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Antifungal therapy in companion animals – A practical approach

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There are many good accounts of the pharmacology of a large number of drugs used to treat fungal infections in companion animals, including a good section in the latest version of Maddison, Page and Church's *Small Animal Clinical Pharmacology* (Second Edition), articles in recent Kirk's Current Veterinary Therapy editions, a section in Greene's text, and another account in Sykes' monograph. For a more detailed account of mechanisms of action, pharmacokinetics (PK) and pharmacodynamics, please refer to these sources.

This article concentrates on (i) what works, (ii) what is affordable, (iii) how to make sure the owners can afford to give the medication and (iv) how to be able to maintain good compliance. So the **emphasis will be on practicality** rather than the theory of drug action and also include some pertinent practical mycology to explain certain aspects of disease pathogenesis which underpin therapeutic decision making.

Dermatophytosis (Ringworm)

Dermatophyte infections are common in companion animal practice, especially in cats. *(Figures 1 & 2)*. Most dermatophytosis in cats and dogs is caused by *Microsporum canis*, which is a zoophilic dermatophyte adapted to life in mammalian hosts. In people, *Trichophyton rubrum* is the equivalent organism. Occasionally soil-dwelling organisms (geophilic dermatophytes) such as *Microsporum gypseum* or *Trichophyton mentagraphytes* can cause infections; these infections are usually more florid. *M. canis* infections are more common in cats than dogs. Dermatophytes live in keratin in the epidermis, and USUALLY do not penetrate into the dermis. Relatively rarely epidermal and dermal invasion occurs, resulting in **kerion** (ulcerative superficial dermatitis with folliculitis and furunculosis) or **pseudomycetomas** (deep cutaneous to subcutaneous pyogranulomatous inflammation; *Figure 3)*. Because dermatophytes colonise keratin, the host response is usually primarily one of hyperkeratosis and epidermal hyperplasia in order to increase turnover of the stratum corneum and 'shed' the organism, usually with a relatively minor inflammatory component (erythema). This means that effective antifungal drugs need to be lipid-soluble to get into the often thickened stratum corneum and stratum germinativum, in order to have an effect on the organism.

In the past, **griseofulvin** was the drug of choice for treating dermatophyte infections. It worked by being concentrated in the keratin of the epidermis, making it impervious to fungal attack. It was well tolerated, safe and cost-effective, although treatment required long courses, and there was a requirement to give the tablets with a fatty meal to enhance drug absorption. However, some cats (a very small minority) developed life-threatening idiosyncratic neutropenia, and for this reason, and the difficulty in getting the drug in modern times, it has largely fallen out of use. It is still an effective drug in the dog and may be available via compounding pharmacists. It is not recommended as the most suitable option.

Figure 1. Area of alopecia caused by Microsporum canis *infection in a cat.*

Figure 2. Dermatophye infection in a steer. These lesions were self-limiting, but pose a zoonotic risk to farmers.

There are currently two drugs that are recommended for systemic therapy of dermatophytosis – **itraconazole** and **terbinafine**. Itraconazole is the only drug that has been registered for veterinary use for treating dermatophytosis in Europe and the UK (but not Australia). Itraconazole is an azole compound, in fact a triazole compound, with a very wide spectrum of antifungal activity, being effective against yeasts and moulds, including dermatophytes. Luckily, it has recently come out of patent, and so as well as the original product **Sporanox®**, there is at least one good available generic formulation made by Mayne in Melbourne.

Sporanox® comes as 100 mg capsules and as a paediatric liquid elixir. The paediatric liquid has superior bioavailability, and it's the formulation which was adapted for veterinary use in Europe. For the last 20 years, Richard Malik (RM) has used Sporanox® capsules for treating dermatophyte infections using the original drug formulation Sporanox® DELIBERATELY because the bioavailability of each formulation depends on who makes it, and itraconazole is a VERY WATER INSOLUBLE drug (or putting the shoe on the other foot, it's a VERY LIPOPHILIC drug) – so using the original formulation by Janssen-Cilag is important. This is one drug where compounded formulations have to be viewed as being unlikely to produce RELIABLE therapeutic drug concentrations. If you open up a Sporanox® capsule, you will find out it is full of hard tiny white spherical beads. These are odourless and have no taste and can be mixed up with any canned food. There are many dosage regimens for treating cats with *M. canis* using Sporanox® – but we recommend 5 mg/kg orally once a day in the food. Typically, ringworm is found in kittens and young cats – and so the dose of itraconazole is small. For a 2 kg cat you need to give 10 mg once a day – which is about 1/10th of a capsule. To give such a small dose, you sprinkle what looks like a 1/10th of a capsule and add it to some tasty canned cat food e.g. Fancy Feast® or Dine®, and make sure that you have used a whole capsule by the end of 7 days (store the capsule in a sterile urine container). It doesn't matter how much the cat gets each day as long as it gets a whole capsule over the course of a week, as the drug accumulates in the lipid of the skin. Treating a 2kg cat for a week takes 1 capsule which costs around $3 to $4 per capsule, and it takes 4 to 6 weeks to clear most infections. Remember, it takes about 7 to 11 days for the itraconazole to build up in the skin, and so the lesions do not clear immediately. Remember also the cat's immune system is helping and their infections tend to be eventually self-limiting.

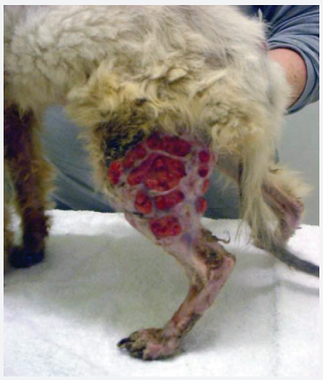


Figure 3. Pseudomycetomas in a long haired cat due to Microsporum canis.

Systemic itraconazole therapy is complemented by topical therapy – and several things can be used. Our recommendation is to wash the kittens using Malaseb™ shampoo because the miconazole is a useful topical antifungal, and washing cats and kittens with this product is easy, and leaves the coat looking good. In most cases it is best NOT to clip the coat, as all you end up doing is contaminating the environment of the veterinary hospital and, most worryingly, the clipper blades. For really bad focal lesions, Lamisil® cream (terbinafine) is a really good spot treatment if given in concert with systemic itraconazole. In the USA and certain other jurisdictions, lime-sulphur garden spray is used as a topical antifungal, but formulations available in Australia are not suitable for use on cats, and Malaseb™ is easier and more pleasant to use. Imaverol™ shampoo marketed for horses and dogs is a potent and useful topical antifungal that can be used for cats, but you need to make sure cats are towel dried and then blown dry (hot air dryer), as it can cause mild toxicity if they groom it off the coat (and therefore ingest the enilconazole).

The other treatment for ringworm which can be effective and inexpensive is terbinafine. This is because the drug is now out of patent and available in several human generic formulations as well as the original formulation Lamisil®. It is given at a dose in the order of 10-20 mg/kg twice a day, and this is a highly effective treatment comparable (although probably not quite as good) as itraconazole. There have been good recent papers by Kim Coyner and Karen Moriello about how useful this drug can be in a shelter situation where it is more cost effective than itraconazole. Lamisil® and its generic equivalents come as tablets which are scored for use in cats. Terbinafine has a cost advantage over Sporanox® which is especially relevant if the patient is a dog.

Fluconazole is not as effective as itraconazole for treating dermatophyte infections because it is water soluble, although it is used successfully in people and has the advantage of low cost. **Ketoconazole** is not as effective as itraconazole. Ketoconazole is no longer made commercially and is relegated to the history books in relation to the

treatment of ringworm, except for some shampoos like Nizoral® which can be used topically in place of Malaseb™. (Nizoral® shampoo is less expensive but not formulated for canine and feline skin).

Finally, environmental decontamination is very important in ringworm. A veterinary hospital is relatively easily to decontaminate (it's the spores that are problematic), and chlorine bleach and F10™ and the like are quite effective when combined with meticulous cleaning. For the premises of shelters and cat breeders, the Clinifarm enilconazole smoke bombs have a lot to recommend them (but they are not really suitable for inside a residential dwelling because a fine powder is left everywhere).

For people REALLY interested in dermatophyte infections, their pathogenesis epidemiology and therapy, there is a hugely rewarding website hosted by Karen Moriello, and if you have a breeder with a problem, consulting this information resource can be very worthwhile. There have also been some very good recent review articles written by the same author and her close colleagues in the *Journal of Feline Medicine and Surgery* and *Veterinary Dermatology* has recently published consensus guidelines which are available as a free download (open access) from http://onlinelibrary.wiley.com/doi/10.1111/vde.12440/epdf. If you commonly see such infections we strongly recommend you consult these resources.

Superficial Malassezia Infections

These yeast infections are not really an area of expertise for the authors, except for combined reasonable experience in Devon Rex and Sphynx cats and yeast otitis externa in dogs, which is common, especially in the setting of atopy. Most vets are now aware about how sticky tape (Scotch Tape™) preparations and characteristic clinical features can suggest the involvement of these lipid-loving-yeasts on the skin surface and ear canals. All Devon Rex and Sphynx cats have these, to a greater or lesser extent, especially in their ear canals and between their toes, and sometimes with a wider distribution of affected areas (including the tongue [black tongue disease] and dental plaque) *(Figure 4)*. As a result of the commonness of these infections, many medicated shampoos manufactured for dogs and cats have miconazole or ketoconazole in the formulation, at the right pH and with additional agents active against this organism and also *Staphylococcus* spp.

When systemic therapy is required for the management of severe yeast infections, itraconazole is generally the drug of choice, because it is a lipid soluble agent that partitions into cutaneous lipids, producing effective concentrations where it is most needed. The doses required are much lower than those used for treating invasive infections (like cryptococcosis and aspergillosis). Thus, for treating confirmed yeast infections, itraconazole (Sporanox®) at a dose of 3-5 mg/kg orally with food once daily is usually effective, if given in concert with medicated shampoos such as Malaseb™, and with dietary measures designed to decrease the production of sebum (reduced fat diet i.e. no premium dry food as such diets are especially high in fat). Some people believe terbinafine given systemically is also effective, although most dermatologists still consider itraconazole to be the drug of choice.

Remember, many Devon Rex cats have pruritic dermatopathy due to *Malassezia* spp infections and a trial of low-dose itraconazole combined with Malaseb™ shampoos weekly can be good empiric therapy before making a diagnosis of atopy (which is also common in this breed). Cases of *Malassezia* otitis externa are usually managed by topical therapy with an otic preparation containing miconazole. Frontline® Spray applied to gauze swabs is a highly effective way to 'defat' the skin and removes yeast impregnated sebum from the toes and ear canals of Devon Rex cats.

Cryptococcosis

Cryptococcosis is an invasive mycosis caused by members of the *Cryptococcus neoformans/gattii* species complex. The species complex is comprised of multiple molecular types (depending on which taxonomy you care to adopt), represented in *Table 1*. A current proposal for division of

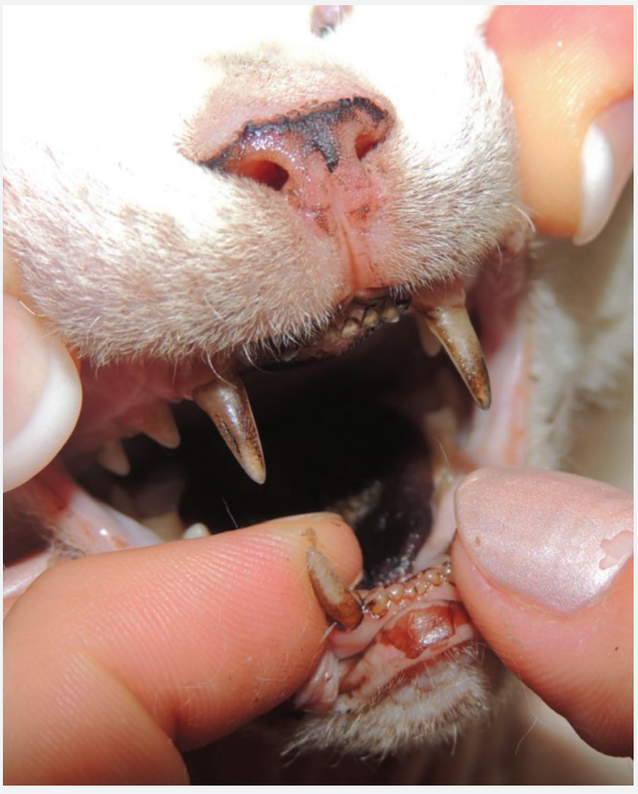


Figure 4. Photograph of a Cornish Rex cat where Malassezia is so abundant on the skin that it has been groomed onto the tongue and dental plaque, causing 'feline black tongue disease'. The brown/black discoloration was greatly improved by treating the cat with itraconazole and topical rinses with chlorhexidine. Photograph courtesy of Martina Načeradská.

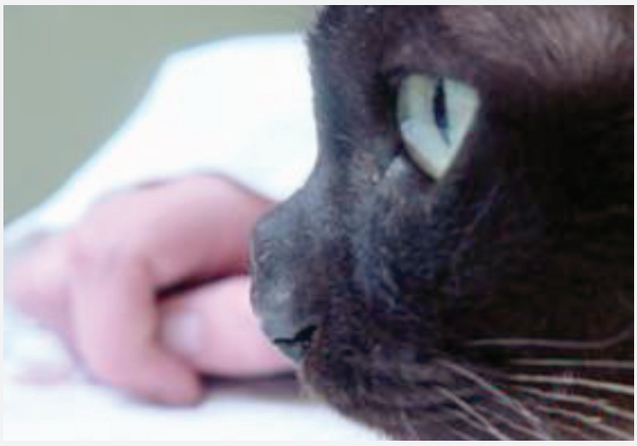


Figure 5. Cat with localised cryptococcal rhinosinusitis. The bump on the nose would be an ideal site to obtain a diagnostic needle aspirate.

Figure 6. Another cat with cryptococcosis, likely disseminated.

these species complexes into 7 different species and 4 hybrids remains controversial, and the current consensus is to manage the taxonomy as species complexes. In Australia, there are two important molecular types of the *C. neoformans* species complex (VNI, VNII) and two important molecular types of the *C. gattii* species complex (VGI, VGII). *C. neoformans* VNI (*C. neoformans* var. *grubii*) is widespread in Australia, while VNII is of sporadic distribution. *C. gattii* VGI is widespread throughout Australia with a strong eucalypt association and VGII is more focally distributed in South-West Western Australia and the 'Top End' of the Northern Territory with only sporadic occurrence elsewhere in Australia.

Subclinical self-limiting disease is actually likely very common in Australia (proven in the case of koalas; suspected for other species e.g. dog, cat and man). The number of symptomatic cases is much smaller, and these are the cases we see as veterinarians. It is an uncommon cause of invasive sinonasal disease in cats *(Figures 5 & 6)*. In dogs it is even more rare, but typically disseminated. The organism multiplies in host tissues as a yeast, budding to create new daughter cells. Infections generally start in the upper respiratory tract, although in Western Australia where *C. gattii* VGII molecular type occurs, it seems to sometimes enter via the alimentary tract (at least in dogs and horses). 'Crypto' can cause disease in all species, including native animals such as the koala *(Figure 7)*.

C. neoformana / C. gattii species complex	PCR Finger Printing / RFLP Type	AFLP Type 3	MLST Type	Proposed Species Names
C. neoformans species complex	VNI	AFLP1	Clade F	*C. neoformans var. grubii*
	VNII	AFLP1A / 1B / VNB	Clade G, H	*C. neoformans var. grubii*
	VNIII	AFLP3		*Cryptococcus neoformans-Cryptococcus deneoformans* hybrid
	VNIV	AFLP2	Clade I	*Cryptococcus deneoformans*
Hybrid		AFLP9		*Cryptococcus neoformans-Cryptococcus gattii* hybrid
Hybrid		AFLP8		*Cryptococcus deneoformans-Cryptococcus gattii* hybrid
Hybrid		AFLP11		*Cryptococcus neoformans-Cryptococcus deuterogattii* hybrid
C. gattii species complex	VGI	AFLP4	Clade D	*Cryptococcus gattii*
	VGII	AFLP6	Clade A	*Cryptococcus deuterogattii*
	VGIII	AFLP5	Clade C	*Cryptococcus bacillisporus*
	VGIV	AFLP10	Clade B	*Cryptococcus decagattii*

Table 1. Cryptococcal Nomenclature. *The easiest and most relevant nomenclature for the clinician is presented in the first column. One needs to be aware that several molecular typing schemes are used. In Australia, the predominant typing is reported as VNI-IV and VGI-IV. In Europe and USA, AFLP and MLST types may be reported by laboratories. In the final column is a proposed nomenclature that is currently somewhat controversial as to whether it should be accepted or not.*

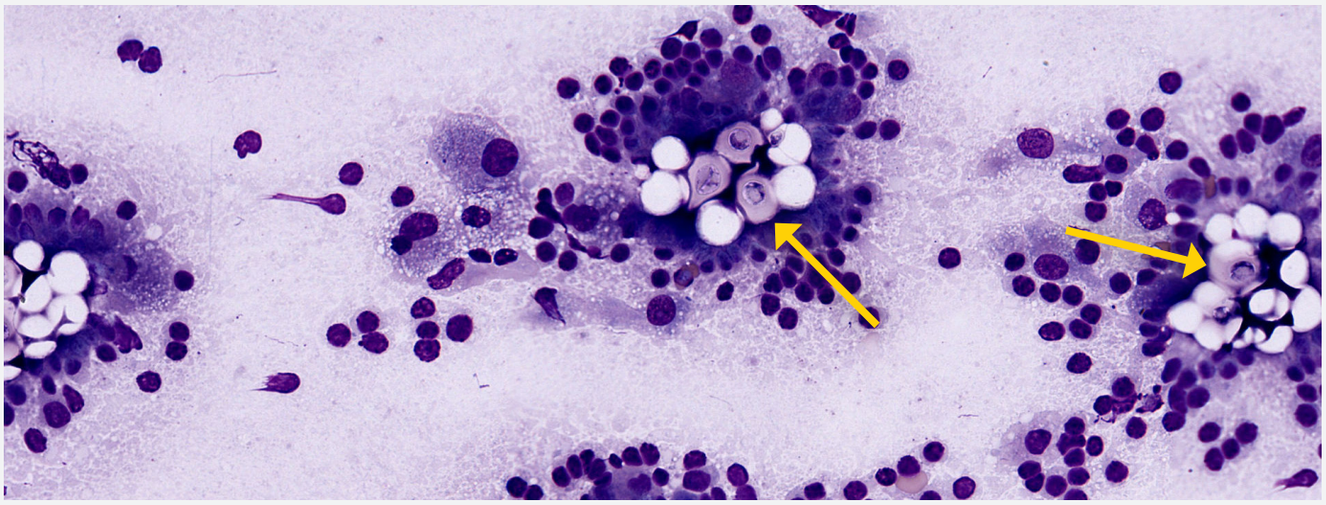


Figure 7. Cytology from a fine needle aspirate of a tracheobronchial lymph node of a koala with cryptococcal pneumonia taken at necropsy. Note the numerous encapsulated yeast cells associated with an inflammatory infiltrate including macrophages. Rapid Diff (Australian Biostain P/L Stains and Reagents for Pathology, Traralgon VIC).

There is a common relatively uniform antifungal susceptibility profile for *C. neoformans* VNI and *C. gattii* VGI isolates. Fluconazole is effective *in vitro* (in the laboratory) in nearly all cases from the east coast of Australia, whether the infection is caused by *C. neoformans* var *grubii* or *C. gattii* VGI. *C. gattii* VGII has a different susceptibility profile, often including substantial intrinsic resistance to fluconazole. Therefore in Western Australia and the Northern Territory susceptibility testing is important.

Fluconazole is the backbone of treatment for most cats and ferrets with cryptococcosis and many canine cases. The drug is safe, effective, inexpensive, suitable for twice daily dosing (once daily dosing is also acceptable although probably not as effective) and we have a sufficient feel for its pharmacokinetics in these species that therapeutic drug monitoring is usually not necessary (although measuring fluconazole levels during treatment is still a sensible thing to do). The feature that make it especially attractive is that many different compounding pharmacies can source good quality raw fluconazole from manufacturers in India or China, so that very affordable compounded capsules can be made up, usually at a fraction of the price of Diflucan®, the original product made by Pfizer. Merran Govendir's group, Mark Krockenberger, and Debbie Marriot's group (at St Vincent's Hospital, Sydney) and RM have looked at blood levels achieved during therapy in a variety of species and the compounded product seems to be bioequivalent to the original formulation. This is presumably because fluconazole is a water soluble drug with quite good absorption from the alimentary tract; so there is no need to have complex formulations to improve its bioavailability. There are also many generic human formulations and these are often even cheaper than compounded formulations, especially in dogs. (It's important to get your nurse or the patient's owner to find the most cost effective source of medication). The bottom line is that every cat and dog owner (even large dog owners) can afford fluconazole therapy for the protracted period required to effect a clinical cure, especially if you write a prescription and let people buy the product directly from the compounding pharmacy or local chemist, rather than mark it up from your own hospital pharmacy.

For the average cat with localised cryptococcal rhinosinusitis, monotherapy with fluconazole at a dose rate of 10 mg/kg orally twice daily will be successful in eradicating the infection, although treatment usually takes in the order of 6-12 months, if you treat past clinical cure, until the cryptococcal antigen test is negative (either Meridian latex agglutination or IMMY lateral flow). In dogs, very few cases are localised at the time of diagnosis; most have disseminated widely, typically with central nervous

The approach to invasive subcutaneous infections *(Figure 7)* is as follows:

1. Obtain good specimens for fungal culture and susceptibility testing
2. Start empiric treatment with itraconazole (dogs) or posaconazole (cats)
3. After preliminary therapy, consider surgical cytoreduction of lesions to debulk fungus-impregnated tissues. Consider use of intralesional or depot formulations (voriconazole in poloxamer reverse thermodynamic gel) at the time of surgery
4. Continue with combination therapy based on susceptibility data and information in the literature using an azole combined with terbinafine
5. Use an echinocandin e.g. caspofungin or anidulafungin or Amphotericin B if there is an inadequate response to oral therapy

system (CNS) involvement. So although fluconazole is still a vital component of therapy, you cannot cure that many dogs using fluconazole alone.

There is a new lateral flow immunochromatography test manufactured by Biosythex called Crypto PS which is very similar to IMMY but used a platform similar to those used for FIV and FeLV testing. Preliminary data from VPDS suggests it compares favourably with the IMMY strips. Each test kit costs $9.20. These point of care tests are useful screening tests, but all positive results should be confirmed using LCAT testing at a veterinary clinical pathology laboratory. Such kits will be very useful for both GP vets and specialists who wish to *rapidly* 'rule in' or 'rule out' cryptococcosis in e.g. in cats, dogs and ferrets with sinonasal disease or CNS disease.

A major limitation of fluconazole is that this triazole is only fungistatic, like most orally administered anti-fungal drugs. So it stops the fungus multiplying, but relies on the immune response of the host (both innate and adaptive immunologic mechanisms) to actually kill the fungus and clear it from host tissues. For this reason, in severe disease, long standing disease (diagnosed late), or where the host's immune response has been weakened by corticosteroids, you need to add in the **fungicidal** drug **amphotericin B.**

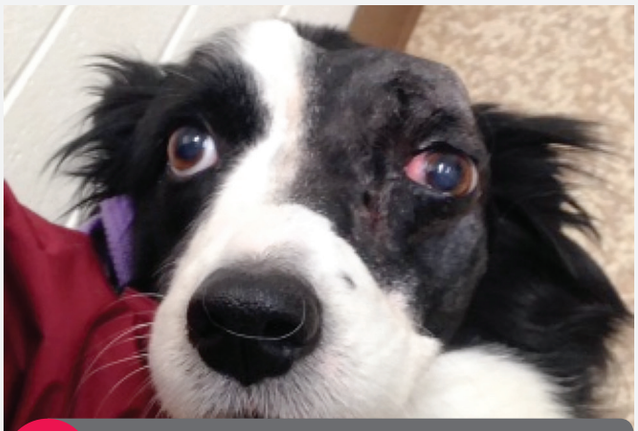
Amphotericin B is a much more powerful antifungal drug than fluconazole, and so we use it for severe infections and especially if there is CNS involvement. It is almost impossible to cure canine or feline cryptococcosis with CNS involvement using fluconazole – you need amphotericin B, although much later in the course of treatment fluconazole is continued for a very long time to consolidate therapy. It's much harder to use amphotericin B, as it is not well absorbed from the gastrointestinal tract*, so it must be given parenterally, either intravenously (IV), or as a subcutaneous bolus infusion. There are three different formulations of amphotericin B – the original bile salt formulation (deoxycholate), a lipid complex formulation and a liposomal formulation – the advantage of the latter two is you get equivalent efficacy with less nephrotoxicity, but at a very great price. In the 1990s we accidentally developed a very cost-effective subcutaneous protocol administering amphotericin B deoxycholate to cats and dogs, and it has been used subsequently to treat cats, dogs, koalas, large felids and other wildlife species. The original amphotericin B formulation is still available from Medsurge in Melbourne (http://www.medsurge.com.au).

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.

Amphotericin B protocol: Cats with advanced, disseminated or severe disease and patients with CNS involvement may improve with oral azole therapy; however, these cases will respond more quickly and have better outcomes if treated using amphotericin B and either or both flucytosine (see next section) and fluconazole, in concert. If the animals are sufficiently debilitated to require intravenous (IV) fluid therapy, amphotericin B should be given IV as a continuous infusion (0.5 mg/kg/day) in 0.45% saline and 2.5% dextrose supplemented with 20 mmol/L potassium chloride/L at twice maintenance rates (approx. 4mLs/kg/w).

Otherwise, we use a protocol in which amphotericin B is administered two or three times weekly as a subcutaneous bolus infusion. Amphotericin B is prepared by adding 10 mL of sterile distilled water to a 50 mg vial of amphotericin B to produce a 5 mg/mL colloidal suspension. Once made up, the amphotericin B suspension can be stored frozen for up to four weeks without loss of efficacy, and probably it is OK to store the vial for 2-3 months or even longer. The amphotericin B stock solution is thawed, when required, and the calculated dose is aseptically aspirated from the vial, which is subsequently refrozen. Work from French investigators indicates that heat-pre-treatment (60 to 70°C for 10 minutes i.e. hot tap water) of standard formulations of amphotericin B prior to administration reduces nephrotoxicity, thereby increasing the dose that can be given safely. To prepare a subcutaneous bolus infusion of amphotericin B, a 500 mL bag of 0.45% saline in 2.5% dextrose is heated to 40°C in a microwave oven, connected

***Note:** There are oral formulations in development that might change this recommendation in the future.*



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to a fluid-administration set, and 100-150 mL discarded. The calculated dose (0.5 - 0.8 mg/kg; typically 0.4-0.8 mL for a cat) of the stock amphotericin B suspension is injected into the fluid bag through its injection port. Fluid is then aspirated and injected back and forth into the syringe, repeatedly, to ensure effective transfer of amphotericin B to the bag.

A 19 gauge needle is then attached to the administration-set, inserted into the subcutaneous space between the scapulae roughly on the midline and the fluid allowed to flow as fast as gravity will allow. Raising the bag of fluid as high as possible facilitates rapid delivery of the infusion, which usually takes 10 minutes or so. I prefer to deliver the entire volume (350 to 400 mL) in one site, although the needle is occasionally repositioned further caudally if the patient shows significant discomfort after half or more of the fluid has been administered. In general, the subcutaneous infusion is well tolerated by cats that do not resent restraint *per se*. In fractious cats, sedation using midazolam/ketamine or light anaesthesia using isoflurane or sevoflurane is necessary. The fluid moves extensively through the subcutaneous space, tending to pool ventrally over several hours prior to being absorbed.

These infusions are continued two to three times per week (usually 0.7mg/kg twice a week) until there has been demonstrable clinical improvement and a corresponding decline in the serum antigen titre. Typically, the cumulative dose of amphotericin B required is in the order of 10 to 20 mg/kg (although some cases require as much as 40mg/kg). Interestingly, it is the cumulative dose that appears to be important, rather than the period over which the drug is administered. The rationale for using amphotericin B subcutaneously as a dilute suspension is to delay its absorption into the systemic circulation. This avoids high peak blood levels that cause renal damage. Delivering amphotericin B together with a large volume of fluid (and sodium) further reduces the tendency towards nephro-toxicity, because of the protective effect of the ensuing diuresis. It is therefore possible to administer larger, and thus more effective, quantities of amphotericin B using this protocol than have been administered traditionally.** It is prudent to monitor serum urea and creatinine concentrations regularly during therapy and to temporarily discontinue therapy if azotaemia develops; usually the urea concentration increases before the creatinine concentration. Owners are also advised to add a small amount of salt to the patient's diet, as this is thought to further minimize renal toxicity.

Amphotericin B is continued on a two or three times a week basis. If animals are hospitalised, treatment is often given on a Monday/Wednesday/Friday basis (0.5 mg/kg/infusion), whereas cases treated on an out-patient basis generally receive two infusions per week (0.5 to 0.8 mg/kg/infusion) for the convenience of the owners, and to minimise stress for the patients and to minimise cost. Once this therapy is embarked upon, it is usually continued for at least 6 to 12 weeks, at which time the patient is usually well enough to be continued on follow-up fluconazole therapy for several months until the antigen titre declines to zero. In some cases, a further course of amphotericin B infusions is required later, e.g. if the decline in the antigen titre is not sustained during fluconazole therapy. If mild azotaemia develops, it is necessary to discontinue combination therapy for a few weeks. This is usually more of a problem in older cats that have lost some renal reserve. Cats are given fluconazole while amphotericin B therapy is temporarily discontinued.

When treating canine cryptococcosis patients, subcutaneous infusions are given either twice or three times per week, and it is even possible to train exceptional owners to do this at home, as dogs are much more tolerant of the procedure than cats. When making up amphotericin B bolus infusions, it is important to remember that the drug is irritant, and that a concentration of 20 mg/L should not be exceeded. Thus, when treating dogs in excess of 20 kg, it is necessary to suspend the amphotericin B in 1000 mL of 0.45% saline and 2.5% dextrose, while dogs weighing less than 20 kg usually require 500 mL. Many dogs develop sterile abscesses at the site of fluid administration but these usually resolve spontaneously. They can be very severe in the occasional dog and in such patients amphotericin B can be given IV as an infusion over several hours preceded by 30-50 mL/kg of crystalloid solution such as Hartmann's solution IV over 1-2 hours.

Flucytosine can be administered orally in concert with the amphotericin B and fluconazole in cats with severe cryptococcosis, including cats with neurological involvement. It is administered at a dose of 250 mg roughly

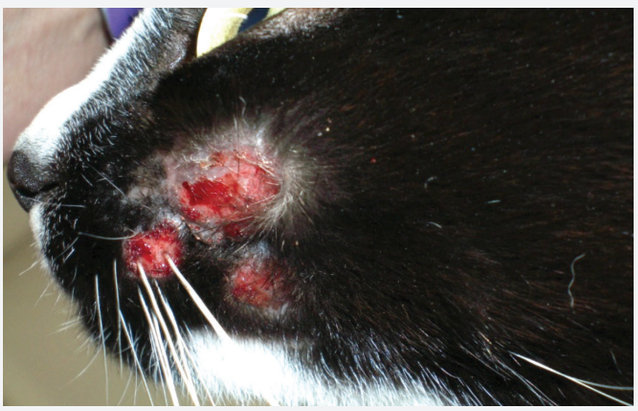

Figure 9. Localised fungal infection in an immune competent cat, probably after a penetrating cat scratch – cats claws are often contaminated by various saprophytic fungi from digging in soil. Photography courtesy of Kim Kendall.

***Note that if liposomal amphotericin is used for preliminary intravenous therapy, much higher doses are required on a mg/kg basis. Note liposomes are probably not absorbed intact after subcutaneous administration.*

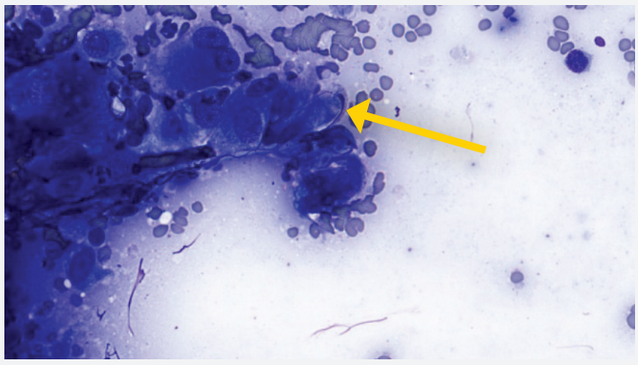

Figure 10a. Cytology from a distal antebrachial lesion of a domestic shorthair cat. Note the small variably sized conidia within macrophages. Rapid Diff. https://imagehub.sydney.edu.au/dih/webViewer.php?snapshotId=15031060848282

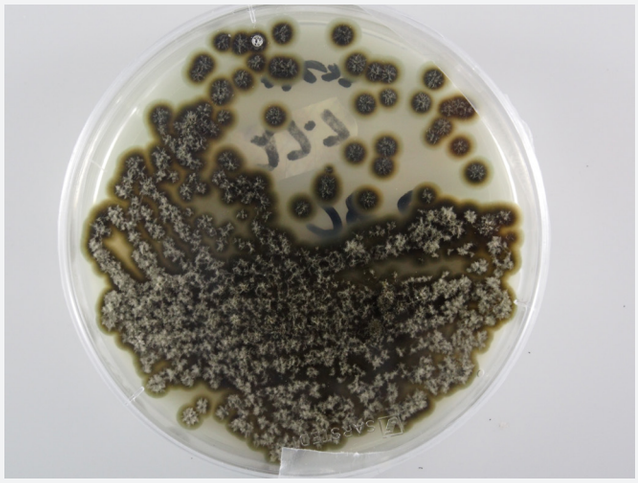

Figure 10b. Culture of a pigmented hyphal fungus from this case needs to be identified at a reference laboratory.

every eight hours (morning, afternoon and night). It can be difficult to source, but can be ordered from compounding pharmacists. This drug is generally well tolerated in the cat, although the occasional cat can become thrombocytopenic during therapy or develop other signs of bone marrow dysfunction. The combination of amphotericin B and flucytosine is truly synergistic, and represents the most effective drug therapy we can offer feline patients with cryptococcosis. Unfortunately, almost all dogs develop toxic epidermal necrolysis as a severe and predictable side effect of flucytosine therapy, and this usually develops about 7-10 days after starting therapy. So generally, we do not recommend using this combination in the dog, although probably it is safe for initial therapy with drug discontinuation after 7 days.

Amphotericin B is the most effective anti-cryptococcal agent, and although time-consuming to give, it is cost effective and well within the scope of an average GP vet. It is the only drug which is unequivocally fungicidal and of proven permanent benefit in CNS infections. It cannot currently be given orally; therefore, its administration requires a hospital visit. It is nephrotoxic, although this is largely reversible. Newer forms of amphotericin B

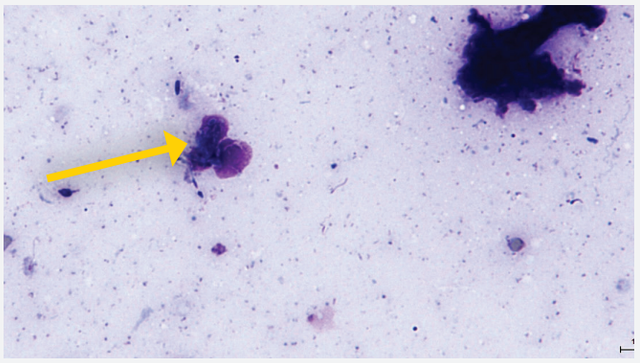

Figure 11a. Cytology specimen from ulcerated tail lesions of a 10-year-old female speyed Ragdoll cat. Note the fine fungal hyphae, also with some negatively stained intracellularly. Small tear drop shaped conidia can be found throughout the smear as well as scattered inflammatory cells and occasional squames and clusters of adnexal glandular epithelial cells. Rapid Diff. https://imagehub.sydney.edu.au/dih/webViewer.php?snapshotId=15031049117547

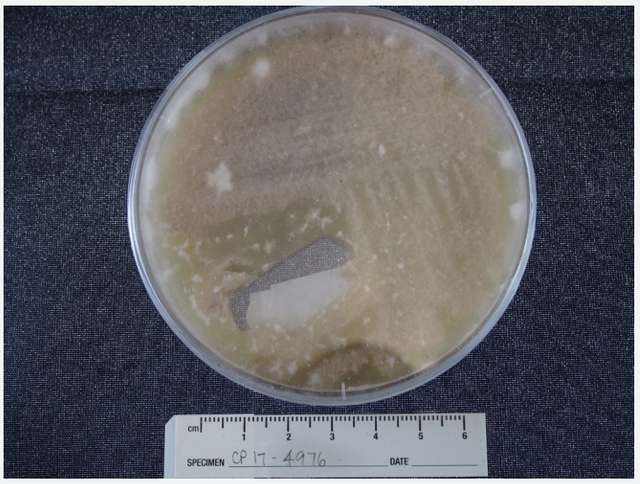

Figure 11b. Primary culture from this case on Sabouraud's agar. Colony morphology is insufficient to identify this fungus in a routine mycology laboratory. Identification at a reference laboratory may include both morphology and molecular identification.

such as liposomal and lipid complex preparations have no greater efficacy *per se*, but are less nephrotoxic, which in a veterinary setting may translate into greater efficacy, although at a substantially increased cost. The problem is there are no well-defined protocols for using these newer formulations in cats, as they are not stable for long periods after reconstitution, and so treating a cat is disproportionately expensive compared to using the original deoxycholate formulation, where a single vial can treat a given cat for many months.

In certain jurisdictions, fluconazole is not an effective drug for treating cryptococcosis because *C. gattii* VGII (Perth and NT) and VGIII (California) strains can be resistant to it. In these cases, itraconazole and terbinafine can be used for therapy. In people, there is evidence **voriconazole** can be more effective than fluconazole in some cases of CNS cryptococcosis, and this is an option in canine patients, but

probably not in feline patients because of the incidence of neurotoxicity during therapy when this drug is utilised at high dose rates.

Localised Invasive Fungal Disease in Immune Competent Hosts

Sporadic fungal disease can occur whenever penetrating injury results in inoculation of hyphae or spores into the subcutaneous tissues of the host *(Figure 9)*. Dogs, cats and other animals can all be affected. The range of fungal pathogens capable of causing disease by direct inoculation is extensive. The fungi implicated all have funky sounding names, and they all have different and often unique virulence properties. When fungi capable of making melanin are involved, they impart a characteristic colour to infected tissues e.g. black, grey, brown, green etc. These infections do occur everywhere, although a busy vet might only see one every couple of years. They can occur after cat scratches, and after bite wounds where the wound is contaminated by dirt. In the cat, the face and distal extremities are predilection sites.

Infections caused by pigmented fungi are collectively referred to as phaeohyphomycoses, regardless of the species of fungus involved. The name simply refers to the pigmented nature of the fungus involved. [Note that when the fungal agent is a pigmented yeast **without** hyphal formation, then the term used by the pathologist may be chromoblastomycosis (these are very rare in Australia)]. The clinical manifestations of these infections can be protean, although characteristically there are either focal pyogranulomatous lesions ('granulomas'), or draining sinus tracts, or both, usually after some history of penetrating trauma. If the feature of pyogranulomatous inflammation with pigmented club colony formation of 'grains' is present, then the pathologist may refer to the disease as a black grain eumycotic mycetoma.

Unlike dermatophyte infections and most cases of cryptococcosis, there are so many different fungi that can be implicated in these infections that you cannot easily make generalisations about which is/are the best veterinary agent(s) for effective therapy. **It is VITAL to get a mycological diagnosis (to the species level) by culture at a lab that is good at doing mycology.** Even then, usually the organism then needs to be forwarded to a Mycology Reference Laboratory e.g. ICPMR at Westmead, National Mycology Reference Centre in SA Pathology and PathWest in WA, where species identification is ascertained through classical mycology and PCR/sequence analysis, and subsequently susceptibility testing can then be arranged. This investment of $100-200 can be very worthwhile in the long term *(Figures 10 & 11)*. Please keep in mind that the 'breakpoints' of antifungal susceptibilities are not well established and the correlation with *in vivo* susceptibility can be sketchy. However, antifungal susceptibility data provides an excellent starting point to know the most likely effective antifungal agent, although the actual clinical response to therapy will need to guide you throughout the course of treatment. Most of these infections are caused by **moulds** rather than yeasts, so fluconazole is generally useless for therapy (there are rare exceptions). Normally, the drugs that can be considered include the 2nd and 3rd generation azoles (itraconazole, voriconazole [in dogs], and posaconazole), terbinafine, amphotericin B and the echinocandins. Having said this, some of these infections are localised to the extent that surgical 'debulking' has an important part to play in therapy, and some cases can be cured using surgery alone on the proviso that the lesion can be removed in its entirety with 'clean margins'.

Most of the newer drugs for treating invasive fungal infections are moderately expensive to hideously expensive, **so what the owner can afford becomes a critical part of drug choice and the selection of treatment regimens.** The investment in time and effort can be worthwhile, as the patients have no underlying immune issues and treatment generally has curative intent, i.e. the aim is to permanently eradicate fungal elements from the host tissues, without the need for ongoing antifungal therapy. In some cases, amputation of a limb is the most expedient way to ensure that the infection is eradicated.

Itraconazole has been around longer than most of the other drugs and most vets are aware of this drug and stock it in their hospital pharmacies. Older vets might still think about ketoconazole, but it is no longer manufactured and there are usually alternatives that work better, and without impacting on appetite and cortisol production by the adrenal glands and the metabolism of other drugs given concurrently. Ketoconazole is still used effectively in certain jurisdictions, such as for treating sporotrichosis in Malaysia and cryptococcosis in Canada (where many *C. gattii* VGII strains are resistant to fluconazole), but it really has little or no place in Australia except for the prevention and treatment of yeast infections in dogs and cats as a topical ingredient in shampoos and ear preparations.

As mentioned earlier, compounded itraconazole is very unreliable, as formulation of this water-insoluble drug is tricky and bioavailability is related to the cleverness of the formulation. Most people have got to know Sporanox® well – because of its efficacy in treating *Malassezia spp,* dermatophytes (especially in kittens) and cryptococcosis (in the days prior to veterinary compounding pharmacists, where Sporanox® had a cost advantage over Diflucan®). Itraconazole has a broad spectrum of antifungal activity, covering most yeasts (*Malassezia* spp, *Cryptococcus* spp, *Candida* spp) and many filamentous fungi including *Aspergillus fumigatus*. When treating invasive infections, doses of the order of 5 mg/kg twice a day or 10 mg/kg once a day are usually used. The drug must be given with food or its bioavailability is reduced, but its absorption

from the gut is still erratic, with considerable variation from individual to individual. Sporanox® recently came out of patent, and there are some generic formulations available. One of the most interesting is **Lozanoc®**, manufactured by Mayne in Melbourne using a patented new technology. This formulation uses a novel method to improve bioavailability, which means the dose required in people is 50% of the dose of the original Sporanox® formulation. We are currently trialling it in koalas with cryptococcosis, using therapeutic drug monitoring to determine its comparative bioavailability in comparison to Sporanox®.

There are three newer azole antifungals, and all have some advantages over itraconazole. The first is **voriconazole**, which has greatly improved efficacy against *Aspergillus* spp, good penetration of the cerebral spinal fluid and CNS, good urinary levels, improved bioavailability, useful activity against *Cryptococcus* spp and most *Candida* spp. Its dose rate is about 6-8 mg/kg orally once daily in dogs with food. The pharmacokinetics of this drug are complex and non-linear, and in time recommendations may change to dose twice daily. In cats, it can be used safely in some individuals, but in others it causes neurological side effects which are sufficiently severe to prevent on-going use. It is currently very expensive, to the extent that only the most dedicated and well-off owners can afford to use it in dogs. A generic form has just become available in Australia and this should help pricing in the future. Most feline specialists are wary of using it because of the possibility of neurological sequelae, but it can be life-saving in some instances, especially when fungal infections involve the urinary tract. Voriconazole is a photosensitising drug and patients should be kept out of the sun when receiving this agent, as it can cause not just actinic dermatitis, but also squamous cell carcinoma (at least in people).

The second new azole is **posaconazole**, which has many of the attributes of voriconazole, but an even broader spectrum of activity, more reliable bioavailability and better tolerance by both human and animal patients. It is available in a variety of preparations, although we are most familiar with the liquid formulation which is convenient for use in cats, as it permits very precise dose titration. The new tablet formulation has a lot of advantages including bioavailability, but the 100 mg tablet size means it is only potentially useful in dogs, as the tablets must not be split. The dose in dogs and cats is in the order of 6-8 mg/kg orally with food once daily. The pharmacokinetics of this drug are complex, with non-linear 'flip-flop' kinetics. For this reason, therapeutic monitoring is recommended during therapy for severe or life-threatening infections. The wonderful thing about this drug is that it is ideally suited to treating fungal infections in cats. It comes in a palatable liquid formulation (40 mg/mL); indeed, some cats will lick it off the dosing syringe. It is easy to titrate the dose in the light of clinical response or therapeutic monitoring (measuring drug levels in serum during therapy). This drug is useful for almost all fungal infections of cats. It is especially useful where a fungal organism is seen in histological or cytological preparations, but when (for whatever reason) it is not possible for fungal identification (ID) to be undertaken. Its spectrum of activity is so wide that it has efficacy against most of the pigmented agents that cause phaeohyphomycosis, zygomycetes like *Mucor* spp and resistant organisms like *Fusarium* spp and *Pythium* spp (a nasty oomycete) and algae like *Prototheca* spp. A 100 mL bottle costs in the vicinity of $800 wholesale and provides sufficient medication to treat a cat for appropriately 3-4 months (0.8 mL daily). The cost of this drug for dogs is prohibitive for all but the most committed and affluent owners. Some insurance companies will, however, cover the cost of voriconazole or posaconazole therapy.

The most recent azole to appear on the market is **isavuconazole**. It has good activity against cryptic *Aspergillus* species but has not been used much in dogs or cats. It has several theoretical advantages over voriconazole but the pharmacokinetics are not well understood in cats or dogs.

The final class of antifungal to mention is the echinocandins. These are potent useful agents that must be given parenterally. Usually they are given IV once daily for a course of therapy lasting 5-14 days, usually dictated by the size of the patient and the amount of drug in each vial. Subcutaneous treatment regimens have not yet been explored, but may have merit. They are especially effective against *Aspergillus* sp including cryptic species such as *A. felis*, and *A. terreus*. Although many of the new generation of antifungal drugs are expensive, these are **especially expensive** and their use is therefore generally restricted to refractory infections that fail to respond to other agents. The newer agent anidulafungin is less expensive than caspofungin, the best known drug of this class, but this cost advantage might make it the most practical drug to be used in veterinary patients.

Disseminated Fungal Disease

You need to consider the possibility of reduced immune function in these cases because it may alter your approach to therapy. In disseminated fungal diseases caused by filamentous (hyphal) fungi, immunosuppression/deficiency is a substantial possibility, especially in certain breeds of cats and dogs.

There are two common types of immune deficiency in dogs. First, there are inherited immune-deficiencies e.g. the predisposition to develop *Pneumocystis canis* pneumonia in Cavalier King Charles Spaniels. [In passing, although this is a fungal disease, it is treated with trimethoprim sulphonamide combinations (initially by the IV route) combined with inhaled pentamidine.] A much more common example of inherited immunodeficiency

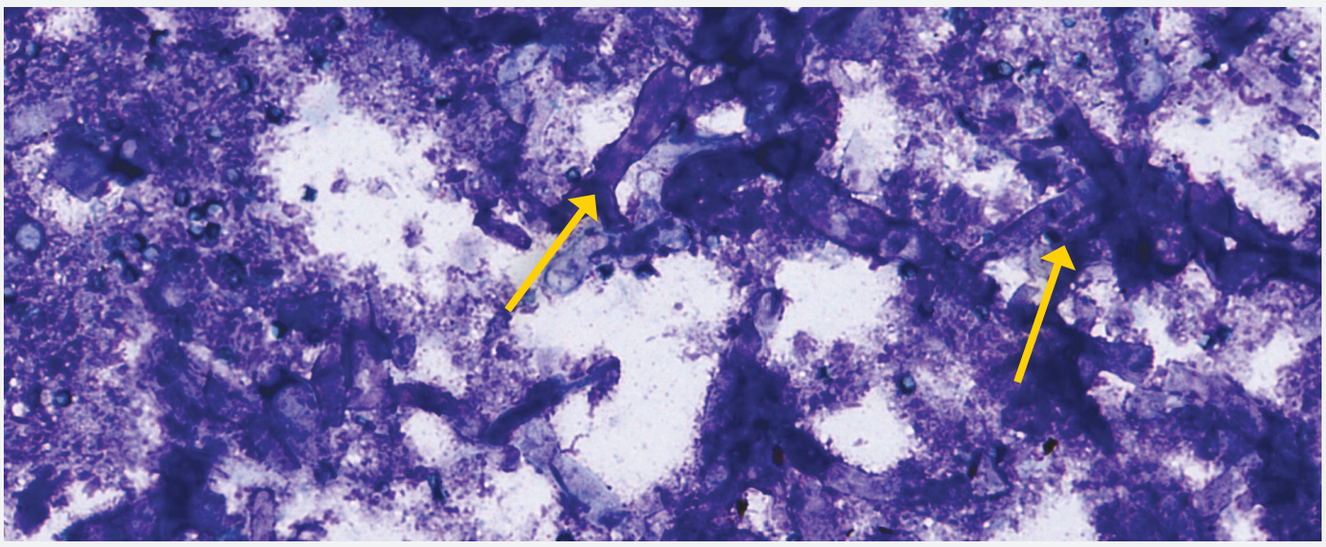

Figure 12. Specimen from the frontal sinus of an 11-year-old Boxer dog. Note the large amount of debris including large branching hyphae. If you go to the virtual microscopy link you may be able to explore the slide to find conidial heads consistent with Aspergillus *spp. Rapid Diff.* https://imagehub.sydney.edu.au/dih/webViewer.php?snapshotId=15029559711079

is the predisposition of German Shepherd dogs (GSD) to *Aspergillus terreus* and a broad range of other fungal pathogens and even organisms generally regarded as environmental saprophytes! The other conceptual category is dogs and cats rendered immunodeficient as the result of treatment of immune-mediated disease or cancer with immunomodulatory drugs such as prednisolone, chlorambucil, azathioprine cyclosporine and mycophenolate. Skin infections due to grass seeds contaminated by fungal organisms are being seen with increasing frequency in Australia and elsewhere.

One of the most challenging diseases an internist will treat is disseminated mycotic disease due to inherited immunodeficiency. This is especially well known in GSD, but occurs in other breeds including Vizslas, other pedigree breeds and pedigree hybrid dogs. These dogs have uncharacterised defects in their immune responses, probably a defect affecting innate immunity or cell-mediated immunity. After inhalation of a large dose of fungal spores from some environmental source, they get focal pneumonia with rapid lymphatic spread to the hilar lymph nodes. The fungal elements or spores then spread haematogenously to tissues which have vascular tortuosity, such as the uveal tract in the eye (causing anterior uveitis), the vertebral endplates, kidneys and the central nervous system. So affected dogs can present for red eye, back pain referable to discospondylitis, peripheral lymphadenomegaly and for signs of CNS dysfunction. **The majority of these cases have fungal hyphae in their urine and a mat of fungal hyphae in their renal pelvis (of one or both kidneys).** This fungal mat for some reason is not always easy to appreciate using ultrasound.

There are numbers of obstacles to treating these dogs. Inherited immune defects cannot be fixed, so the dog is always at risk for disease recurrence or the development of sequential opportunistic infections with other saprophytic pathogens. In dogs being given therapeutic immunosuppressive medication, you risk flare-up of the primary immune-mediated disease if you discontinue the immunomodulatory drugs too quickly, even though this facilitates successful treatment of the fungal infection.

Let's take the case of a young (2-year-old) female GSD with disseminated *A. terreus* infection. Consider the scenario in which the dog has multiple discospondylitis lesions, uveitis and fungal hyphae in the urine. We need high blood (and tissue) levels of anti-fungal agents to clear the vertebral/disc infection, but we also need effective urine concentrations to cure the mycotic pyelonephritis. *A. terreus* is not susceptible to amphotericin B *in vitro* or *in vivo*; furthermore, because of the mycotic pyelonephritis, the use of nephrotoxic agents is relatively contraindicated. So the ideal combination would be caspofungin (IV once a day for 1-2 weeks), plus 6 months of terbinafine plus either voriconazole or posaconazole orally, using therapeutic drug monitoring to ensure effective blood levels of the azole are being obtained in serum and urine. This will usually cure the soft tissue and bone lesions, but often fungal infection of the renal pelvis persists, so to have curative intent you need to think about topical therapy for the renal pelvises *viz.* 'wash outs' using indwelling nephrostomy tubes. This exercise in a 30 kg dog would probably cost somewhere in the order of $20,000 to $40,000 depending on how you mark-up the drugs. An alternative is not to have curative intent, but to rather control the infection indefinitely using a combination of generic terbinafine and Lozanoc®; this option is expensive, but possible for many committed owners. A far better way forward is to archive DNA from affected dogs with a view to performing a genome-wide association scan (GWAS) or whole genome sequencing with a view to using bioinformatics to find the underlying genetic defect. Prevention is far better than cure, in this scenario.

This is currently happening via research at UC Davis where Jane Sykes in internal medicine is working together with Danika Bannasch the medical geneticist at UC Davis.

Mycotic Rhinosinusitis

This subject is really sufficiently large for another article, but we think it should be mentioned for completeness. Our ideas are actually at odds with some of what is written in the literature, as we consider this to be an invasive mycotic infection in an immuncompetent host in both cats and dogs. Dogs that get this are likely unfortunate and suffer a really heavy load of fungal spores from some environmental source or possibly have focal breaches of respiratory tract mucosal barriers, whereas we believe cats probably have compromised host-immune defences (mucosal barriers) due to acute or chronic feline Herpesvirus-type 1 (FHV-1) infection or potentially other upper respiratory tract pathogens. In some cases, disease is limited to the sinonasal cavity, but fungi like *Aspergillus* species like to grow into blood vessels and cause turbinate atrophy (Figure 12). In other cases, the fungi 'punch out' of the nasal cavity to involve contiguous tissues such as the orbit or the brain (olfactory lobes), and this is more of a feature of the disease in cats. We can certainly see a similar occurrence with nasal cryptococcosis in all species.

Most of the literature over the last 20 years has emphasised topical therapy using 'soaks' and 'flushes' using topical agents such as enilconaozle and clotrimazole in what are pretty nasty solvents. Although we see a place for this, especially using less injurious formulations and depot formulations such as the reverse thermodynamic gel poloxamer, our view is that systemic therapy has been underutilised, and that combination therapy using terbinafine and either voriconazole or posaconazole will have a big role to play, especially when these drugs come out of patent and are thus more affordable. In the interim, a combination of Lozanoc® and terbinafine can be highly effective for treating early cases, although trephination of the frontal sinuses to clean out all the accumulated infected tissues and mucus and fungal mats can be very helpful at the outset. The problem with the literature is that it is dominated by long-standing cases that have been treated for protracted periods using antibiotics and sometimes corticosteroids, so that extensive changes are present by the time a definitive diagnosis is made.

Treatment of feline nasal aspergillosis due to a variety of cryptic species is especially problematic, although combination therapy using posaconazole and terbinafine following initial therapy with an echinocandin currently offers the best hope for survival. The new agent isavuconazole might also prove useful in these cases.

Fungal infections are tricky. Tricky to diagnose and tricky to treat. Mark Krockenberger and Richard Malik are very happy to assist colleagues in practice to manage these cases. Submissions can be made to the VPDS Laboratory at the University of Sydney, Building B14. We also happily talk to colleagues on the phone or by e-mail about management of these cases. We work with Catriona Halliday at Westmead Hospital, Sarah Kidd at SA Pathololgy in Adelaide and Ian Arthur at PathWest Laboratory Medicine, Perth, the best diagnostic molecular mycologists in Australia, and have strong links to research labs at Westmead Hospital, and human infectious diseases clinicians that are world authorities in the diagnosis and management of fungal pathogens.

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