Infections of cats attributable to slow growing or 'non-culturable' mycobacteria

Richard Malik [1],
Carolyn O'Brien [2]
& Janet Fyfe [3]

1. Centre for Veterinary Education, The University of Sydney NSW 2006
2. Faculty of Veterinary Science, The University of Melbourne, Werribee VIC 3030
3. Victorian Infectious Diseases Reference Laboratory, North Melbourne VIC 3051

Cats are susceptible to a range of different mycobacterial infections. Tuberculosis has not been seen in domestic species living in Australia (including the cat) for decades. Mycobacterial infections most commonly develop in cats subsequent to penetrating injuries (typically inflicted by other cats) that become contaminated with soil or dirt. Most of these infections are caused by rapidly growing mycobacteria, especially *Mycobacterium smegmatis* and related species, although occasionally other species such as *Mycobacterium avium* and *Mycobacterium ulcerans* are involved. In this report we briefly review infections caused by some novel mycobacterial species, which are either impossible or very difficult to grow *in vitro* using the usual range of liquid and solid media available in reference laboratories. Our understanding of these infections, sometimes referred to as 'feline leprosy-like syndromes', has increased greatly since the application of molecular techniques and the systematic investigation of affected cats.

Mycobacterium lepraemurium infections

M. lepraemurium causes a systemic disease in rats and mice. Cats may become infected by fighting with or ingesting infected rodents. Young, immunocompetent cats are at greatest risk. Initially, lesions are localised to the head, face and extremities – presumably sites where cats are bitten by rodents (Figure 1). In some cats, lesions expand and ulcerate. Satellite lesions can develop. Histologically there is a tuberculoid response, with conspicuous necrosis within lesions. Generally, there are sparse to moderate numbers of acid fast bacilli (AFB), with organisms most obvious in areas of necrosis. AFB do not stain with haematoxylin, are approximately 2-4 μm and are generally difficult to grow (except rarely in specialist laboratories).

These infections are especially challenging to treat, as lesions can spread rapidly, are often refractory to aggressive combination drug therapy and may recur after surgical excision. Disease is more common in regions where rats are prevalent. The condition has been described in many places around the world. PCR and sequence analysis of 16S rRNA and internal transcribed spacer (ITS) regions can provide a reliable molecular diagnosis in these cases [1-4].

Infections with an un-named novel east coast mycobacterial species

The most common cause of nodular mycobacterial granulomas along the east coast of NSW is a novel mycobacterial species. Infections have also been seen in cats in Brisbane, Melbourne

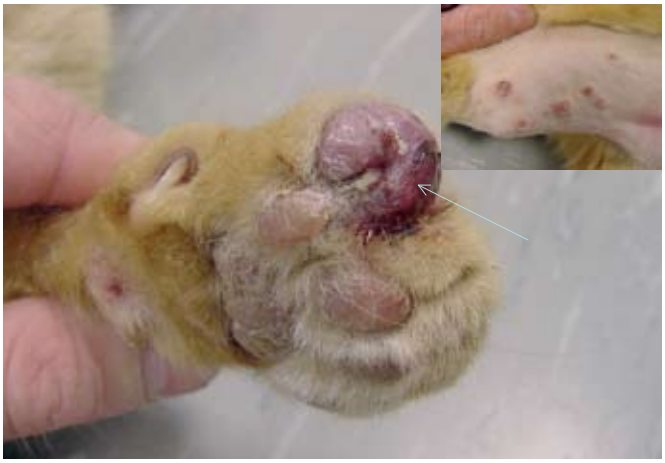

Figure 1. *M. lepraemurium* infection of the digit of a young cat. Note the lesion is beginning to ulcerate (arrow). The inset shows that multiple lesions are present elsewhere (flank and proximal hind limb) presumably as a result of multiple infected rat bite injuries. This cat was successfully treated with a multi-drug treatment regimen.

and New Zealand. Despite numerous attempts, we have been able to propagate this organism on solid media in only one instance; further sub-cultures have failed. Hence diagnosis rests on PCR and sequence analysis.

Clinically and epidemiologically, the disease is quite distinct from that caused by *M. lepraemurium.* Affected cats are old and generally have co-morbidities. Lesions consist of numerous subcutaneous granulomas of different sizes (Figure 2), suggesting possible haematogenous dissemination. There may also be involvement of internal organs. Infection has an indolent course, with the lumps increasing in number and size over many months. Lesions do not ulcerate, and necrosis is not observed histologically.

Microscopically, there is a lepromatous response, with sheets of epithelioid macrophages containing enormous numbers of AFB (Figure 3A). Organisms take up haematoxylin weakly and are thus evident in H&E-stained sections. AFB are 4-6 μm and in cytological preparations they are seen as negatively-staining bacilli (Figure 3B). To date, reported cases are isolated to the east coast of Australia or in New Zealand, although overseas conceptually similar infections have been observed with the closely related organism *M. visibile* [1]. Analysis of ITS and 16S rRNA sequences from these two novel mycobacterium species suggest they show close genetic relatedness with *Mycobacterium leprae* [3] and the newly described species *Mycobacterium lepromatosis* that caused diffuse lepromatous disease in human patients [5]. These infections respond to standard combination therapy with rifampicin, clarithromycin and clofazimine, although cats generally develop other problems (neoplasia, infections, kidney failure) after successful therapy [2-4]. These infections are thus considered to be a marker of immunodeficiency, the causal organism being considered a saprophyte of low virulence. The environmental niche for this organism and mode of transmission remain unknown.

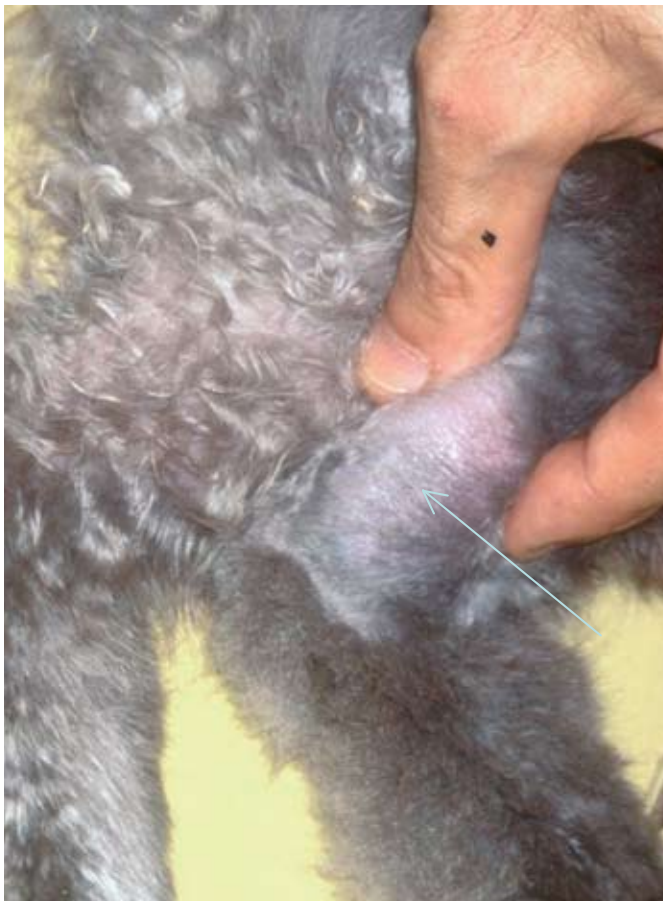

Figure 2. Granuloma on the hock of an 11-year-old, FIV-negative cat infected with the east coast novel mycobacterium species. Although this was the largest lesion evident (arrow), numerous similar lesions were present elsewhere over the integument.

Infections in rural Victoria due to *Mycobacterium* sp strain Tarwin

A different pattern of feline leprosy-like disease is seen in cats from rural Victoria [6]. Affected cats tends to be young adults. Male cats are over-represented, suggesting a pathogenesis associated with territorial aggression. Lesions tend to be in regions subjected to scratch and bite injuries, such as the cornea, periocular tissues and distal limbs. In addition to non-ulcerated subcutaneous granulomas, affected cats may suffer from mycobacterial keratitis or conjunctivitis.

Microscopically, lesions are lepromatous and multibacillary, organisms do not stain with haematoxylin and are 2-4 μm. The strong geographical restriction of patients is reminiscent of the behaviour of *M. ulcerans.* It has so far been impossible

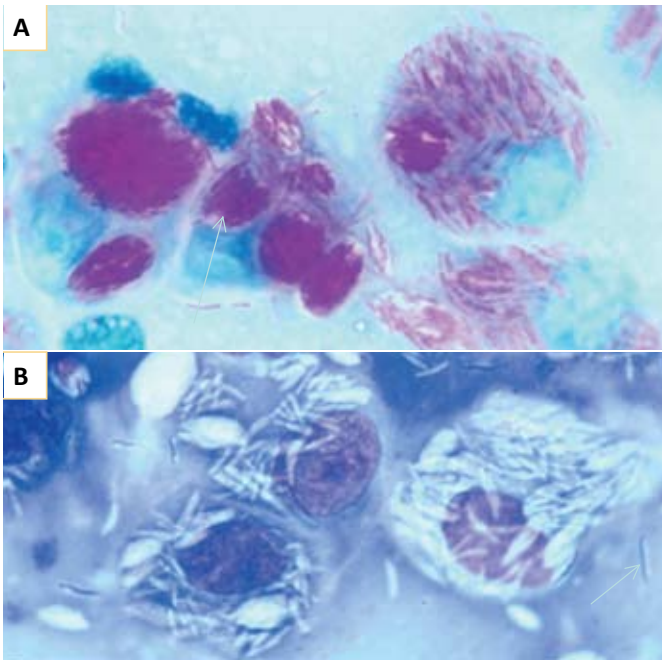

Figure 3. (A) Acid-fast stain of a smear from a needle aspirate of the lesion in Figure 2. Macrophages laden with abundant intracellular AFB appear pink as a result of taking up the carbol fuchsin stain. The AFB are often grouped in ovoid bundles called globi (arrow). (B) Diff Quik-stained smear from the same lesion. Negatively-stained bacilli are evident individually (arrow), and in bundles, predominantly within macrophages.

to propagate this organism *in vitro,* with diagnosis relying on PCR and sequence analysis. Interestingly, the organism appears related genetically to the mycobacterium that causes leproid granuloma syndrome in dogs [7].

Concluding comments

There is much to learn about the mycobacteria species discussed above. Can they be grown on synthetic media? What's the trick? Can they be grown in tissue culture, in the foot pads of mice or in cultures of Acanthomoeba? What drugs are effective against these slow growing organisms? What is their environmental niche? How do they gain entry to the body? Why aren't dogs and horses susceptible? As we have recently amplified the ITS sequence for the east coast organism in the lymph node of a human patient thought to have had relapsing tuberculosis, the answers may have implications for human health!

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Richard Malik is a small animal medical specialist with a life-long interest in infectious diseases of companion animals, especially infections caused by saprobes, including fungi and mycobacteria. He sees cases in the eastern suburbs of Sydney and at the RSPCA, does collaborative research and organises veterinary continuing education for colleagues in practice.

Carolyn O'Brien Carolyn O'Brien is a Registered Specialist in Feline Medicine. After completing a residency in Small Animal Medicine and a Masters degree in the epidemiology of cryptococcosis in dogs and cats at the University of Sydney she moved back to Melbourne to undertake work in referral practice in both the private setting and at the University of Melbourne. She is a director of the Cat Clinic in Prahran, Victoria and is also involved in the continuing education of veterinarians in the field of feline medicine. She is currently taking time out from a PhD investigating *Mycobacterium ulcerans* to raise a family.

Janet Fyfe is the senior molecular biologist at the Victorian Mycobacterium Reference Laboratory based at the Victorian Infectious Diseases Reference Laboratory (VIDRL) in North Melbourne, Victoria. Her main research interests are in the detection and molecular characterization of novel mycobacterial species causing disease in humans and animals. She has also has a major interest in the ecology and transmission of *Mycobacterium ulcerans* to both humans and Australian native wildlife species.