs and radiographic findings. Unguided BAL followed by cytological examination and culture is a simple, cost-effective procedure to facilitate differentiation of these different disorders.

Acknowledgments

JA Beatty is supported by a Research Career Development Fellowship awarded by the Wellcome Trust, UK. Richard Malik is supported by the Valentine Charlton Bequest administered by the Post Graduate Foundation in Veterinary science of The University of Sydney.

References:

1.Farrow CS. The thorax. In: Farrow CS, Green R, Shively M, editors. *Radiology of the cat*. Mosby, St Louis, 1994;74.
2. Lautenslager JP. Internal helminths of cats. *Vet Clin North Am* 1976;6:353-362.
3. Malik R, Martin P, Wigney DI et al. Nasopharyngeal cryptococcosis. *Aust Vet J* 1997;75:483-488.
4. Malik R, Martin P, Wigney DI, Love DN. Cryptococcosis in cats: diagnosis and treatment. In: *Feline practice*. University of Sydney Postgraduate Committee in Veterinary Science Proceedings No 243 1995;49-54.
5. Malik R, Wigney DI, Muir DB et al. Asymptomatic carriage of *Cryptococcus neoformans* in the nasal cavity of dogs and cats. *J Med Vet Mycol* 1997:35;27-31.
6. Jacobs GJ, Medleau L. Cryptococcosis. In: Greene CE, editor. *Infectious diseases of the dog and cat*. 2nd edn, Saunders, Philadelphia, 1998;383-390.
7. Hamilton TA, Hawkins EC, DeNicoa DB. Bronchoalveolar lavage and tracheal wash to determine lung involvement in a cat with cryptococcosis. *J Am Vet Med Assoc* 1991;198:655-656.
8. Jacobs GJ, Greene CE, Medleau L. Feline and canine cryptococcosis. *Waltham Focus* 1998;8:21-27.
9. Malik R, Wigney DI, Muir DB, Gregory DJ, Love DN. Cryptococcosis in cats:clinical and mycological assessment of 29 cases and evaluation of treatment using orally administered fluconazole. *J Med Mycol* 1992;30:133-44.
10. Medleau L, Jacobs GJ, Marks MA. Itraconazole for the treatment of cryptococcosis in cats. *J Vet Intern Med* 1995;9:39-42.
11. Malik R, McPetrie R, Wigney DI, Craig AJ, Love DN. A latex cryptococcal antigen agglutination test for diagnosis and monitoring of therapy for cryptococcosis. *Aust Vet J* 1996;74:358-364.
12. Mitchell TG, Perfect JR. Cryptococcosis in the era of AIDS - 100 years after the discovery of *Cryptococcus neoformans*. *Clin Microbiol Rev* 1995;8:515-548.

13. Robertson ID, Robinson WF, Alexander R, Shaw SE, Sutherland RJ. Feline immunodeficiency virus and feline leukaemia virus in cats. *Aust Vet Pract* 1990;20:66-69.
14. Malik R, Kendall K, Cridland J et al. Prevalences of feline leukaemia virus and feline immunodeficiency virus infections in cats in Sydney. *Aust Vet J* 1997;75:323-327.
15. Walker C, Malik R, Canfield PJ. Analysis of leucocytes and lymphocyte subsets in cats with naturally occurring cryptococcosis but differing feline immunodeficiency virus status. *Aust Vet J* 1995;72:93-96.
16. Hartmann K. Feline immunodeficiency virus infection: an overview. *The Vet J* 1998;155:123-137.
17. Burkhard MJ, Hoover EA. Feline immunodeficiency virus (FIV): immunopathogenesis. Part 1. *Feline Practice* 1998;26:10-13.
18. Grencis RK. Th2-mediated host protective immunity to intestinal nematode infections. *Philosophical Transactions of the Royal Society of London - Series B: Biological Sciences.* 1997:352:1377-1384.
19. Holmes PR, Kelly JD. *Capillaria aerophila* in the domestic cat in Australia. *Aust Vet J* 1973;49:472-473.
20. Wilson-Hanson SL, Prescott CW. A survey for parasites in cats. *Aust Vet J* 1982;59:194-195.
21. Milstein TC, Goldsmid JM. Parasites of feral cats from southern Tasmania and their potential significance. *Aust Vet J* 1997;75:218-219.
22. Bowman DB. Nematodes. In: Bowman, DB, editor. *Georgi's parasitology for veterinarians.* 7th edn, Saunders, Philadelphia, 1999;144-221.
23. Pechman RD. Respiratory parasites. In: Sherding RD, editor. *The cat:disease and clinical management*. 2nd edn. Churchill Livingstone, New York, 1994:613-622.
24. Barrs VR, Swinney GR, Martin P, Nicoll RG. Concurrent *Aelurostrongylus abstrusus* infection and salmonellosis in a kitten. *Aust Vet J* 1999; 77:11-14.
25. Herman LH. *Capillaria aerophila* infection in a cat. *Vet Med Sm An Clin* 1967;62:466-468.
26. Norsworthy GD. Feline lungworm treatment case report. *Feline Practice* 1975;5:3,14.
27. Endres WA. *Capillaria aerophila* infection in a cat. *Vet Med Sm An Clin* 1976;71:1553.
28. Evinger JV, Kazacos KR, Cantwell HD. Ivermectin for treatment of nasal capillariasis in a dog. *J Am Vet Med Assoc* 1985;186:174-175.
29. King RR, Greiner EC, Ackerman N, Woodard JC. Nasal capillariasis in a dog. *J Am An Hosp Assoc* 1990:26:381-385.
30. Blagburn BL, Hendrix CM, Lindsay DS, Vaughan JL. Anthelmintic efficacy of ivermectin in naturally parasitized cats. *Am J Vet Res* 1987;48:670-672.
31. Gruffyd-Jones TJ. Investigation of the coughing cat. In:*Feline Practice.* The University of Sydney Post Graduate Foundation Proceedings 1995;243:171-173.

(Accepted for publication 7 September 1999)

Successful treatment of invasive nasal cryptococcosis in a ferret

R MALIK[a], P MARTIN[b], J McGILL[c], A MARTIN[d] and DN LOVE[b]

Invasive cryptococcal rhinitis due to *Cryptococcus neoformans* var *gattii* was diagnosed in a castrated, 5-year-old, albino ferret with subcutaneous swelling of the nasal bridge. The diagnosis was based on histology, needle aspirate cytology and positive culture on Sabouraud's dextrose agar and birdseed agar. The ferret was successfully treated using a long course of itraconazole (25 to 33 mg orally once daily with food) subsequent to surgical debulking of the lesion, using sequential cryptococcal antigen titre determinations to guide therapy.
Aust Vet J 2000;78:158-159

LCAT	Latex cryptococcal antigen agglutination test
MIC	Minimum inhibitory concentration

A 5-year-old castrated albino ferret from the Central Coast of New South Wales was surrendered to the NSW Ferret Welfare Society because it had developed a lump on its nose. The lump had been present for several months and the ferret was otherwise well. A subcutaneous swelling on the nasal bridge immediately caudal to the nasal plane was excised surgically and submitted for histological examination. Cryptococcosis was diagnosed on the basis of characteristic organism morphology in tissue sections.[1,2] The ferret was referred to the University Veterinary Centre, Sydney, for treatment.

Physical findings included residual swelling of the bridge of the nose and a dry unkempt coat. The carer who had taken over responsibility for the patient observed lethargy, coughing and sneezing. Appetite, body weight (1.2 kg) and neurological function appeared normal.

The ferret was anaesthetised using isoflurane in a 2:1 mixture of nitrous oxide and oxygen delivered using a tight-fitting face mask connected to a coaxial non-rebreathing (Bain) system. Blood was collected to determine the baseline LCAT titre (1024) and a fine needle aspirate was obtained from the nasal swelling for cytology and fungal culture.

Diff-Quik stained smears from the aspirates demonstrated numerous spherical capsulate yeasts, many macrophages and fewer neutrophils and lymphocytes. A moderately heavy growth of *Cryptococcus neoformans* was observed on Sabouraud's dextrose agar and birdseed agar at 28°C. Colonies on birdseed agar were mucoid and demonstrated the brown-colour-effect characteristic of *C neoformans*. The strain was identified as *C neoformans* var *gattii*,[2,3] and showed in vitro susceptibility to amphotericin **B**, flucytosine, itraconazole (Etest MIC 0.023 mg/L) and fluconazole (Etest MIC 0.5 mg/L).

The ferret was treated using orally administered itraconazole (Sporanox, Janssen Cilag)[5] initially at 25 mg once daily. Dosing was facilitated by mixing a quarter of the contents of a 100 mg capsule with a palatable nutritional supplement gel (Energel, Veterinary Companies of Australia). After 3 weeks of therapy the itraconazole dose was increased to 33 mg daily. The ferret did well during treatment: appetite was unchanged or increased, nasal cavity signs improved and then resolved, and overall physical status improved concurrently. At 171 days after starting itraconazole, there was no sign of nasal cavity disease, swelling of the nasal bridge was absent, the LCAT titre had declined (128) and serum alanine

[a]Department of Veterinary Clinical Sciences, The University of Sydney, New South Wales 2006
[b]Department of Veterinary Anatomy and Pathology, The University of Sydney, New South Wales 2006
[c]New South Wales Ferret Welfare Society, 56 Percival Street, Lilyfield, New South Wales 2040
[d]Hornsby Veterinary Hospital, 11 Leonard Street, Hornsby, Sydney, New South Wales 2077