ADRENALS:
What you won't find in a textbook

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PART 4b: UCCr and CALP

As part 4 of this series looks at diagnostic tests, we can't escape some statistics. So, some very simplistic explanations relative to hyperA are as follows:

Sensitivity: the likelihood that the test will detect hyperA

Specificity: the chance that a positive test is truly hyperA

Then, there are predictive values which take into account the prevalence or likelihood of a disease in addition to sensitivity and specificity.

Positive predictive value (PPV): the chance of a positive result being indicative of hyperA in dogs with signs of hyperA (e.g. Can we confidently diagnose hyperA when we get a "positive" result?)

Negative predictive value (NPV): the likelihood that a negative results eliminates the possibility of hyperA in dogs with signs of hyperA (e.g. Can we rule it out with a negative result?)

Urine corticoid: creatinine ratio (UCCR)

It is commonly stated that this test is highly sensitive but poorly specific, that is, the test picks up most dogs with hyperA but is also positive in lots of other disease states. It is widely advocated as a screening test to RULE OUT hyperA but should we be doing that?

The literature confirms that the test has good sensitivity and poor specificity[1-4] (as low as 21%)[1]. In addition, one study had a positive predictive value of only 3%[2] (i.e. only 3% chance that a dog with increased UCCR actually had hyperA!) so we certainly can't use UCCR for diagnosis.

What about the negative predictive value: can we use the test as a "rule-out".

NPVs range from 0.96[1] (Smiley and Peterson) to 0.99[2] (Soffner and Reusch) so it would seem that a normal UCCR should rule out hyperA. However, these papers were all written in the early to mid 1990s and not in Australia. In Jody Braddock's Master's Thesis (2002), the sensitivity of the UCCR was only 66%, as 13 of 38 animals with rigorously confirmed hyperA had a UCCR ≤15.[6] It is unclear whether this was because the cases were being picked up earlier (in the 2000s compared to the 1990s) or whether the dog population in Braddock's thesis is different to that in the other studies.

This statistic would confirm my impression at Vetnostics that we do seem to see "false negatives" at a much higher rate than the 2-4% suggested in the early studies. Thus this test cannot be used as a rule-out test.

Given that UCCR cannot be used to diagnose hyperA (poorly specific) and cannot be used as a rule-out either, there is little point in performing this diagnostic test. UCCr does have a role in monitoring treatment with trilostane (to be addressed in a later newsletter).

Corticosteroid-induced alkaline phosphatase (c-ALP)

Increased serum ALP, the most common routine laboratory abnormality in hyperA is due mainly to the induction of a specific ALP isoenzyme by glucocorticoids. The corticosteroid-induced isoenzyme of ALP can be measured by electrophoretic separation, heat inactivation or more usually in commercial laboratories, by levamisole-inhibition. The levamisole inhibition explains why c-ALP is sometimes referred to as l-ALP but this terminology can be confusing as sometimes l-ALP is used to describe the liver isoenzyme; it is also referred to as CAP (corticosteroid-induced ALP) or SIAP (steroid-induced alkaline phosphatase).

The sensitivity of c-ALP has been reported to be 0.81-0.95.[7-9] Specificity is poor (0.18-0.44)[7-9] and PPV in one study was as low as 21.4%[7] thus this test cannot be recommended as a diagnostic test. Interestingly, only 50% of glucocorticoid-treated dogs had increased c-ALP in one study and that same study found that absence of c-ALP increase does not rule out spontaneous or iatrogenic hyperA (Solter et al 1993).[7]

Percentage c-ALP is often discussed. Wilson and Feldman[9] found that c-ALP comprised 25% or greater of ALP in hyperA dogs and that hyperA could not be distinguished from exogenous glucocorticoid administration, liver disease or diabetes mellitus by percentage in this study.

Similar to UCCr, c-ALP results cannot be used to rule in or rule out hyperA.

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Partners in Practice

Welcome...

Hello again! In this final Vetnostics newsletter of 2012, we have the penultimate installment in the Cutaneous mycobacterial diseases in dogs and cats series – a source of useful and current information for when dealing with this increasingly common disease category. Additionally, we continue the series' on Adrenal disease and frustrating histopathology cases/diagnoses.

We at Vetnostics hope you have a festive end to 2012 and look forward to an ongoing fruitful partnership ('Partners in Practice') with you in 2013 and beyond.

As always, please contact me (ph 02 9005 7272 or email doug.hayward@vetnostics.com.au) if you have any requests/ideas for future newsletters or any other queries.

CUTANEOUS MYCOBACTERIAL DISEASE IN DOGS AND CATS (PART 2):

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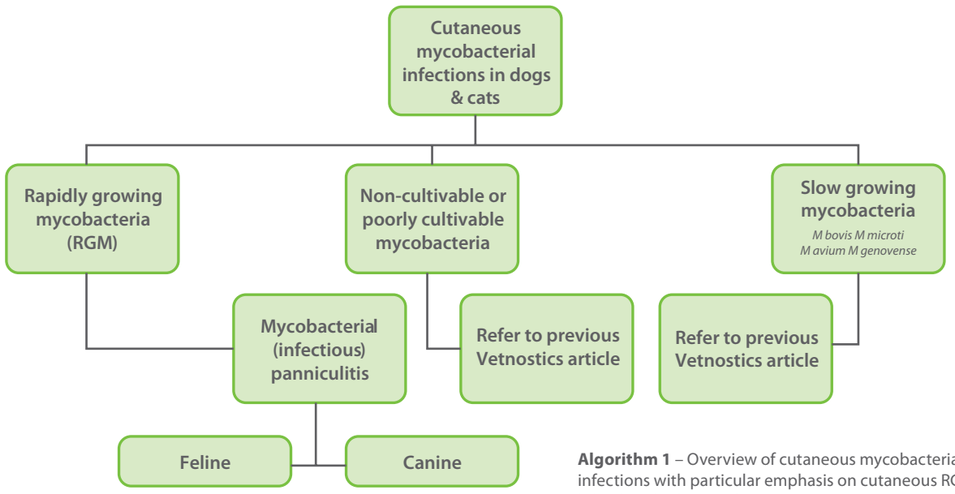
Laboratory diagnosis and treatment of cutaneous infections caused by rapidly growing mycobacteria in dogs and cats.

Introduction:

Here we continue the discussion on ancillary laboratory testing currently used to diagnose the various manifestations of cutaneous mycobacterial infections in dogs and cats and the therapeutic modalities currently available for their treatment. In this article we focus upon cutaneous mycobacterial infections in dogs and cats caused by rapidly growing mycobacteria(RGM) (Algorithm 1).



Algorithm 1 – Overview of cutaneous mycobacterial infections with particular emphasis on cutaneous RGM infections in dogs and cats

Cutaneous infections caused by rapidly growing mycobacteria (RGM):

Rapidly growing mycobacteria (RGM) (formerly Runyon Group IV or atypical mycobacteria) are by definition, characterized by the ability to form colonies on solid media within 7 days of incubation (Runyon 1970).

Infectious panniculitis is one of three different syndromes of disease that RGM can produce in cats and dogs. It is also known as "mycobacterial panniculitis" and is associated with chronic infection of the subcutis and skin with any RGM. The other 2 syndromes which can be involved in RGM disease include pyogranulomatous pneumonia, and disseminated systemic disease.[1]

Feline mycobacterial panniculitis:

Panniculitis is the most common clinical presentation of infection with RGM[2]. Numerous solitary feline case reports and smaller case series have been reported in the veterinary literature. In Australia, organisms from the M. smegmatis group[2, 3] (particularly M. smegmatis sensustricto [R. Malik unpublished observations]) account for the majority of feline cases, whereas in the USA, infections with members of the M. fortuitum group predominate[6].

The aetiopathogenesis of feline mycobacterial panniculitis usually involves the seeding of the causative mycobacterial agents into anatomical locations which are well endowed with fat, following penetrating injuries (e.g. via bite wounds, penetrating foreign bodies, injections or surgery) which breach the integument.[5] Consequently RGM are able to overcome the normal host defenses and establish infection in these "fatty" subcutaneous tissue regions (e.g. subcutaneous panniculus and especially the inguinal fat pad of cats) - particularly in obese individuals (often desexed females) in which there is a greater tendency for this disease to occur. In cats, infection often tends to start in the inguinal region (although sometimes it can begin in the axillae, flanks or dorsum). The disease may subsequently spread to contiguous areas of the lateral and ventral abdominal wall, perineum and tail-base.

Mycobacterial panniculitis in cats initially tends to present with a circumscribed plaque or nodule of the skin and subcutis at the site of injury, although trauma is not reported in every case.6 Often the initial clinical suspicion is for a catfight abscess, although the absence of the foetid odour and turbid pus should alert the clinician to the possibility of an unusual infectious aetiology. Indeed, many such lesions treated with surgical drainage and antibiotics effective against obligate anaerobes are followed by wound breakdown and development of a non-healing suppurating tract surrounded by indurated granulation tissue. Later in the clinical course the subcutaneous tissue becomes thickened and the overlying skin becomes adherent, alopecic and punctuated with fistulae (Figure 1), which discharge a watery exudate (the so called "pepper pot" appearance). Thin areas of epidermis overlying subcutaneous collections of pus lead to characteristic focal purple depressions intermingled with the fistulae. Over time, the depth and breadth of the infected area increases and may eventually involve the entire ventral abdomen, adjacent flanks and limbs.

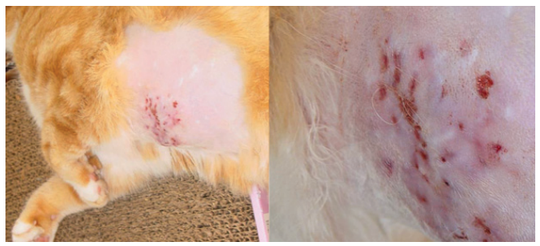


Figure 1: Typical clinical presentation of a cat with mycobacterial panniculitis

The problem usually remains localized to the skin and subcutis, and although adjacent structures such as the abdominal wall can be affected eventually, spread to internal organs or lymph nodes is very unusual. Even cats with extensive cutaneous lesions may have few signs of systemic illness. Severely affected cats may develop constitutional signs of malaise, pyrexia, inappetance, weight loss, and reluctance to move. Occasionally, cats develop hypercalcaemia of granulomatous disease, although this is rarely, if ever, symptomatic.

Canine mycobacterial panniculitis:

There are substantially fewer reports of RGM infections of the skin and subcutis in dogs where the majority of cases represent localized infections in immunocompetent individuals.[8,9,4,10,11] As in the cat, canine cutaneous RGM cases occur following a penetrating injury of the integument, such as road vehicle trauma, bite wounds, stick injury, injection sites (especially with the use of multi-dose vials) or previous surgical intervention.[1,4,11] In many cases the initial clinical suspicion when a dog presents with a chronic non-healing cutaneous wound is that of a subcutaneous foreign body (for example a grass awn or wood splinter), however the possibility of a mycobacterial infection should be suspected if the lesion is not responsive to surgical drainage and conventional antimicrobial therapy and if a foreign body is not detected using advanced imaging techniques.[1]

In dogs, lesions are usually non-painful, non-pruritic, firm to fluctuant, solitary or multiple subcutaneous nodules that may or may not have ulcerations and draining sinus tracts (Figure 2). New lesions may appear as "satellites" at the edges of older lesions.11Anatomical sites in affected dogs are variable, presumably reflecting the site of inoculation and may include the dorsum, cervico-thoracic and flank regions. Most animals are systemically well, however a few may demonstrate pain, fever and lameness.[9,10,11]

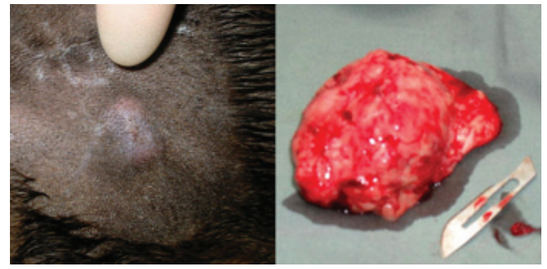


Figure 2: Dog with mycobacterial panniculitis – lesion as it appears in-situ and after excisional biopsy.

Diagnosis of feline and canine mycobacterial panniculitis:

Diagnosis of cutaneous RGM infection is relatively uncomplicated, provided the clinician has an adequate index of suspicion and the laboratory has been informed that a mycobacterial infection is suspected so that special procedures can be employed. Diagnostic material can be collected via fine-needle aspiration or biopsy. Cytologic and histologic preparations can be stained to look for acid fast bacilli (AFB). PCR testing can also be performed on cytological specimens as well as histopathology specimens, although it is important that clean slides are used as mycobacteria can be present in dust. Pus and/or tissue homogenates can be cultured using routine mycobacterial media.

CUTANEOUS MYCOBACTERIAL DISEASE IN DOGS AND CATS (PART 2) *continued...*

Specimen Collection and Submission from skin and soft tissue infections:

In our experience, samples of pus obtained from aspirates of affected tissues through intact skin provide the best specimens. This material can be obtained from a palpably abnormal portion of the subcutis. The overlying skin should be disinfected with 70% ethanol prior to obtaining material to preclude isolation of saprophytic mycobacteria from the skin surface. It may be necessary to carefully move the needle in the subcutaneous space while applying constant negative pressure, until a pocket of pus is encountered. In dogs, the use of high resolution ultrasound can be helpful in locating suitable pockets of pus. Aspirated fluid should be submitted for cytology and mycobacterial culture. Aspirated fluid samples (>0.5ml in volume) can be placed directly into a small sterile 5ml screw top tube (Vetnostics order code: 646792). Aspirated fluid samples (<0.5ml in volume) can be inoculated into a paediatric blood culture bottle (Vetnostics order code: 667193) with a couple of drops also placed onto the end of a sterile microbiology transport swab (Vetnostics order code: 641078) which has been very mildly pre-moistened with saline (to be prevent elution of any microbes into the cotton swab) and placed back into the transport media (Figure 3). Material from draining sinus tracts is usually unsuitable due to the high numbers of contaminating secondary bacteria. Surgical tissue samples collected aseptically from the depths of the lesion may also be submitted for culture.

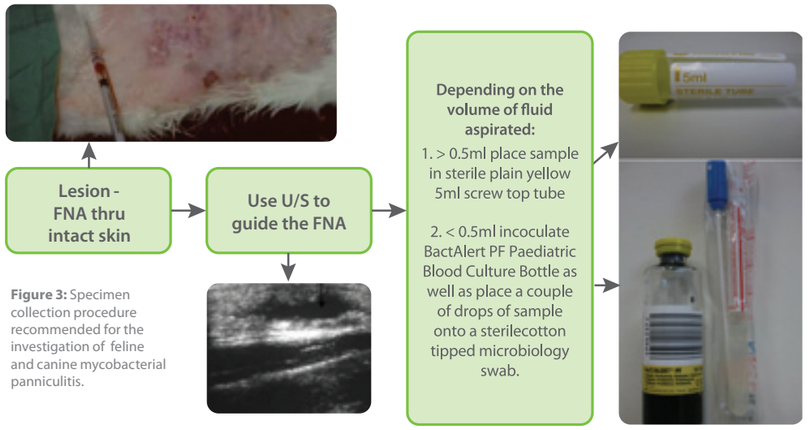


Figure 3: Specimen collection procedure recommended for the investigation of feline and canine mycobacterial panniculitis.

Cytologic Examination:

Smears should be stained using Diff Quik®, Burke's modification of the Gram stain, and a modified acid-fast procedure (decolorizing with 5% sulfuric acid for only 3 to 5 minutes because RGM are not as acid-fast as other mycobacteria). A pyogranulomatous cytologic picture predominates and Gram positive, acid-fast, or in the case of Romanowsky-stained samples, "negatively-staining" organisms are sometimes visualized within foamy macrophages and lipid vacuoles (Figure 4).

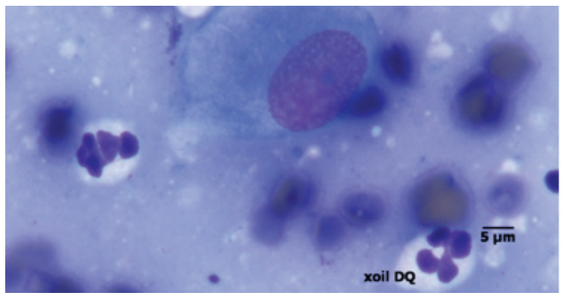


Figure 4: Canine mycobacterial panniculitis with scant, negative-staining, mycobacterial organisms in the cytoplasm of a large macrophage (x oil Diff-Quik stain).

Unlike canine leproid granuloma and feline leprosy, the organisms may be much harder to visualise and an exhaustive search of several smears is sometimes required. PCR testing could also be attempted off the Romanowsky-stained cytology slides (but not other stains) using a generic Mycobacterium PCR (MYCPCR) assay to identify the RGM. Attempting culture of the lesion in question once a cytological diagnosis or suspicion of RGM has been raised is preferred (see below).

Organism Cultivation and Antimicrobial Susceptibility Testing:

There is great value in obtaining RGM species identity and susceptibility data in every case, as this has a big impact on antimicrobial strategies. Susceptibility testing of RGMs is not only useful for clinical purposes, but historically has also been used to provide phenotypic data for typing of isolates (for example, susceptibility to trimethoprim, polymyxin B, and tobramycin). This information can be gained from a mycobacterium reference laboratory following primary isolation at a general Veterinary Microbiology Laboratory like Vetnostics, and may well provide additional data with respect to newer human anti-infective agents (for example, moxifloxacin, tigecycline and linezolid).Minimum inhibitory concentrations (MICs) for moxifloxacin, enrofloxacin, pradofloxacin, amikacin, gentamicin, clarithromycin, tigecycline, minocycline and doxycycline can be determined easily using the E-test method or broth microdilution.

Advanced molecular techniques for mycobacterial species identification:

Species identification off culture plates which have grown RGM, taking into account a number of phenotypic and biochemical features, can be carried out in a well-equipped veterinary bacteriology laboratory such as Vetnostics. Specimens may also need to be sent to a Mycobacterium Reference Laboratory, following primary isolation, for further identification using PCR techniques which Vetnostics can organise. PCR testing for identifying RGM species utilizes 16S rDNA and/or ITS sequencing and sequencing of other regions if necessary to provide species identification.

Pathologic Findings:

Histopathological lesions are typically characterized by pyogranulomatous inflammation of affected tissues. Usually, few acid fast bacilli (AFB) are visible using appropriate stains such as modified ZN. AFB are most often found in lipid vacuoles (Figure 5)

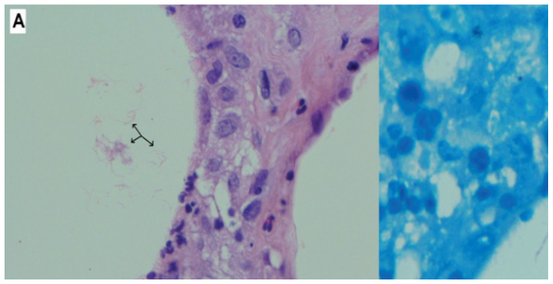


Figure 5: Histological sections (A- Haematoxylin/Eosin stain & B- modified Ziehl Neelsen) of mycobacterium panniculitis showing: Pyogranulomatous inflammation surrounding a lipid vacuole in which numerous slender bacterial organisms are present (arrows) and modified Ziehl-Neelsen stained section revealing scant acid-fast bacilli.

Therapy:

Although the medical and surgical management of mycobacterial panniculitis in both dogs and cats is well described[1] the introduction of new antimicrobial agents such as the fourth generation fluoroquinolones, moxifloxacin and pradofloxacin, and new tetracycline derivatives (for example, tigecycline) means that therapeutic recommendations continue to evolve (R. Malik unpublished observations).

Historically, these infections were considered very difficult to treat and problems in making a prompt diagnosis have been proposed as a reason for the chronicity, severity, and refractoriness of these infections. The more recent use of lipophilic antimicrobial agents directed by susceptibility data and, if appropriate, aggressive surgical resection and reconstructive techniques has greatly improved the prognosis for the majority of cases. Despite this, some cases remain frustratingly refractory to treatment, particularly those caused by M. fortuitum and M abscessus, which are more common in the United States[6] than in Australia.

Briefly, treatment should commence with one or two oral antimicrobials (doxycycline, a fluoroquinolone, and/or clarithromycin) initially chosen empirically (considering local knowledge on the most common species) but subsequently based on in vitro susceptibility data. Dosages for these drugs are listed in Table 1. Long-term administration of such an agent or agents is sometimes sufficient to effect a cure over several months, but in more severe cases, surgical resection of recalcitrant tissues is eventually necessary. Given the extent and severity of the pathology in many of these cases, understandably, adequate levels of antimicrobials may not be achieved throughout all affected tissues. In such instances, the best chance for a successful outcome is to remove as much infected tissue as possible following preliminary antimicrobial therapy.[12] Residual foci of infection can then be targeted by the high concentrations of antibiotics achieved during and after surgery. Peri- and postoperative antimicrobial therapy is vital to ensure primary intention healing of the surgical incision.

Specific Recommendations

Once a tentative diagnosis of mycobacterial panniculitis is made, it is desirable to start treatment immediately. Because positive primary culture takes 3 to 4 days, with an additional similar period required for susceptibility testing, the initial choice of one or more antimicrobials must be guided by retrospectively acquired microbiology data. This data is different in different regions.

M. smegmatis group isolates (most common in Australia) are susceptible to a wide range of antimicrobial agents suitable for treating chronic infections, except for clarithromycin to which a majority of strains show inherent resistance. In contrast, *M. fortuitum* group isolates generally demonstrate resistance to one or several agents and often have higher MICs for agents to which strains are susceptible, while *M. chelonae-abscessus* group isolates tend to be resistant to all common agents available for oral dosing apart from clarithromycin and linezolid.[13] In Australia, doxycycline and/or a fluoroquinolone (such as marbofloxacin or moxifloxacin, but not enrofloxacin in cats due to the risk of retinal toxicity) are thus sensible choices for first-line therapy. Recommendations from human infectious disease experts emphasize the possibility of RGM developing resistance to quinolones during a course of therapy.[13] Thus using quinolones strategically after surgical debulking or using them initially in concert with another effective antimicrobial may be prudent to reduce the likelihood of resistance developing. Such considerations are said not to be applicable to doxycycline or clarithromycin.[13]For this reason, many veterinary dermatologists in Australia routinely use combination therapy with doxycycline and a fluoroquinolone from the outset. Emphasis should be made that, although some RGM strains show in vitro susceptibility to amoxicillin-clavulanate, this drug combination has no efficacy in vivo.

Once susceptibility data becomes available, the optimal drug or drugs are selected. The in vivo response to a drug (or drugs) known to be effective in vitro can then be assessed. In general, using doses as high as possible is necessary because affected subcutaneous tissues are not well perfused, and considerable diffusion barriers prevent blood levels of antibiotics from reaching organisms in fat. Treatment should commence using standard dose rates. Subsequently, the dose is increased slowly (over several weeks) until adverse side effects (inappetance, vomiting) suggest the need for slight dose reduction or until a convincing clinical improvement is observed.

Some animals treated in a preliminary fashion using orally administered agents respond progressively to such an extent that surgery becomes unnecessary. These animals can be cured using medical therapy alone, although treatment with oral antimicrobials for 3 to 12 months may be required. As a generalization, lesions that resolve without the need for further surgical intervention involve a lesser depth of tissues than those that require surgery. Some lesions are so severe, however, that only a limited improvement can be achieved with antimicrobial therapy alone, and surgical intervention is required. Because predicting which cases will require operative debridement is impossible, our recommendation is to start empiric therapy, determine the in vitro susceptibility pattern, then reassess the patient every 3 to 4 weeks to decide if continued improvement is occurring or whether therapy has plateaued and surgery is required.

Preliminary medical therapy is of great benefit because, firstly, it reduces the amount of tissue requiring resection, and secondly, it minimizes the possibility of wound dehiscence.

Surgical resection, drainage, or debulking of large pyogranulomatous masses is becoming increasingly accepted in managing both cats and dogs with RGM infections. However, recurrence may occur at the wound margins, especially in cats. Obtaining more radical surgical margins has proved to be of benefit and the likelihood of success is possibly correlated with the skill and imagination of the surgeon, who most commonly uses these techniques in excising soft tissue tumours and reconstructing the resulting tissue deficits. If surgery is required, a drug with known efficacy against the causal strain that can be administered by injection for example, gentamicin, should be administered intra-operatively (2 mg/kg every 8 hours or 6 mg/kg every 24 hours; IV or SC) and in the early postoperative period (ideally for several days if economically possible). Gentamicin is a good choice because it is bactericidal, available in a parenteral form, inexpensive, and displays good in vitro activity against all RGM. Amikacin is superior to gentamicin, although it is substantially more expensive in Australia. Another option would be moxifloxacin (10mg/kg slowly IV once daily), as it is available as a parenteral formulation (Avelox, Bayer).

The critical surgical consideration is to remove as much abnormal subcutaneous tissue as possible, which in some animals may necessitate the removal of very large portions of infected tissue. Severe cases benefit from the radical excision technique developed by Hunt in which infected tissues are resected en bloc followed by rearrangement of nearby skin to fill the often substantial tissue deficits created.[14,15] In some cases, however, panniculitis is so extensive that this technique is not feasible. Advanced cases with extensive lesions optimally require the skill of an experienced soft-tissue surgeon to reconstruct the resulting wound without undue tension, particularly in feline cases. The large amount of dead space created by the debridement requires judicious use of latex (Penrose) or closed suction (Jackson-Pratt) drains for several days post-operatively. Vacuum-assisted wound closure has been utilized by some centres when confronted with challenging cases.[16]

Following surgery, drugs thought to be of greatest theoretical efficacy against the causal organism are used in the postoperative period to ensure that primary intention healing occurs. Residual bacteria at the wound margins are thus targeted by high levels of the effective agent or agents. Because of cost considerations and other practicalities, the choice is generally reduced to one or a combination of a fluoroquinolone, doxycycline, or clarithromycin based on in vitro susceptibility. Dosages for these drugs are listed in Table 1.

CUTANEOUS MYCOBACTERIAL DISEASE IN DOGS AND CATS (PART 2) *continued...*

Of the agents suitable for post-operative therapy, the fluoroquinolones (particularly moxifloxacin or pradofloxacin [available in Europe, but not yet in Australia]) and doxycycline are generally the agents of choice for treating RGM infections in Australia, where *M. smegmatis* and *M. fortuitum* strains predominate.

Fluoroquinolones are bactericidal, penetrate well into tissues (including fat), and are concentrated in polymorphs and macrophages. Current concerns about the retinotoxic potential of enrofloxacin when given to cats in daily doses exceeding 5 mg/kg probably preclude its use in this species, whereas moxifloxacin, or other veterinary quinolones may be safer choices at the high doses likely to be required for these infections. Pradofloxacin is a veterinary quinolone chemically related to moxifloxacin. Pradofloxacin (3-6 mg/kg once daily depending on the formulation) has comparable efficacy to moxifloxacin, and will likely become the quinolone of choice for treating RGM (and other mycobacteria) in countries where it is registered. The authors have had no experience using marbofloxacin or orbifloxacinin the treatment of mycobacterial infections, and no published data on their use is available. We have used compounded moxifloxacin (10mg/kg PO once daily, or 5 mg/kg twice daily in those cats that vomit with the higher dose) in a growing number of cats and found it to be safe and effective. Doxycycline has a cost advantage over the quinolones and, based on our experience, has similar efficacy and is equally suited to long-term oral therapy. Doxycycline monohydrate is the tetracycline of choice for use in small animal patients because it is well tolerated, is present in a readily available form (VibraVet tablets; Pfizer Animal Health, Sydney, NSW, Australia), and has good lipid solubility. This drug is not readily available in many other countries, which is problematic because other doxycycline salts are more irritating, causing vomiting or, worse, oesophageal ulceration.[17] For this reason, doxycycline should be either given immediately before meals with butter or margarine, or it should be followed by a small amount of liquid. The commercial monohydrate formulation does not have a strong taste and can be ground into a powder and given in a small amount of tasty canned food for cats that are difficult to medicate. Clarithromycin, a macrolide with an extended spectrum of activity and prolonged pharmacokinetics, has proven extremely useful in treating RGM infections in human and veterinary patients. **Clarithromycin is very effective for *M fortuitum* and *M chelonae/abscessus*, but does not work for over 70% cases with *M smegmatis* group infections.** Its major disadvantage is its high cost, which becomes an issue in the treatment of large dogs. Information is insufficient to recommend routine combination therapy in these animals, but the possibility of resistance emerging during therapy should be considered in animals in which a favourable response (especially to fluoroquinolones) is not sustained during a course of therapy or if relapse occurs.

The total duration of therapy should be at least 3 to 12 months. Agents should be administered for at least 1 to 2 months after affected tissues look and feel completely normal. In occasional refractory cases, clofazimine,18cefoxitin, or amikacin may be used for monotherapy or in conjunction with other agents shown to be effective in vitro. Cefoxitin and amikacin can be given only by injection. Several new oral agents for treating refractory RGM infections have become available, including linezolid and tigecycline.[13,19] Although these agents hold great promise for some previously untreatable mycobacterial infections, high cost tends to precludes their routine use.

Table 1: Drug Dosages for Treatment of Rapidly Growing Mycobacterial Infections

DRUG [a]	SPECIES	DOSE [b] (MG/KG)	ROUTE	INTERVAL (HOURS)	DURATION (WEEKS)
Gentamicin	B	2 mg/kg	SC, IM	8–12	2–4[c]
Amikacin	B	5–10 mg/kg	SC, IM	8–12	2–4[c]
Doxycycline	B	5–10 mg/kg	PO	12	12–52[d]
Trimethoprim-sulfonamide	D	15–30 mg/kg	PO	12	4–6[e]
	C	10 mg/kg	PO	12	4[e]
Ciprofloxacin	B	10–20 mg/kg	PO	12	12–52[f,g]
Enrofloxacin	D	5–15 mg/kg	PO	24	12–52[f,g]
	C	5 mg/kg	PO	24	12–52[f,g,h]
Clofaziminei	B	8–12 mg/kgi	PO	24	12–5[g]
Clarithromycin	B	10–15 mg/kg	PO	12	12–52
	C	62.5 mg total	PO	12–24	12–52

B, Both dog and cat; C, cat; D, dog; SC, subcutaneous; IM, intramuscular; PO, by mouth.

[a] For specific information about each drug consult Drug Formulary, Appendix 8.

[b] Dose per administration at specified interval.

[c] Monitor blood-urea-nitrogen weekly for evidence of nephrotoxicity; often combined with other drugs. Cannot use long term.

[d] Use monohydrate salt, if available; to minimize oesophageal irritation give before or with food, or followed by a small amount of water.

[e] Must check haemogram weekly for evidence of myelosuppression. Cannot use long term.

[f] For the dosage of other quinolones, see Drug Formulary.

[g] Avoid in young animals.

[h] Avoid higher doses or parenteral use in cats because of risk of retinal toxicity.

[i] Because of difficulty of fractionating liquid in capsules, cats are usually given one 50-mg capsule per dose. The contents of capsule may be cut into halves with a scalpel blade while wearing disposable gloves and dividing it into two gelatin capsules. Alternatively a compounding pharmacist can provide the optimal dosage size.

Conclusion:

Mycobacterial panniculitis is an eminently treatable disease. Diagnosis is straightforward, especially for practitioners familiar with the syndrome. The prognosis is good, even in cases with severe, extensive, and longstanding disease. Treatment involves long courses of antimicrobials chosen on the basis of laboratory testing, sometimes combined with extensive surgical debridement and wound reconstruction. Furthermore, the routine prophylactic use of doxycycline following treatment of penetrating injuries in obese dogs and cats may prevent the development of these deep-seated infections.

Finally, some centres are evaluating vacuum-assisted wound closure as an adjunct to cytoreductive surgery, as mentioned earlier. Hyperbaric oxygen therapy may also have a place in the management of refractory cases, but is not widely available except in New Zealand. The new parenteral tetracycline, tigecycline may prove useful in certain patients, and may be useful for intra-lesional and transdermal therapy especially in severe or refractory cases.

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The most difficult and frustrating histopathological diagnoses

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Hepatic disease

There are many indications for hepatic biopsy in dogs and cats, including high hepatic enzyme activities for 30 or more days, ultrasonographic lesions, and disease staging in patients with neoplasia. Interpreting liver biopsies is all about correlating histology, signalment and biochemical alterations. Interpretation become very frustrating for me when I'm made to "guess" the species, breed, age, clinical findings, what the liver looked like, or precisely how high were the enzyme levels. For example, congenital vascular disorders tend to have stereotypic histologic appearance and the final diagnosis can only be made with clinical information indicating the presence or absence of a shunt vessel.

The method of biopsy can have a significant influence on interpretation. Comparisons of needle biopsy with wedge biopsies from the same lobe have revealed discordant diagnoses in about 50% of cases. Nodular lesions and many multifocal processes can easily be missed or the relative proportion of the liver affected can be over or under estimated. In general "more is better" and if I can make this clear to you we can save frustration in selected cases.

I have a few guidelines for biopsy collections that should ensure that samples are not collected at the wrong time or for the wrong reason, leading to non-diagnostic biopsies.

- **Abnormal serum enzymes and function tests for 30 days or more**
- **Hepatomegally/microhepatica of undetermined cause**
- **Hepatic involvement in systemic disease**
- **Staging of neoplastic disease**
- **Evaluation of response to therapy or progression of disease**

When these guidelines are not followed a mismatch between your expectations and pathologic findings can result. The decision to biopsy an animal on the basis of a single observation of elevated transaminases is a good example. Take the following scenario: A dog ingests a hepatic toxin and liver injury develops in 24-48hrs. The dog is ill and taken to you and you learn that liver-related enzymes are increased, so you schedule a biopsy the next day. Given the half-life of ALT (2.5 days) the dog may have significantly elevated enzymes three days after the injury, but the hepatic repair process can clear the necrotic hepatocytes and replicate hepatocytes to replace the lost cells yielding a relatively normal looking biopsy. You point out that the ALT is 1000 IU/L, yet I say the liver is normal and you suggest that not all Board certified pathologists are competent.

Finally, proper handling of the biopsy at the time of collection is important. Avoid excessive use of forceps to collect the sample as they can introduce significant crush artifact. Rapid formalin fixation will avoid drying and early degeneration.

Next Newsletter… Inflammatory bowel disease

Vetnostics