Perspective No.157

FUNGAL DISEASE: AN UPDATE

[1]Richard Malik DVSc PhD FACVS FASM
[2]Patricia Martin MVSc
[3]Patrizia Danesi DVM PhD
[2]Lydia Tong BVSc MACVSc
[2]Mark Krockenberger BVSc PhD FACVS
[1]Centre for Veterinary Education
[2]University of Sydney Veterinary School
[3]Istituto Zooprofilattico Sperimentale delle Venezie – Legnaro (PD) – Italy

Introduction & Our Conceptual Framework

Veterinarians in Australia see cryptococcosis cases and dermatophyte infections (ringworm) like most of the world, but we do not have the endemic river valley mycoses like blastomycosis and coccidiomycosis that are such an important problem in the USA. We do have pockets where histoplasmosis occurs, but they are quite localised. So, in this *C&T* perspective, when we talk about fungal diseases, we generally refer to random fungal diseases which might be seen anywhere in the world.

The infections we wish to concentrate on in this essay are sporadic fungal diseases, usually in immune competent hosts, but sometimes in hosts that are immune deficient because of drugs administered to treat auto-immune diseases. We will also briefly cover dogs and cats with some inherited immune defects which predispose them to disseminated fungal infections with pathogens that rarely produce disease in normal hosts. We will also touch upon diseases caused by oomycetes and algae, which clinically can resemble fungal infections.

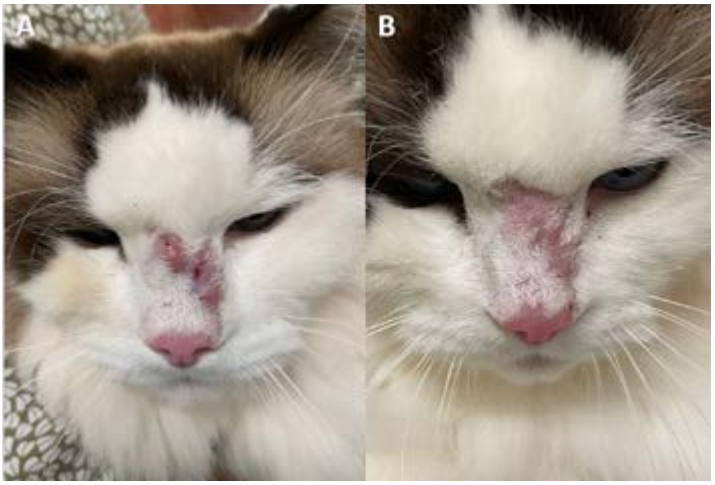

Figure 1. (A) This cat has a ***Fusarium solani*** infection in the naso-ocular region. This is a common site for deep fungal infections in cats, presumably as cat scratch injuries result in inoculation of fungal elements that are located on the nail of the perpetrator. A variety of fungi can be isolated from these types of infections, with a range of pathogens that are often refractory to commonly used antifungal drugs. Of course, you can also get unusual soil bacteria like ***Corynebacterium***, ***Nocardia*** and ***Mycobacteria*** in this location, and herpetic dermatitis is included in the differential diagnosis. This cat responded to long term oral treatment with posaconazole (B) during therapy. Some of these cases require intralesional amphotericin B, or debulking surgery followed by reconstruction of the resulting surgical wound to effect a cure.

Many fungal infections of soft tissues occur after penetrating trauma. This might be a stick, grass awn or a metallic object. It might also occur after a scratch or after a bite injury where two combatants roll around in the dirt. **In all these scenarios, we are dealing with an immune competent host in which a heavy inoculum of fungal elements is introduced into the subcutis, with variable destruction of local tissues caused by the inciting trauma.** In some situations, foreign material coated with a fungal biofilm is left in the wound, whereas in others the penetration is transient, but results a heavy inoculum of fungal spores or vegetative fungal elements being deposited in the subcutis.

Normally, an immunocompetent mammalian host is protected from potential fungal pathogens in the environment by an intact epidermis, usually covered by protective fur, and the action of the innate immune system. **When these protective mechanisms are circumvented by traumatic inoculation, a localised fungal infection can develop.** Cleansing wounds will of course decrease the chance of this occurring.

This local infection may be: (i) constrained by innate and adaptive immunity, or (ii) it might spread via the lymphatics (so called sporotrichoid spread) or (iii) by local extension to contiguous tissues, or (less likely) (iv) it might undergo widespread haematogenous dissemination, although this usually only happens in immune deficient animals.

It is not just fungi that can cause disease in this manner. The somewhat related oomycetes *Pythium* and *Lagenidium* can do the same. Indeed, both can actually set up infection even without penetrating injury, as they have motile zoospores which can penetrate skin softened by maceration. A chlorophyll-deficient algal organism called *Prototheca* is also capable of giving rise to subcutaneous disease after penetrating trauma.

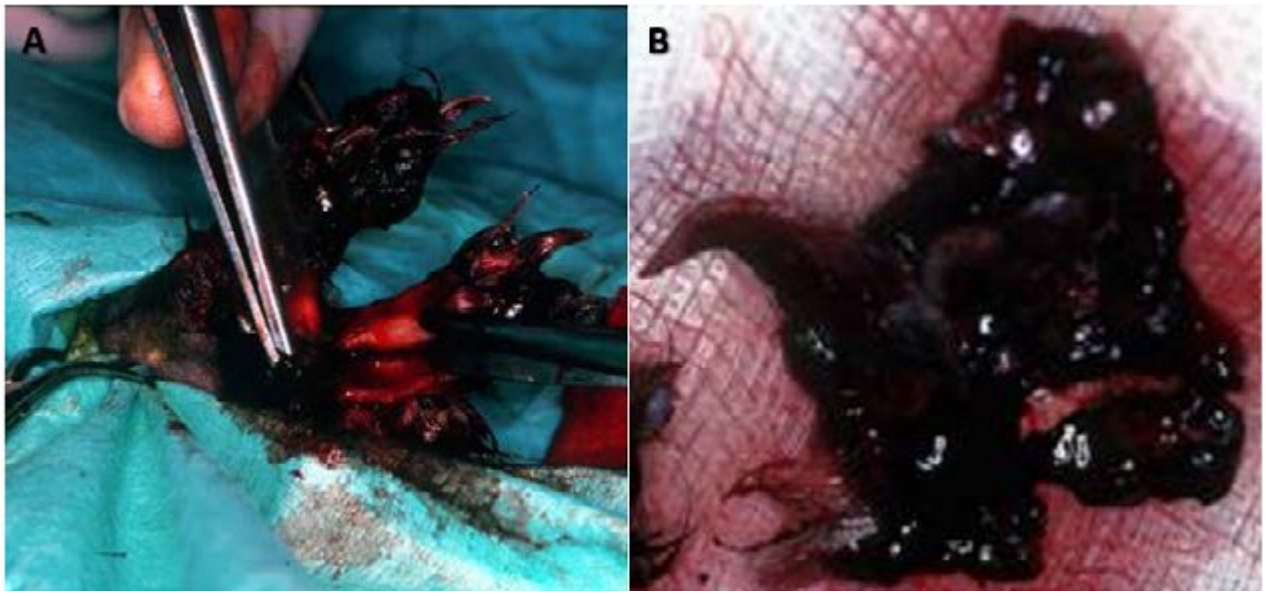

Figure 2. This cat has a deep soft tissue infection with a dematiaceous (pigmented) fungus – ***Wangiella dermatitidis***. This group of fungi normally live in the soil. A specialist surgeon is exploring the area and resecting all tissues which have a dark appearance from melanin imparted by the fungal pathogen. The surgeon has unfortunately placed the biopsy material on a gauze swab, which can be OK, but will sometimes prevent the growth of Mucormycetes such as ***Mucor*** as the large hyphae are easily fragmented by the cotton fibres. Better to just put the biopsy in a sterile urine container with a little sterile saline.

Lesions caused by saprophytic fungi introduced by penetrating trauma tend to have certain stereotypical anatomical distributions. For example, in cats, scratch injuries to a cat's naso-ocular region (face) can be contaminated by fungi, and also by mycobacteria and *Nocardia* spp—all having in common a domicile in the soil and dirt. Another common site for saprophytic fungal infection in cats is the distal limb, in the vicinity of P3 or the digital pads.

Although not the major focus of this article, mycotic rhinosinusitis (nasal aspergillosis) is another example of a localised infection (at least in the dog) in an immune competent host; although the exact pathophysiology has not been determined with certainty, it likely involves inhalation of a heavy load of fungal spores, or fungal elements on a grass seed foreign body.

A quite different scenario occurs when the immunity of the host has been compromised. This might be an acquired problem. Dogs with immune-mediated haemolytic anaemia, immune-mediated thrombocytopenia and polyarthritis are all generally treated with a combination of corticosteroids and immunomodulatory agents such as azathioprine, cyclosporine, or mycophenolate, which compromises innate and adaptive immunity, both cell mediated, and antibody mediated.

Dogs on combination immunosuppressive regimens are not infrequently afflicted by multifocal fungal infections of the skin and subcutis, possibly as a result of abrasions from grass awns and similar penetrative vegetable matter, where the foreign sharp object is often coated with fungal spores or vegetative fungal elements. A different type of immunosuppression occurs in the breed-associated immune deficiency states, where some as yet to be discovered chink in the normal immune response permits normally commensal fungi or environmental saprophytes to 'take off'. In some lines of Cavalier King Charles Spaniels and certain miniature Dachshunds, some defect in antibody or cell mediated immunity allows *Pneumocystis canis*, present as trophozoites in the lung of normal dogs in exceedingly small numbers, to multiply and give rise to what is eventually life-threatening pulmonary disease, often in association with cutaneous demodicosis and *Bordetella bronchiseptica* pneumonia. Recently, a Shih Tzu with *Pneumocystis* pneumonia was shown to have an X-linked CD40 ligand deficiency.

In German Shepherd dogs, Vizslas and a variety of other pedigree and pedigree hybrid dogs, filamentous fungi such as *Aspergillus terreus* or *A. deflectus*, the capsulated yeast *Cryptococcus* and a wide variety of other fungi, some of which are better known as plant pathogens, give rise to disseminated disease. The likely pathogenesis of these infections is that infectious propagules, most likely fungal spores, give rise to focal mycotic pneumonia, which is clinically silent, followed by lymphatic spread to the hilar lymph nodes, and subsequently haematogenous dissemination to tissues with a good blood supply but with some vascular tortuosity, such as the bony vertebral endplates, certain other bones, the anterior uveal tract in the eye, and the central nervous system. Treatment of such infections can be especially

challenging as certain immunological mechanisms that prevent fungal disease developing and help eradicate fungal pathogens are lacking in these breeds, which makes a successful outcome much harder to achieve. Furthermore, there is always the possibility for disease recurrence or the development of similar but unrelated infective insults.

Diagnostic Tips

1. **Get a really good sample. Handle it carefully!**
   It is optimal to collect a sample which contains the fungal elements (yeast cells or hyphae) without contamination from normal bacterial skin flora present in the overlying skin. This is because bacteria generally grow faster than fungi, although you can to some extent circumvent this by adding antibiotics to the fungal media to inhibit their growth.

   So, to procure a good biopsy, first cleanse the biopsy site by washing thoroughly but gently with an iodinated soap, and then 70% ethanol. Allow sufficient time for the ethanol to evaporate. Then make an incision, debride the tissues, and obtain representative deep material for biopsy. This might be an open surgical biopsy (e.g., *Figure 2*) or a core biopsy (using a skin biopsy punch (usually 6 mm or 8 mm diameter) or a spring-loaded core biopsy gun), or even just a fine needle aspirate biopsy.

   Often, of course, we do not know at first if a specimen will be fungal, oomycete, algal, bacterial or neoplastic.

   Under such circumstances, cut the biopsy specimen and cut it in half. Put half in formalin. Put the other half in a sterile urine container containing a few drops of sterile saline (to keep the biopsy from drying out). Critically, keep the unfixed portion of the sample in the practice refrigerator at 5°C (while you wait for the results of the examination of the formalin-fixed tissues).

   Do NOT wrap the biopsy in a gauze swab, as this will fracture the hyphae of certain fungi and stop them growing later when they are plated out on Sabouraud's dextrose agar in the laboratory.

   You can start off the microbiological investigation of the lesions by using a normal histological examination of the formalin-fixed tissues (initially stained with haematoxylin and eosin (H&E) and adding, when appropriate, special stains such as periodic acid Schiff (PAS) or Grocott-Gomori's methenamine silver stain (GMS)).

   ½ in formalin for histology; ½ in a sterile pot in the practice fridge

   This 2-step investigative process can save the owner some money!

   Keeping half the sample in the fridge (NOT freezer!) is an effective way to preserve fungi or other infectious agents in a viable state until it is clear that you need to submit a fresh tissue specimen for mycological culture. Freezing often kills eukaryotic organisms such as fungi, although their DNA remains intact for PCR and sequence analysis.

   If you are sure the infection is fungal (perhaps after seeing fungal elements in a preliminary fine needle aspirate), or if money is no object, then submit both specimens at the same time. However, when doing so, it is vital to adequately seal the formalin-containing specimen, or send it in a separate plastic container, as it is very easy for leaky formalin to contaminate the sterile sample and make it useless for fungal culture.

2. **Try to submit the sample to a reference mycology laboratory, either directly, or indirectly.**
   Although veterinary laboratories can often do a respectable job at handling fungal specimens, if you see a reasonable number of fungal cases, there is benefit in developing a relationship with a specialist human or veterinary mycology reference laboratory. They have expertise in both classical mycology, with excellent microscopy skills and a broad range of fungal media and special stains at their disposal as well as in the most modern molecular mycology methods, including panfungal PCRs with sequence analysis and many rapid tests (IMMY lateral flow for cryptococcal antigen, galactomannan index, β-glucan, etc.).

   If you use a veterinary laboratory for primary fungal isolation, it's often much better to ask them to forward the positive culture plates to a reference laboratory for specialist procedures such as definitive species identification (ID) and fungal susceptibility testing. Reference laboratories, in addition to employing scientists specialised in mycology to work at the bench, often have an association with infectious disease clinicians who may be willing to give you very good advice about case management.

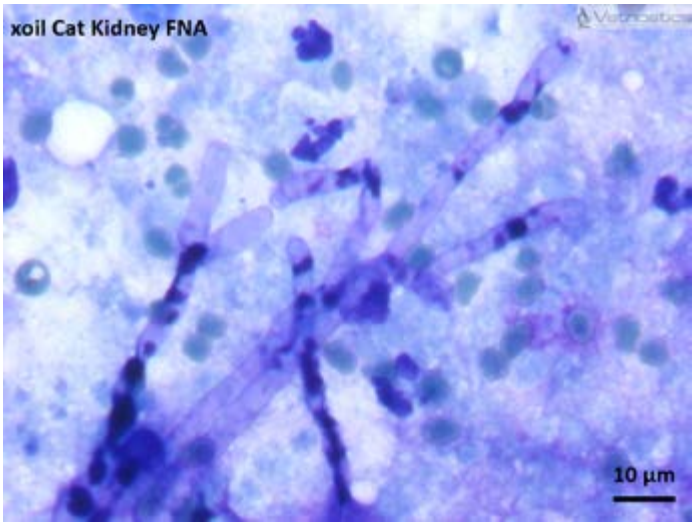

Figure 3. Fine needle aspirate from the renal pelvis of a cat with mycotic pyelonephritis. Diff-Quik stain of a cytospin preparation. Note the very broad ribbon-like hyphae, suggestive of a Mucoromycete. Culture PCR and sequence analysis was diagnostic of *Lichtheimia corymbifera*. The cat was treated with posaconazole based on susceptibility testing, although the cat succumbed. Most antifungal drugs do not reach sufficient concentration in the glomerular filtrate to effectively treat mycotic pyelonephritis.

Figure 4. Fine branching septate hyphae from a pellet after centrifugation of a urine specimen from a German Shepherd with mycotic pyelonephritis. The morphology of this organism is highly suggested of an Aspergillus species. 400X magnification; Diff-Quik stain.

An alternative is a veterinary lab in a veterinary teaching hospital that has a special interest in fungal infections. Veterinary teaching hospitals will often have academic staff who make fungal disease their research area.

In Australia, there are several human hospital laboratories that will process veterinary specimens for a fee. The key contact people are senior hospital scientists at Westmead Hospital (Dr Catriona Halliday catriona.halliday@health.nsw.gov.au), Adelaide Women's and Children's Hospital (Dr Sarah Kidd sarah.kidd@sa.gov.au) and Concord Hospital (Evanthia Tambosis). Veterinary Pathology Diagnostic Services at the SSVS at the University of Sydney is also worth an enquiry.

3. **Try to get the lab to fully describe the fungal morphology in cytology or histology specimens.**
Some labs just say, `fungal elements detected'. This is not helpful!

We need to know: Are there yeasts or hyphae? Do the yeasts have a capsule? Is there budding? Are the hyphae branched? What angle do they branch? Are the hyphae septate? Are the hyphae thin with parallel walls, or thick and irregular or ribbon like?
(See *Figure 3)*

If you have a good morphological description you can often guess the general class of fungus, which will help you select the best empiric agents for therapy.

If the lab will not do this for you, find another lab!

4. **Choosing empiric therapy and obtaining susceptibility data.**
Once you get a definitive identification of the fungal pathogen, ideally from a reference laboratory, do a key word search in Google Scholar or a PubMed search engine to find some recent veterinary or human papers on management of the pathogen. If they are behind a paywall—just fire me an e-mail and I will get them for you.

Each fungal pathogen behaves similarly in animals and man so both human and veterinary papers are equally germane to your cause. Even a horse paper might be informative for a companion animal clinician, and vice versa. For some unusual fungi, only human papers may be available. The reason why the host species is less important is that most fungi are environmental in origin so their biology, resistance pattern and behaviour are very much a feature of their normal environmental niche rather than the host in which they cause disease.

Based on the published information for the particular fungal species, you will be in a position to choose empiric therapy. It is ideal, however, to ask the reference laboratory to perform susceptibility testing, if possible (susceptibility testing may be difficult / impossible for poorly sporulating isolates). This takes time (often 5-10 working days, or more) and, in general, you cannot afford to delay therapy. So, start empiric therapy with one or more agents. You can change your drug choices in 1-2 weeks when susceptibility data becomes available. **In severe or acute cases, it is vital to be aggressive and use at least two agents, as then at least one is likely to be effective.**

Delay in appropriate therapy is a common reason for treatment failure. Unfortunately, the best drugs are often the most expensive and sometimes high efficacy is associated with injectable-only agents or comes at a cost of some toxicity.

When you are sure about ID and susceptibility, it is then appropriate to de-escalate and use the most cost-effective drug(s) that are likely to work.

It is bad medicine and generally poor value to choose a drug because it's less expensive and hope it might help. It is much better value in the long term to choose a drug which is expensive but likely to be effective against almost all fungal pathogens. In 2022, usually that is drug posaconazole.

5. **Learn about rapid tests for endemic fungi likely to be encountered where you practice.**
In Australia, cryptococcosis is the most common systemic mycoses in cats and probably dogs. The same is probably true in California and British Columbia in Canada. It is even more common in native Australian animals such as koalas. Obtaining representative material from nasal exudate, nasal washings, bronchoalveolar lavage fluid specimens or fine needle can often provide a rapid diagnosis because of the very characteristic organism morphology in smears stained with rapid Romanowsky stains such as Diff-Quik (see *Figure 5*).

There are exceptionally good rapid tests for this pathogen, of which the IMMY lateral flow was the first to appear on the market and to date its performance appears to be superior to the many imitators that have appeared subsequently. This test uses immunomigration technology (immunochromatography) to detect nanomolar concentrations of polysaccharide fungal antigen. The authors strongly recommend that this test be used before doing expensive testing such as cross-sectional imaging (CT, MRI) or endoscopy, as cryptococcosis is sufficiently common in endemic areas that an inexpensive screening test is worthwhile. The IMMY lateral flow test is highly sensitive but only moderately specific, making it a useful screening test. By this, we mean that false positives do occur. Positives need therefore to be confirmed by latex cryptococcal antigen agglutination testing, or by obtaining material for cytological examination and/or culture.

IMMY style tests can be done cage side in the practice. A similar test is available to diagnose histoplasmosis using urine as the diagnostic specimen. Histoplasmosis is rare and sporadic in Australia, so this test is rarely used, although it is widely used in the USA in places where histoplasmosis is endemic.

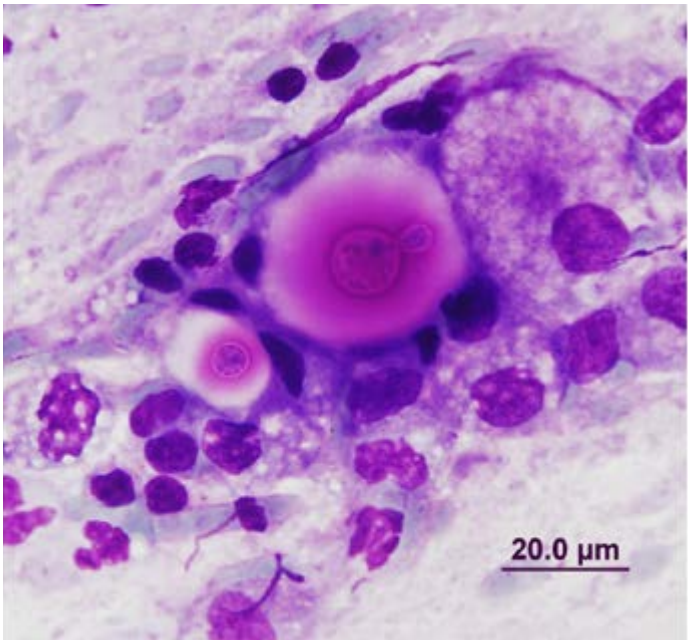

Figure 5. Diff-Quik stained smear of an aspirate from a nasopharyngeal mass that turned out to be a granuloma caused by *Cryptococcus gattii* in a cat. The presence of an abundant capsule that may or may not take up the stain, and narrow-necked budding are characteristic and thus many smears are pathognomonic for cryptococcosis.

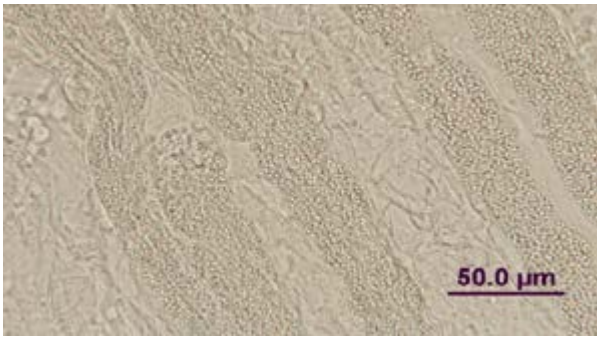

Figure 6. Ectothrix arthrospores of *Microsporum canis* in a KOH preparation of a hair plucked from a cat.

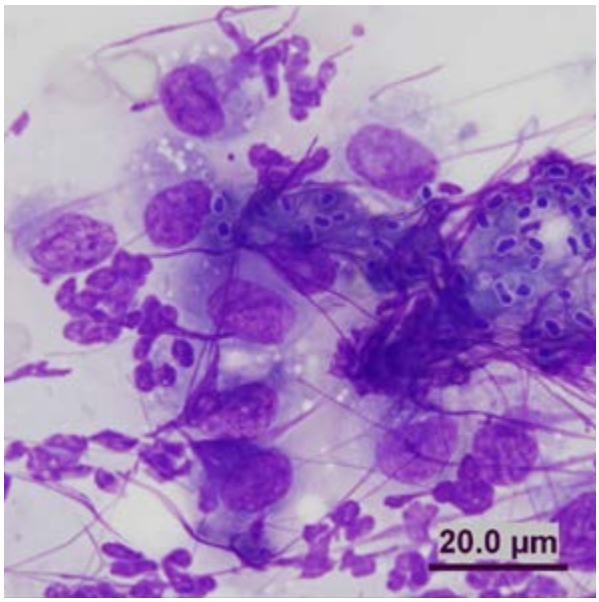

Figure 7. Diff-Quik-stained fine needle aspirate from a skin mass caused by the geophilic dermatophyte *Nannizzia gypsea*.

Treatment Tips

1. **Treating dermatophyte infections**
   Perhaps the most common fungal infections of cats and dogs, especially young patients, is dermatophytosis. The most common dermatophyte involved is the animal-adapted organism *Microsporum canis*. Much less common are soil-associated organisms such as *Nannizzia gypsea*, (formerly known as *Microsporum gypseum*). The diagnosis and management of dermatophyte infections deserves a full article. It is worth mentioning in passing, that diagnosis can now be performed by PCR testing as well as the more traditional fungal culture in the practice (Fungassay®) or in the diagnostic laboratory (using selective medium).

   There is an incredibly good general article on management of dermatophyte infections by Karen Moriello, Kim Coyner, and colleagues available as an open access free download at onlinelibrary.wiley.com/doi/full/10.1111/vde.12440.

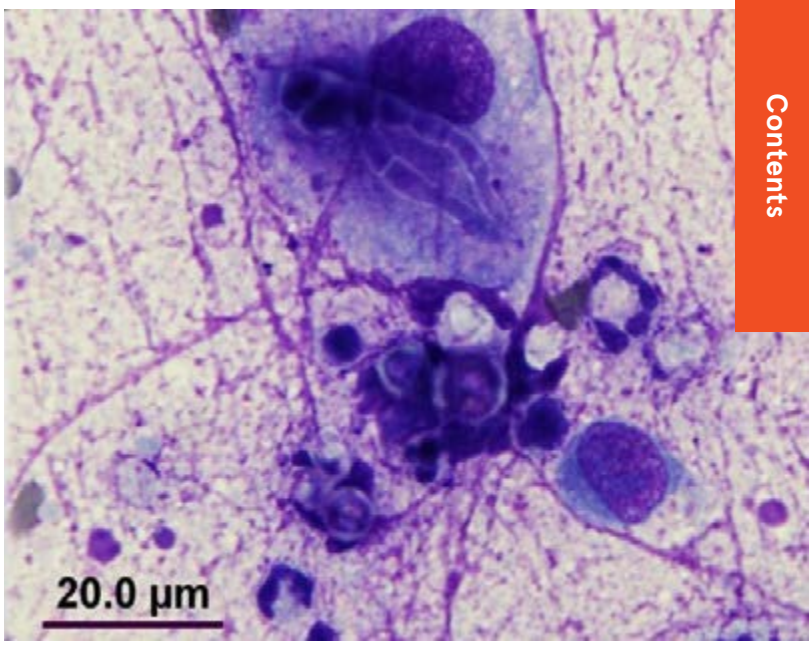

Figure 8. Diff-Quik stained fine needle aspirate biopsy from a subcutaneous mass in a cat caused by the plant pathogen *Microsphaeropsis arundinis* often thought to be associated with the garden escape weed elephant grass.

Note that the doses of itraconazole used for treating dermatophytes (typically 5 mg/kg of Sporanox) are substantially lower than those used for systemic mycoses, as dermatophyte infections are usually limited to the skin, and itraconazole is concentrated in the skin because of its high lipid solubility.

Therefore, do NOT use low dermatophyte dose regimens for itraconazole when treating more invasive infections. Use at least 5 mg/kg twice a day, or 10 mg/kg once a day.

Itraconazole should be given with food to enhance its absorption.

2. **Treating soft tissue infections following penetrating trauma with posaconazole ± terbinafine.**
   Subcutaneous and cutaneous infections can occur in dogs and cats after penetrating injuries with sharp objects, such as sticks, plant awns and barbs, sharp metallic objects, and after cat scratches and animal bites contaminated by dirt. A wide range of fungi can be implicated.

   The most common fungal pathogens encountered are likely influenced by geographical considerations such as soil types. For example, in Sydney, NSW, Australia a very unusual dematiaceous (pigmented) fungal pathogen *Microsphaeropsis arundinis* is the most common cause of soft tissue infections in cats. It also occurs in human patients, and to date, 1 dog. It is thought to be common here

because of its relationship as a pathogen of the garden escape weed known colloquially as Elephant grass.

Many different fungi can be involved, however, including the especially nasty pathogen *Fusarium*. The range of potential pathogens is so large that it is impossible to choose a single antifungal agent that will cover all possibilities. **Our considered advice is that posaconazole is a particularly good agent to use for empiric therapy while awaiting culture and susceptibility results.**

Posaconazole is a second-generation triazole agent. It is an exceptionally safe drug and tends to be very well tolerated. It is generally given orally once daily. Cats and very small dogs are best treated using the suspension, whereas in dogs the sustained release tablets represent the most cost-effective way to administer this agent. It has a very broad spectrum of activity. It used to be very expensive, almost prohibitively expensive, but it came out of patent in 2022 and several generic formulations of the sustained release tablets that are affordable and cost-effective are now available. THIS IS REALLY A GAME CHANGER—as it means posaconazole is affordable therapy for long term treatment in large dogs. Note that the tablets cannot be scored in any way—to dose smaller animals, use the 100 mg dose but give it every second day or even every third day.

The suspension is generally used in cats and small dogs at a dose rate of 8 mg/kg once a day, whereas the dose of the sustained release tablet is 5 mg/kg every day, or every other day, depending on whether you need to achieve really high blood concentrations. The drug levels obtained in individual patients can be unexpectedly high or low, such that measuring blood levels is a very cost-effective exercise. This is known as therapeutic drug monitoring (TDM). TDM is available in the pharmacology department of St Vincents Hospital in Sydney and is highly recommended for all antifungal drugs, especially expensive drugs, and drugs with potential toxicity.

Drugs commonly assayed for TDM include fluconazole, itraconazole, voriconazole and posaconazole. TDM is not widely used for amphotericin B, flucytosine or terbinafine.

To further broaden the spectrum of antifungal activity, terbinafine is often added to posaconazole in a combination therapeutic regimen. Terbinafine is available as a generic drug and is therefore much cheaper.

Table 1 – Current costs of posacoanzole

Original products
Noxafil Oral Suspension MSD $888.90 plus 10% GST
Noxafil tablets MSD 100 mg 24 $838.95 plus 10% GST
Generics
Pharmacor tablets 100 mg 24 $169.99 plus 10% GST
ARX MR tablets 100mg 24 $332.65 plus 10% GST

In many instances, surgical debulking has a place after preliminary antifungal therapy. It is generally best delayed until after you have obtained susceptibility data for the pathogen, so you can make sure you have established effective blood levels of an appropriate antifungal during surgery, and CRITICALLY, during the healing stage following reconstruction of the surgical wound.

Debulking fungus impregnated tissues, followed by wound reconstruction, is a highly effective way to progress therapy in cases with extensive disease.

Sometimes the surgery can also remove foreign plant material which actually introduced the fungal inoculum into the host tissues.

By cytoreducing the extent of infection, the infective agent is better exposed to high levels of antifungals in the patient's blood.

A top tip for treating most fungal diseases is the drug levels in actual patients can be unexpectantly high or low, such that measuring blood levels is a very cost-effective exercise, and this is known as therapeutic drug monitoring (TDM).

Level too high – sometimes risks toxicity for some drugs.

Level too low – drug will not work!

3. **Intralesional amphotericin B as an adjunct to oral antifungal therapy.**
What do you do when the infection is present on an extremity, such as the nose or a distal

Figure 9. Deep subcutaneous infection in a cat presumably after penetrating injury. *Fusarium oxysporum* was cultured from the lesion.

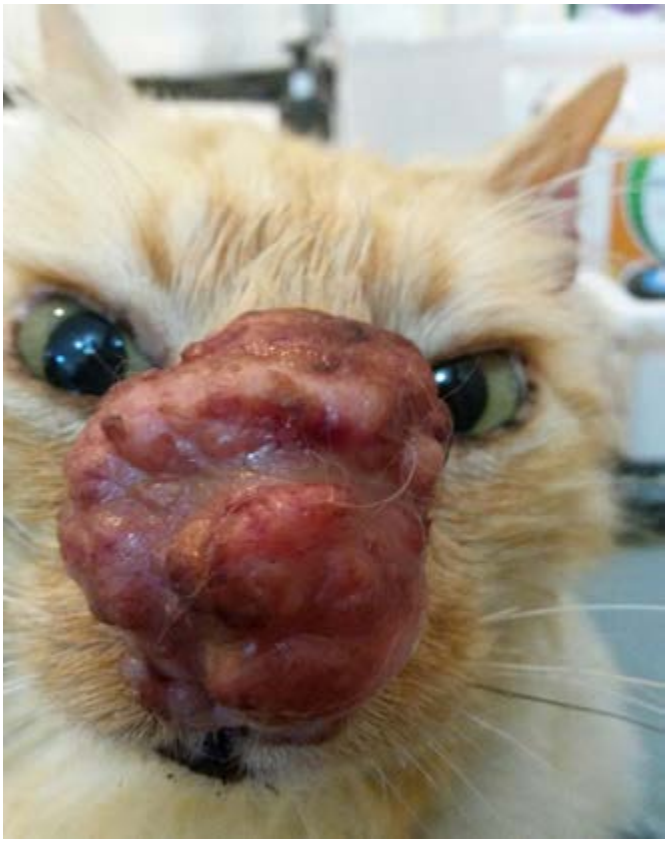

Figure 10. Sporotrichosis in a cat from Brazil caused by *Sporothrix brasiliensis*. This cat would be an excellent candidate for debulking surgery followed by intralesional amphotericin B therapy as an adjunct to systemic therapy with oral itraconazole.

limb, where debulking surgery is difficult to impossible, without recourse to amputation? This is not an unusual scenario!

A specialist surgeon has the ability to use an Esmarch's bandage as a torniquet and resect infected tissues and reconstruct the wound via fusion podoplasty, but this technique is possibly beyond the ability of an average practitioner.

A 'trick' which can be useful in such a setting is the use of intralesional amphotericin B. This agent is traditionally used systemically for life-threating fungal infections such as cryptococcosis, but Brazilian veterinarians have adapted a human procedure for treating refractory cases of feline sporotrichosis on the face near vital structures such as the nose and the eyes. Intralesional amphotericin, using a concentration of 2.5 to 5 mg/mL of amphotericin diluted to a volume of 2-3 mL can be used to infiltrate any fungal infected tissues, administered on a once weekly basis (or more often if necessary) under heavy sedation or light general anaesthesia. This has a potent local antifungal effect and is a nice intensive treatment for extra efficacy on top of oral therapy with posaconazole and terbinafine for a variety of fungal pathogens capable of causing lesions of the distal limbs or naso-ocular region.

4. **What about fluconazole, itraconazole, voriconazole and isavuconazole for systemic therapy of fungal disease?**
Fluconazole and itraconazole were the first two triazole antifungal agents. The original azole antifungal ketoconazole is no longer available, except from compounding pharmacists.

Fluconazole is active largely against yeasts, such as *Cryptococcus* spp and *Candida* spp. Its leading role is therefore in treating *Candida albicans* and other susceptible *Candida* species, and in the management of cryptococcosis. It is a narrow spectrum antifungal. It is very safe, and widely available as generic formulations which are

Itraconazole is an exceptionally difficult drug to compound, and treatment failures are often a direct result of using compounded itraconazole formulations that have poor bioavailability.

DO NOT USE COMPOUNDED ITRACONAZOLE – IT IS A FALSE ECONOMY

cost effective, but it generally has almost no efficacy against filamentous fungi like *Aspergillus* spp and other soil-dwelling fungi likely to be seen after penetrating trauma. It is a very important drug in the treatment of cases of cryptococcosis that are mild to moderate and in which there is no CNS involvement. In such cases, its low cost, safety, efficacy and good penetration of the CNS and eye make it the backbone of therapy. It has very little use for most other fungal infections. It is occasionally used for dermatophytes where its major advantage is low cost, but it does not concentrate in the skin like itraconazole.

Itraconazole has a much wider spectrum of activity compared to fluconazole and is effective for treatment of many fungal infections including sporotrichosis, blastomycosis, histoplasmosis and coccidiomycosis. Indeed, treatment regimens have been developed for treating these so-called river valley mycoses using itraconazole based on compelling evidence in the form of extensive case series. The original drug was developed by Jansen Cilag, with a capsule formulation and a liquid formulation, the latter having superior pharmacokinetics in companion animals. The capsules when opened contain many specially formulated little white spheres which can be mixed in with a cat or dog wet food, and this is a convenient way to administer therapy. There are some good human generic formulations of itraconazole but beware compounded itraconazole.

In Australia, we have a unique formulation of itraconazole called Lozanoc® with superior pharmacokinetics as a result of improved bioavailability; there is much greater consistency in achieving therapeutic blood levels, and a dose of 5 mg/kg of Lozanoc is used equivalent to 10 mg/kg of Sporanox. There are, however, some potential problems with itraconazole. When treating invasive fungal disease, doses of the order of 10 mg/kg once a day or 5 mg/kg twice a day are commonly used. It is not uncommon for such doses to cause clinically significant hepatotoxicity during a long course of therapy. This can be detected early by monitoring the ALT activity in plasma, which rises gradually before icterus and anorexia develop. Many internists administer liver tonics such as S-adenosyl methionine at the same time as itraconazole in an attempt to prevent liver injury, and anecdotally, this strategy appears to be successful. It is the view of this author, however, that in most clinical situations it is better to use voriconazole or posaconazole at recommended doses based on *in vitro* susceptibility testing combined with TDM, rather than using this older agent. Itraconazole can occasionally cause severe cutaneous vasculitis.

Voriconazole despite a slightly narrower spectrum and potential toxicity issues, is an important antifungal especially in the dog with systemic disease. Like posaconazole, it is often combined with oral terbinafine, and in some cases with parenteral amphotericin B.

Voriconazole is a second generation triazole agent with much enhanced activity against many fungi (including most members of the genus *Aspergillus*) compared to itraconazole. It is water soluble, and out of patent, so there are cheaper generic formulations available which are more affordable than the original drug developed by Pfizer. Voriconazole is a highly effective drug against *Aspergillus* species, with comparable efficacy to amphotericin B. But voriconazole is not as forgiving an agent to use as posaconazole. High doses can cause delirium and visual hallucinations in human patients, and in cats, high doses can cause serious neurological signs including seizures. Most likely, this could be avoided by careful TDM and gradual dose escalation, but many feline practitioners prefer to just not use voriconazole. It is a useful dose in the dog, but drug levels are hard to predict, even when using studies of pharmacokinetics in normal dogs as a guide, and in the opinion of the authors, TDM is mandatory. The drug is also known as a potent photosensitiser, and in human patients its use combined with UV exposure can result in actinic damage and even cutaneous neoplasia, so dogs on voriconazole should probably be kept indoors during the heat of the day, especially breeds not afforded the protection of a generous hair coat. Another advantage of voriconazole is that it is good at reaching effective CNS levels because it readily crosses the blood brain barrier, a conspicuous advantage over posaconazole.

5. **Amphotericin B – an old drug but a particularly important agent for severe disease.**
When fungal infections are severe, advanced, disseminated or involve the CNS, amphotericin B remains an important drug, and indeed, often the most important agent.

It was one of the first drugs to be used for systemic fungal infections and comes with

a reputation for nephrotoxicity. This can be largely circumvented by using the liposomal formulation AmBisome®, although this is a very expensive drug and beyond the resources of most owners and requires intravenous access for administration. Liposomal amphotericin B continues to be expensive due to high manufacturing costs even though its patent has expired. There are also lipid complex formulations that are available for IV use in the USA; however, they are not used in Australia, and we have little experience with them.

The original deoxycholate preparation of amphotericin B is much more affordable and can be given by other routes as well as intravenously. This is the formulation with which we have had the most experience and, although this experience is anchored in cats and dogs, it extends to native animals and zoo patients, including koalas with cryptococcosis.

In patients with severe disease, such as disseminated cryptococcosis (often with ocular and/or CNS involvement), amphotericin B is usually given intravenously for the first week of therapy. The protocol for its use is widely accessible in infectious disease textbooks. Usually, daily doses of 0.5 to 1.0 mg/kg are given as a continuous rate infusion intravenously (IV). If owners are exceptionally wealthy or the animal is generously insured, liposomal amphotericin can be used with less risk of acute kidney injury, at a daily dose of 2-3 mg/kg. Note that all formulations of amphotericin B are equally effective. The advantage of liposomal and lipid complex formulations is just reduced kidney damage as the lipid liposomal wall prevents the kidney 'seeing' the active drug which is delivered to the fungus inside macrophages.

Once the dog or cat is well enough to go home, we generally swap from IV therapy to subcutaneous infusions twice weekly. The rationale of the subcutaneous bolus infusion is based on slow absorption of amphotericin from a dilute subcutaneous reservoir. This largely circumvents the propensity towards nephrotoxicity, especially in the dog, which is usually young and therefore tends to have more resilient kidneys compared to an older cat.

The subcutaneous amphotericin B regimen works very well in the dog and in young cats, but in older cats that usually have lost some renal reserve, you need to be careful to 'back off' therapy when the serum urea and creatinine concentration creep up. For some reason, it is the serum urea concentrations that usually increases first rather than creatinine.

Over the last few years, we have been giving amphotericin B infusions INTRA-PERITONEALLY (IP), usually under sedation. The advantage of this IP route is that the fluids can be given faster (but make sure you microwave the fluids first so they are at about 37°C) and that sterile subcutaneous abscesses which can occur with subcutaneous infusions are circumvented. We have also used higher concentrations of IP amphotericin B for treating invasive sparganosis.

As an aside, vets who train in Brazil often use IP fluid therapy rather than SC fluid therapy for the same reasons, speed of administration and faster absorption of the fluids.

Over the last few years, we have started using intraperitoneal (IP) dosing rather than subcutaneous administration in some patients, usually larger dogs. This can be facilitated by sedation in unruly patients. IP administration is faster than IV, and you avoid the propensity for subcutaneous lumps or sterile abscesses developing, although there can be mild chemical peritonitis. It is a worthwhile option in some patients, especially dog breeds with thin skin such as Boxers and greyhounds, that seem more likely to get subcutaneous reactions.

6. **What about 5-flucytosine (5FC) & potassium iodide (KI)?**

5FC is an extremely useful drug in human and feline patients with cryptococcosis, as the drug acts synergistically with amphotericin B. Its downside is that it is expensive, can be hard to source, and it must be given at least three times a day and possibly four times a day.

5FC is a useful drug in cats with severe cryptococcosis but cannot safely be used in dogs together with amphotericin B for more than 7 days because of the development of a skin eruption resembling toxic epidermal necrolysis.

We find it extremely helpful for cats with cryptococcosis that is disseminated or if they have CNS involvement. Unfortunately, it is not a helpful drug in the dog, as almost all

dogs given this drug develop a drug reaction resembling toxic epidermal necrolysis after about 7-10 days of combination therapy. This is a severe cutaneous disease which represents a substantial set back during early therapy, and basically it precludes the use of 5FC in canine patients.
Saturated potassium iodide is an old-fashioned antifungal and antibacterial agent used to treat diseases such as actinobacillosis (wooden tongue) and actinomycosis (lumpy jaw) in large animals (cattle), and fungal diseases such as sporotrichosis and *Conidiobolus* and *Basidiobolus*. It has proved a cost-effective treatment for sporotrichosis in Brazil where *S. brasiliensis* is a hot feline and human pathogen. KI is usually combined with other antifungals such as itraconazole. Later, we will talk about the place of KI in treating pythiosis, as it has been successfully used for treating ovine pythiosis in some settings.

7. **Beware spurious isolation of fungi from clinical specimens.**
Fungal spores are everywhere!

If you do not clean your air-conditioners regularly, fungal spores will be present all throughout the veterinary hospital. They can also be widely present in veterinary microbiology laboratories. As a result, it is not difficult for fungal spores to make their way into clinical specimens that are then plated out on fungal media such as Sabouraud dextrose agar, or even on routine media such as blood agar. (After all, this is how Alexander Fleming discovered penicillin!).

Sometimes the growth of these spores in culture can result in an erroneous diagnosis of fungal infections when no infection exists.

In our experience, contamination is most common in samples obtained from the respiratory tract by bronchoalveolar lavage or deep unguided bronchial washings. A recent case the author consulted on was a small dog where *Purpureocillium lilacinum* (formerly known as *Paecilomyces lilacinus*) was cultured from a deep bronchial washing in which eosinophils were the predominant inflammatory cell present. The dog had nodular lung disease. We wasted time and money treating this alleged infection with voriconazole, only to eventually conclude it was likely a contaminant. To be certain, we repeated the bronchial washing—and on the second occasion eosinophils persisted, but no fungi were cultured. The dog responded rapidly to prednisolone, the presumptive diagnosis being eosinophilic pneumopathy. *Mea culpa!*

The authors are also aware of a scenario in a tertiary referral centre where the samples from several endoscopes were contaminated with various fungal species. This problem was resolved when hospital infection control officers and nursing staff were made aware of the problem by the diagnostic laboratory and improved routine cleaning practices.

To diagnose a fungal infection, it is critical to establish that fungal elements are present in the original smears from the site of infection, in this case smears made from cytospin preparations of BAL fluid. If a true fungal infection exists, there should be yeasts or hyphae in evidence (occasionally this is not the case when samples are collected from sterile sites such as CSF).

Contaminating spores in small numbers are usually not evident in smears, but they will grow on culture. Do not be too fast to blame the laboratory for growing a contaminant, as in our experience most contamination actually occurs in the veterinary practice. A common site of contamination is endoscopes used for bronchoscopy or rhinoscopy that are not cleaned appropriately. To avoid this problem, check that your scope is not contaminated by always submitting for culture a sample of sterile saline aspirated through the endoscope prior to the procedure as well as the actual BAL sample.

8. **Are there any new treatments for pythiosis and lagenidiosis (oomycete infections) in dogs, cats and horses?**
Pythium and Lagenidium species are oomycetes that are distinct from fungi in terms of their basic biology, being parasites of plants. They both are capable of producing refractory disease of the skin and/or alimentary tract. They establish infections using motile zoospores as their infectious propagule, and infections are most common in warm moist environments such as Texas and Louisiana in the USA and far north Queensland and the Northern Territory in Australia. Infections can involve the gastrointestinal tract, the skin and subcutis, or both these tissues.
Oomycetes have broad irregular hyphae which are somewhat characteristic, and they grow well on routine media. These infections traditionally had a very guarded prognosis, with treatment involving a combination of debulking

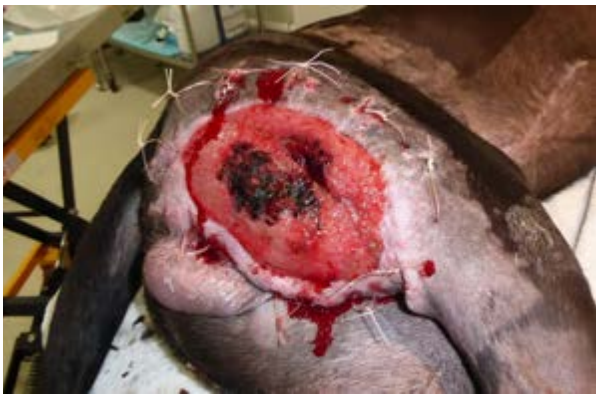

Figure 11. *Lagenidium* infection of the subcutis of a dog from the Northern Territory of Australia. The organism is pigmented, and the presence of melanin imparts a black colouration to the infected tissues.

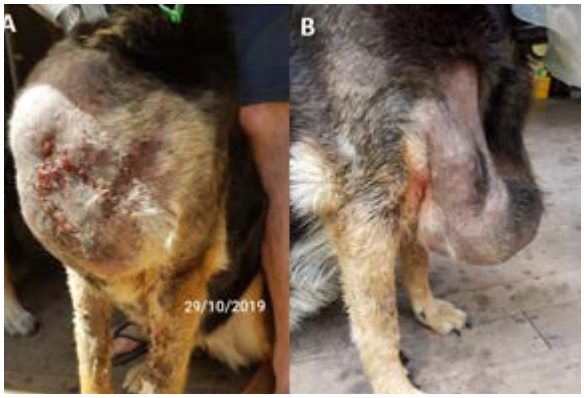

Figure 12. A oomycete in a German Shepherd dog from far north Queensland, before (A) and during (B) treatment with monotherapy using Metalaxyl. The lesion has improved to the extent that surgical resection which was initially impossible is now feasible.

surgery and azoles such as posaconazole combined with terbinafine.

Recently, the use of Metalaxyl or its optically active stereoisomer mefenoxam has greatly improved he prognosis for pythiosis in animals, although publications are scant.

Metalaxyl and its active optical isomer mefenoxam are used to treat oomycete infection in plants, so they are very inexpensive compared to antifungal drugs designed for human patients. This in itself is a huge advantage for therapy, especially in larger patients as posaconazole and terbinafine can be cost prohibitive.

Metalaxyl or mefenoxam can be used alone or combined with triazoles or terbinafine. There is also an experimental literature that suggests azithromycin, minocycline and linezolid (conventional antibiotics) are effective at killing *Pythium*. Corneal infections in people have been managed with combinations of topical linezolid and azithromycin combined with systemic azithromycin, and sometimes voriconazole topically. In sheep with nasal pythiosis, oral potassium iodide has been used successfully as therapy. Although a definitive treatment regimen has not been developed for disease in dogs and cats, the combination of mefenoxam plus KI and azithromycin is attractive as all the medications are inexpensive.

Our experience has been mainly in cats and dogs with cutaneous and subcutaneous disease, and we have used Metalaxyl or mefenoxam both topically and systemically (5 mg/kg orally twice daily). Topical treatment

Figure 13. Suspect early pythiosis lesions in a horse exposed to the floods on the north cast of NSW. A definitive diagnosis for this case was not obtained, but topical Metalaxyl was effective at treating the lesions. The protocol developed by Rosemary Cumings and Oliver Liyou, is set out below:

involves using concentrated Metalaxyl soaked swabs bandaged over the affected regions, with bandages changed daily or every other day.

A highly effective immunostimulant vaccine is also available from Dr Mendoza in the USA and from Dr Mark White in Australia. In jurisdictions in which this is obtainable, it can be very useful. The vaccine is most effectively prepared from the actual isolate causing the infection and can be sourced by contacting Dr Mark White at mark@treidlia.com.au. Ideally, Mark needs a pure culture of the oomycete from the patient to be sent to him. He formulates the vaccine using that isolate, a bespoke vaccine.

Topical Metalaxyl Handling Protocol

Developed by Rosemary Cumings & Oliver Liyou for use in Horses with swamp cancer but applicable to all species.

Wear safety goggles and gloves when preparing and applying and advise owners to do the same!

The ocular toxicity is the highest rating (like for many household cleaners/agricultural chemicals we use frequently) and it is not worth risking a splash injury. If any gets in a person's eye, they should immediately wash the eye out then go to the hospital for high volume lavage. Similarly, do not spray it on the horse's face if they have a lip lesion, just dab it on and I would not apply this near a horse's eye in case it dripped in.

1. Dilute 1:1 with water. It is stable in water for a prolonged period of time and is not degraded by light so you could mix up 150mL, 500mL or 1L depending on the lesion size you are dealing with and either store it in a spray bottle or in a sealed container full of gauze swabs for ease of use. I would use distilled water, water for injection or even just bottled drinking water ideally in case of contaminants in the tap water after the floods that could grow in the container.
2. Clean any debris/discharge off the surface of the swamp cancer lesion with saline or water.
3. Put a rim of Vaseline around the lesion if the skin is already flood damaged and doesn't have hair left to protect it.
4. Place 1-2 gauze swabs soaked in the solution on the lesion and bandage in place OR spray the lesion surface it is too large/not in a spot that can be bandaged.
5. Repeat application daily.
6. Continue to monitor the swamp cancer and treat as you normally would with your usual medical therapy/immunotherapy and, if the lesions are failing to respond, consult a surgeon re excision/an ongoing treatment plan. It will likely take a long time for lesions to completely resolve but you would hope to see some initial shrinking after 3-5 days' treatment.

So, it is highly likely that over the next few years the guarded to grave prognosis for oomycete infections will improve substantially.

For some reason, we very rarely see the gastrointestinal cases of pythiosis that occur in the USA and Hong Kong, although one dog from The Northern Territory that initially had cutaneous disease later developed colitis.

Many animals were impacted by the floods that we have had on the north coast of NSW, with several horses developing lesions suspicious of 'swamp cancer' (pythiosis or lagenidiosis) on their distal limbs. In collaboration with Oliver Liyou (well-known equine dentist) and Rosemary Cumings (an equine internist at Scone Equine Group), we have used Metalaxyl or mefenoxam as topical spray to prevent pythiosis developing or to treat early cases with good effect.

9. **New drugs on the horizon.**
Isavuconazole is the most recently released azole antifungal. It is a safe agent with an extremely broad spectrum of activity, with efficacy against some fungi where other azoles are ineffective. It's expensive and its kinetics are unknown, but it might prove to be a useful agent in cats and dogs with unusual fungal infections. In people, it is useful for aspergillosis and mucormycosis.

There is a pile of exciting new antifungal agents that should soon be on the market, and these will improve our ability to deal with many resistant and hard to treat pathogens. Amongst these, fosmanogepix and olorofim are two of the stand-outs. They will of course be expensive, but they should prove particularly useful for cryptic *Aspergillus* species such as *A. felis* and for *Scedosporium* and *Lomentospora* infections which can be extremely challenging to treat using current agents.

Some Unusual Fungal & Algal Infections

1. **Diagnosis and treatment of pneumocystis pneumonia (PCP)**
Pneumocystis canis is a host adapted fungus that is part of the normal lower respiratory mycobiome in many dogs and cats. Indeed, just about every mammalian species has a host adapted *Pneumocystis* species. With a normal immune system, small numbers of trophozoites or cysts (ascii) persist in the alveolar spaces but do no harm. However, in certain breeds with inherited immune deficiency states,

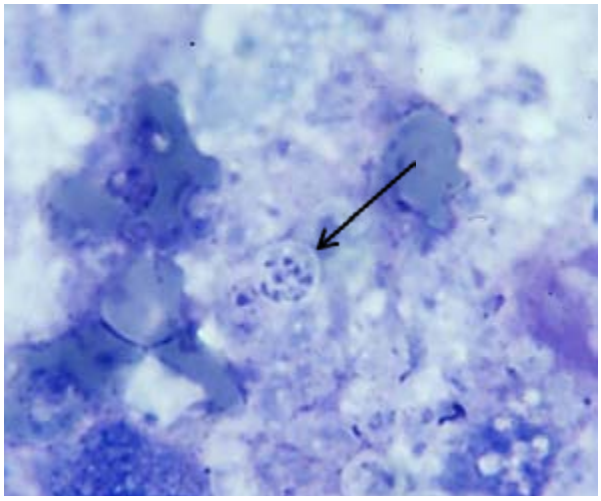

Figure 14. Cyst or ascii (arrow) of *Pneumocystis canis* in a cytocentrifuged BALF specimen from a young Cavalier King Charles spaniel.

especially Cavalier King Charles Spaniels, miniature Dachshunds, and their hybrids, the trophozoites can multiply unchecked and cause disease. This can also occur in dogs of a normal genetic makeup when subjected to immunosuppressive therapy, including drugs like Apoquel.

In dogs, PCP is mainly seen in Cavalier King Charles Spaniels, miniature Dachshunds, and their hybrids. An increased suspicion for this diagnosis in young members of these breeds with lower respiratory issues is a key to early diagnosis. Concurrent infection with *Bordetella bronchiseptica*, or a history of demodectic mange either currently or historically may be present.

Diagnosis is usually suggested by the breed, sometimes by the antecedent administration of immunosuppressive medications (corticosteroids, toceranib, cyclosporine), and by the presence of characteristic radiological changes in chest radiographs and CT scans. Chest X-rays tend to show a characteristic dense interstitial pattern, sometimes accompanied by right sided heart enlargement (cor pulmonale) due to pulmonary hypertension. CT shows a characteristic ground glass appearance of the pulmonary parenchyma.

Having large animal formulations of trimethoprim sulpmethoxazole suitable for slow intravenous injection is extremely helpful for treatment of PCP in dogs. Parenteral therapy offers important advantage in terms of rapidly achieving high blood and tissue levels in critically ill oxygen-dependent patients with PCP pneumonia.

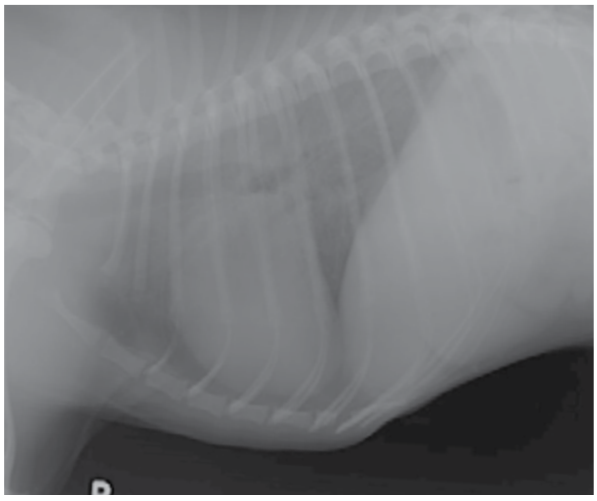

Figure 15. A hazy but dense interstitial pattern is highly suggestive of PCP pneumonia in one of the susceptible breeds. Cor pulmonale is usually also present.

Confirmation of a diagnosis of PCP pneumonia typically relies on seeing the organisms, yet only the cysts (ascii) (rather than the trophozoites) are readily seen.

Panfungal PCR and PJP PCR (for the human organism *Pneumocystis jirovecii*) do not detect *P. canis*. Canine specific PCR primers have, however, been published by Patrizia Danesi and colleagues and, recently, a pan-Pneumocystis PCR for all Pneumocystis species has been developed at the National Institute of Health in the USA.

Real time qPCR for PCP is available at Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy. Hopefully, specialist veterinary PCR labs will offer this as a test in the near future, ideally multiplexed into their respiratory PCR panels.

Figure 16. If you see dyspnoea without coughing in a young Cavalier King Charles Spaniel—immediately consider PCP pneumonia in the differential diagnosis. Photograph courtesy of Linda Abrahams.

Treatment is well worth attempting. High dose trimethoprim sulphamethoxazole remains the drug of choice, and ideally, it should be given intravenously to achieve high blood levels. It is important that this large animal formulation be available for companion animals as parenteral therapy offers important advantages over tablets for preliminary intensive therapy. In time, the use of intravenous echinocandin therapy (e.g., caspofungin, micafungin, anidulafungin) might also find a place in treatment of these cases. Monitor Schirmer tear test readings as keratoconjunctivitis sicca due to TMS can develop during the course of therapy.

If there is a strong index of suspicion for PCP pneumonia in a dog due to *P. canis*, do not feel an obligation to confirm a diagnosis by BAL fluid examination and PCR. These procedures typically require general anaesthesia which can be sufficient to decompensate this type of patient. A fine needle aspirate biopsy from a severely affected portion of lung can be a safer way to obtain material for cytological examination or PCR, and, perhaps, throat swabs might be sufficiently sensitive if subjected to a qPCR assay.

There is nothing wrong with making a strong presumptive diagnosis and embarking on therapy, when characteristic imaging findings are present in breeds that are predisposed to this condition. Remember that co-infection with *Bordetella bronchiseptica* is common, so it is often worthwhile to add doxycycline to the therapeutic regimen.

2. **Disseminated fungal disease in German Shepherd dogs, Hungarian Vizslas and other breeds.**

One of the most vexing entities for companion animal veterinarians to treat is widely disseminated disease due to a variety of fungi, most commonly *Aspergillus terreus*. German Shepherd dogs (GSD) and possibly Hungarian Vizslas possess some inherited immune defect which makes them especially susceptible to fungal pathogens. Sporadically, the same problem is seen in individuals of other breeds.

It is thought that infection starts by inhalation of a large dose of fungal ascospores which lodge in the alveoli. After germination and early hyphal formation, the infection spreads to the hilar lymph nodes.

From there infection spreads to a variety of well perfused tissues characterised by vessel tortuosity. Often the lesions in the lungs have healed by the time clinical signs emerge. **Clinical signs reflect the site of dissemination—vertebral and appendicular osteomyelitis, discospondylitis, anterior uveitis, meningoencephalitis and mycotic pyelonephritis are the most common clinical manifestations.**

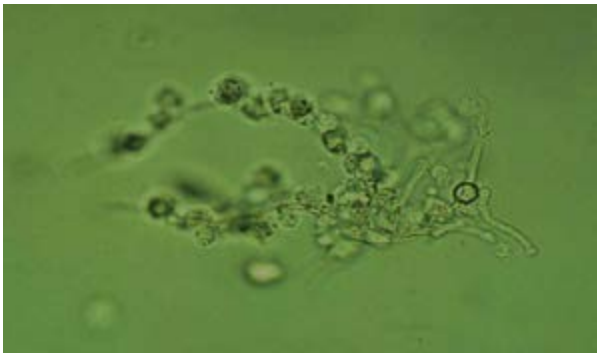

Figure 17. Hyphae of *A. terreus* in a wet preparation of the urine of a 2-year-old German Shepherd bitch.

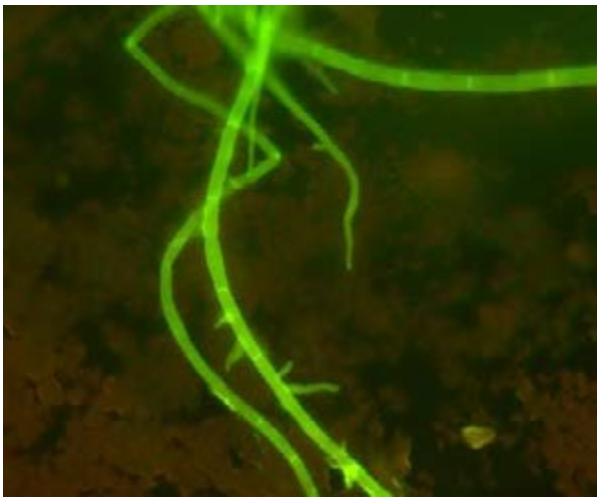

Figure 18. Calcofluor white stain of a positive blood culture from a dog with disseminated aspergillosis due to *A. terreus*. Photograph courtesy of Charlotte Webster, Concord Hospital.

Aspergillus, Scedosporium and related fungal species have a predilection for blood vessel walls and infection can track along large arteries giving rise to aneurysmal dilatations. The diagnosis of these infections is not difficult if one has a good appreciation of the 'illness script' and a high index of suspicion for a fungal aetiology.

Cytological examination of urine for fungal hyphae, either in wet preparations of urine sediment or in Diff-Quik stained cytocentrifuged smears of urine sediment can be a very cost-effective way to obtain a diagnosis. Determining the serum galactomannan index can also be very efficient

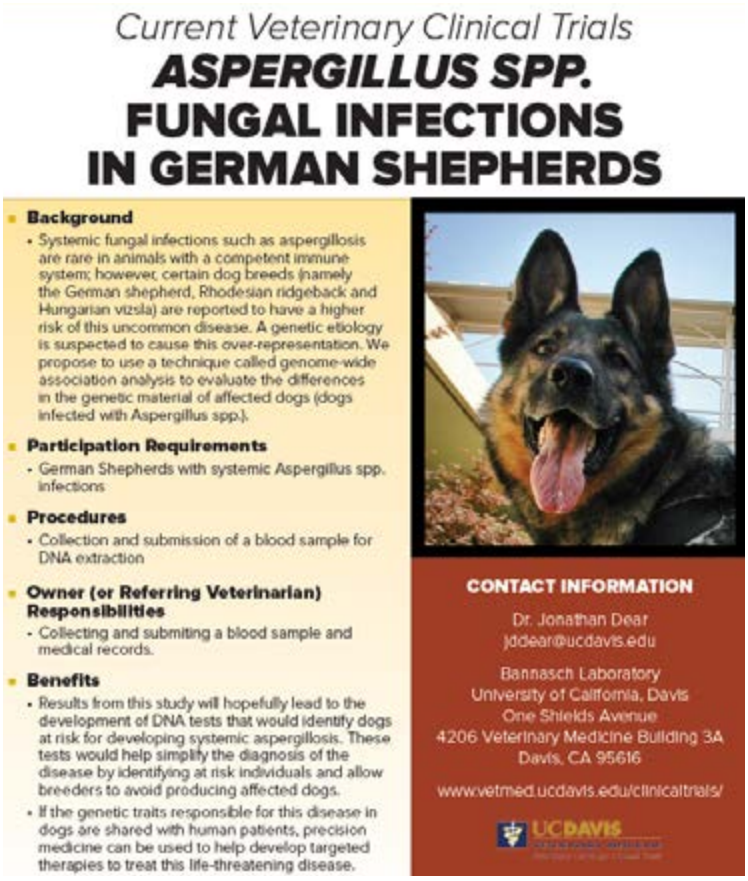

Figure 19. Poster requesting DNA samples from GSD with disseminated aspergillosis for WGAS and whole genome sequencing studies.

diagnostically. The diagnosis can be more difficult if an unusual breed is affected or if the fungal pathogen is unusually exotic.

Management of these cases is challenging. The underlying immune defect cannot be fixed, so there is complete reliance on antifungal therapy for disease control. The presence of unilateral or bilateral mycotic pyelonephritis mitigates against the use of amphotericin B, although perhaps liposomal formulation can be used at reduced doses.

Voriconazole or posaconazole in concert with terbinafine usually represent the most effective therapy, although treatment is expensive because (i) the drugs are expensive, (ii) long to life-long treatment courses are required and (iii) because dogs are large, high absolute doses are required. The only good news is that voriconazole and posaconazole are now out of patent, and generic options are far more affordable than the originator brands.

Infections in well perfused tissues such as the eye and bone can often be eliminated;however, the failure of almost all fungal drugs to reach high levels in the urine makes eradication of pyelonephritis futile unless treatment utilises nephrostomy tubes with topical instillation of antifungal agents into the affected renal pelvis.

Echinocandin therapy can be a useful adjunct in these cases, but, currently, these drugs are almost prohibitively expensive to use for more than several days at the start of treatment.

Truthfully, the best hope for managing these cases is by concentrating on developing a PCR test for whatever genetic defect underlies the condition. This is being undertaken by Danika Bannasch and colleagues at UC Davis (see *Figure 19*). My impression is that this condition is more common in Australia than any other jurisdiction, and we really should be biobanking DNA from all these affected dogs with a view to conducting a whole exome scan or a whole genome association scan (GWAS).

3. **Diagnosis and treatment of protothecosis**

Protothecosis is an algal organism that has lost the ability to make chlorophyll, but we touch on it here as it behaves somewhat like a fungal pathogen. It is a notable cause of refractory colitis in dogs, with the propensity for more virulent species to disseminate to the eye, CNS, and other vital organs. There would appear to be a strong breed predilection towards Boxer dogs and their crosses.

There appears to be some sort of link or association between the granulomatous colitis of Boxer dogs (a.k.a. 'Boxer colitis' or canine histiocytic ulcerative colitis) caused by adherent invasive *E.coli* and protothecal colitis *(Figure 20)*.

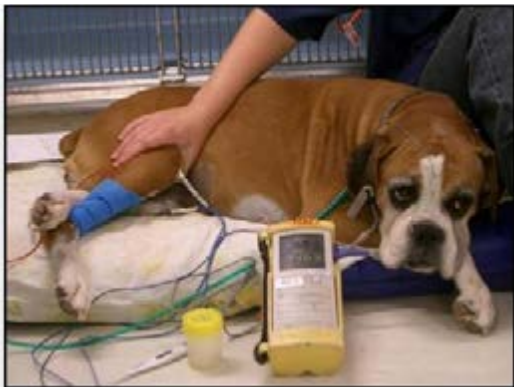

Figure 20. Boxer dog with disseminated protothecosis. Photo courtesy of Bruce Mackay and Vicki Stenner.

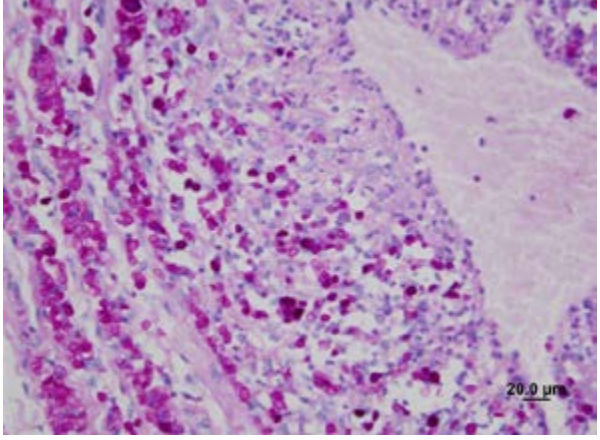

Figure 21. Protothecal algal cells stained with Periodic acid-Schiff (PAS) in the wall of a colonic vein. All the round pink cells are *Prototheca* cells.

Figure 22. Ulcerated lesion on the pad of a cat referable to protothecosis.

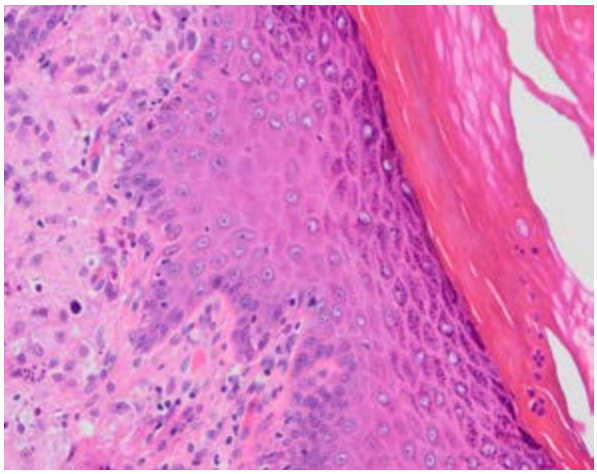

Figure 23. *Prototheca wickerhammi* organisms are evident in the stratum corneum of the the cat in Figure 22. Photo couresy of Allan Kessels.

Disease in cats is much rarer and takes the form of granulomatous cutaneous disease *(Figure 22)*.

Think of *Prototheca* when treating dogs with colitis signs that do not respond to standard therapy, and in Boxers with a combination of colitis and ocular and/or CNS disease. Diagnosis is not difficult once the 'illness script' is contemplated, as rectal scrapes are usually positive for characteristic organism morphology, and the organism can also be present in urine, ocular aspirates, or CSF. The taxonomy has undergone considerable revision by Polish researchers, and the two species we see in Australia are *P. bovis* (formerly *P. zopfii*) which is the 'nasty' one, and *P. wickerhamii*, which is milder and less likely to disseminate. Patrizia Danesi has developed an excellent PCR test for this organism which can be applied equally to fresh tissue and formalin fixed paraffin embedded (FFPE) tissues.

Currently, optimal treatment of protothecosis in dogs consists of amphotericin (whatever formulation is affordable and accessible; usually amphotericin B deoxycholate given by subcutaneous or IP infusions) plus posaconazole. Posaconazole seems the most effective of the oral azoles that have so far been used, with much better clinical efficacy than itraconazole. The availability of generic sustained release tablets means that daily therapy at 5 mg/kg is affordable and generally very well tolerated. Life-long therapy is often required to keep the disease under control or in remission. Professor Rui Kano in Japan is trialling a new azole only available in Japan.

In cats, the optimal treatment consists of wide surgical resection of lesions, with follow up therapy with posaconazole suspension at a dose rate of 8 mg/kg once a day for several months.

If a dog has signs of colitis that do not respond to standard therapy, think immediately of protothecosis and do a rectal scrape to look for the characteristic organism morphology in Diff-Quik stained smears.

That way you might diagnose it early when it's restricted to the colon, before it disseminates to the eye and CNS!

4. **What about cases of mycotic keratitis?**
Fungal disease of the cornea is much more of an equine problem than a dog or cat problem, but occasionally a cat scratch or grass seed foreign body can abrade the corneal epithelium and let a fungal pathogen establish itself in the corneal stroma. In this scenario, topical application trumps systemic therapy because of the high concentrations that can be attained by drops and ointment, and the two most effective agents are natamycin which is available commercially as an ophthalmic formulation, and voriconazole which can be compounded as drops and ointment by compounding pharmacists◆