Hotter Topics in Feline Medicine - Challenging Cases for Advanced Practitioners
Mini Series

Session 1: Dyspnoea and coughing

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Tuberculosis in Cats and Dogs

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Introduction

Mycobacteria of importance to companion animals, i.e. cats and dogs, include i) obligate pathogens which can cause tuberculosis (TB), and ii) non-tuberculous mycobacteriosis (NTM), these can be divided into iia) facultatively pathogenic opportunistic saprophytes that can be grown in laboratory, and iib) a subgroup of NTMs that are difficult to grow, so their environmental niche cannot be determined - feline leprosy syndrome (FLS) and canine leproid granuloma syndrome (CLGS). Regardless of which mycobacteria are involved, most cats present with cutaneous disease which can sometimes progress to pulmonary or systemic disease; only occasional cases present with primary systemic disease. In comparison, most canine cases of TB have disseminated disease at the time of diagnosis, while CLGS are cutaneous.

There is little data on the prevalence of feline and canine TB around the world. Mycobacterial infections are seen in companion animals more frequently in some counties than others e.g. Australia and parts of Africa and America (North and South) see cases of NTM, including FLS and CLGS, while New Zealand (NZ) used to see TB (\textit{Mycobacterium bovis}) and still sees NTM and FLS (Malik et al 2006\textsuperscript{a, b}). In Great Britain (GB) these infections are now relatively common in cats, being reported in \textasciitilde 1\% of all feline tissue samples submitted to diagnostic laboratories for routine histopathology (with \textasciitilde 0.3\% being Ziehl Neelsen [ZN]-positive) (Gunn-Moore et al 2013). When cultured, \textit{Mycobacterium microti} was cultured from 19\%, \textit{M. bovis} from 15\%, \textit{M. avium} 7\%, \textit{M. malmoense} 1\%, and unclassified mycobacterium 4\% (Table 1; Gunn-Moore et al 2011\textsuperscript{a}). A positive culture was only gained in 47\% of samples, despite them having histopathological changes indicative of mycobacterial infection. This may have been, in part, because the culture system used was optimised for \textit{M. bovis}, or because some NTM are very difficult to grow, even in optimised systems. In addition, \textit{M. microti} can take many months to grow, and may give a false negative result, especially with paucibacillary samples (Smith et al 2009, Gunn-Moore et al 2011\textsuperscript{a}). Interestingly, canine mycobacterial infections are not common in GB.

Diagnosis and treatment are challenging because there are no pathognomonic histopathological changes for TB and many mycobacterial species fail to culture, so molecular diagnostics are needed to confirm the cause of the infection. Importantly, until the species has been identified it is not possible to tell whether the infection is TB or NTM (including FLS and CLGS). Treating TB is contentious because of zoonotic risk and the potential for generating drug-resistant mycobacteria. Where treatment is undertaken, it typically involves giving two or three drugs for \textasciitilde six months (Greene and Gunn-Moore 2012), with a current prognosis that only \textasciitilde 40\% of feline cases will achieve an apparent cure (Gunn-Moore et al 2011\textsuperscript{b}), (although this is higher for cutaneous and/or pulmonary TB in cats).
Table 1. Mycobacterial culture results. The samples had histopathological changes indicative of mycobacterial infection and were submitted to the Animal and Plant Health Agency (APHA) for mycobacterial culture between January 2005 and December 2008 (Gunn-Moore et al 2011a).

<table>
<thead>
<tr>
<th>Culture results</th>
<th>Number</th>
<th>Percentage (%) of total</th>
<th>Percentage (%) of cultured</th>
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<tbody>
<tr>
<td><strong>Tuberculous mycobacteria; tuberculosis (TB) complex group</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>M. microti</td>
<td>63</td>
<td>19</td>
<td>40</td>
</tr>
<tr>
<td>M. bovis</td>
<td>52</td>
<td>15</td>
<td>33</td>
</tr>
</tbody>
</table>

| Non-tuberculous mycobacteria (NTM) | | |
| M. avium | 24 | 7 | 15 |
| M. malmoense | 4 | 1 | 3 |
| M. fortuitum | 4 | 1 | 3 |
| M. celatum | 1 | <1 | <1 |
| M. intracellulare | 1 | <1 | <1 |
| Unclassified | 10 | 3 | 6 |

| Cultured total | 159 | 47 |
| No growth      | 180 | 53 |
| Grand total    | 339 | 100 |

**Epidemiology**

Tuberculosis can be caused by a number of different, but closely related, bacteria. Relevant members of the TB complex group include *M. tuberculosis*, *M. bovis* and *M. microti* (the vole bacillus). *M. tuberculosis* causes over 90% of tuberculosis in man, but rarely infects other mammals, although occasional cases are seen in captive elephants, pet dogs, and very rarely, pet cats (Parodi et al 1966, Aranaz et al 1996, Erwin et al 2004, Lobue et al 2010, Mikota and Maslow 2011, de la Fuente et al 2012, Parsons et al 2012, Martinho et al 2013, Botelho et al 2014, Engelman et al 2014). *M. bovis* has the broadest host range of the members of the TB complex group and is globally distributed (Snider 1971, Lobue et al 2010). It is the main cause of TB in cattle, but it can also infect other mammals, including humans (where it causes ~1% of cases of TB), badger, deer, llama, cats, dogs, sheep, goats and pigs, amongst others (Francis 1958, Francis 1961, Smith 1965, Cousins 2001, de Lisle et al 2001, Rastogi et al 2001, Delahay et al 2002, de Lisle et al 2002, Biet et al 2005, Corner 2006, Delahay et al 2007, Une and Mori 2007). *M. microti* has been found in many countries (GB, Germany, the Netherlands, Switzerland, South Africa, and South America), and been seen in voles, wood mice, shrews, cats, llama, pigs, ferrets, squirrel monkeys, rock hyrax, dogs, a badger, a bull and humans (Wells and Oxon 1937, Wells 1946, Huitema and Jaartsveld 1967, Pattyn et al 1970, Cousins et al 1994, Gunn-Moore et al 1996, Kremer et al 1998, van Soolingen et al 1998, Deforges et al 2004, Jahans et al 2004, Oevermann et al 2004, Lutze-Wallace et al 2006, Taylor et al 2006, Henrich et al 2007, Lyashchenko et al 2007, Xavier Emmanuel et al 2007, Rufenacht et al 2011). In cats, *M. microti* infection was previously, rather confusingly, termed *M. microti*-like as it was unclear at the time that it was actually the same organism (Huitema and Jaartsveld 1967, Gunn-Moore et al 1996, Kremer et al 1998, van Soolingen et al 1998). In addition, a number of reports have discussed cases where the infection was reported to be *M. tuberculosis* (Kipar et al 2003) or *M. tuberculosis var. bovis* (Orr et al 1980) which on further investigation were probably *M. microti*.

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Historically, TB used to be common in cats and dogs, with prevalence levels in necropsy studies from Europe and other countries of 1-13% in cats and 0.1-13.5% in dogs (Snider 1971). In cats, over 95% of TB was caused by *M. bovis* (Francis 1961, Orr et al 1980, Pedersen 1988) with only occasional cases of *M. tuberculosis* (Parodi et al 1966), and *M. microti* (Huitema and van Vloten 1960, van Dorssen 1960, Gunn-Moore et al 1996). In dogs, *M. tuberculosis* caused approximately 75% of cases, with most of the rest being due to *M. bovis* (Francis 1961, Parodi et al 1966, Liu et al 1980).
Most cases of TB in cats and dogs were believed to result from ingestion of milk from tuberculous cattle (Jennings 1949, Parodi et al 1966, Snider 1971) or arose secondary to living in close proximity to \textit{M. tuberculosis}-infected people (Parodi et al 1966, Snider 1971, Liu et al 1980). Hence, with the reduction of TB from national herds, the pasteurisation of milk, and the reduction of human TB, there has been a marked decline in the prevalence of disease seen in cats and dogs (Jennings 1949, Smith 1965, Parodi et al 1966, Snider 1971, Anon 2007, Gunn-Moore et al 2010, 2011a).


Analysis of the \textit{M. microti} isolates has led to the recognition of two different types of \textit{M. microti} based on their growth characteristics and their molecular specificity (spoligotyping); the vole type and the llama type (van Soolingen et al 1998). The llama type has so far been recognized in humans, llama, a cat, and a dog (van Soolingen et al 1998, Horstkotte et al 2001, Deforges et al 2004, Xavier Emmanuel et al 2007), while the vole type has been recognized in voles, cats, and a badger (van Soolingen et al 1998, Xavier Emmanuel et al 2007).

In GB and Ireland, TB is being seen with increasing frequency in cats (Smith et al 2009, Gunn-Moore et al 2010, Gunn-Moore et al 2011a, Broughan et al 2013a, Murray et al 2014). In GB, infection has a geographical distribution: \textit{M. bovis}-infected cats are found in the South West of England (co-incident with the areas where cattle, badger, mice and other small rodents are infected with \textit{M. bovis}), while \textit{M. microti} is found in the South East of England, the North of England and Scotland, (where \textit{M. microti}-infected rodents have been detected) (Figure 1; Gunn-Moore et al 2011a).
Figure 1. Map of GB showing the location of 326 feline samples from between January 2005 and December 2008 for which the APHA tried to culture mycobacteria. Successfully cultured samples were divided into the species isolated or grouped as unclassified mycobacteria. Also indicated is whether the position on the map is from a complete postcode (●) or the mean easting and northing of the postcode district (■). The coloured shaded areas correspond to predominance by one species and the coloured circles the spatial clusters identified by the SaTScan analysis. (Gunn-Moore et al 2011a).

While most infections occur in a single pet within a household, clusters have been seen where a number of animals within the same house or within a close geographical area have been infected with *M. bovis* or *M. microti* (Rüfenacht et al 2011, Gunn-Moore 2014, Murray et al 2014, Roberts et al 2014). Of a recent 115 feline cases, 55% were caused by *M. microti* and 45% by *M. bovis*, with these two infections accounting for 34% of all cases of feline mycobacteriosis in GB (Gunn-Moore et al 2011a). Tuberculosis is seen less commonly in dogs in GB: only seven cases were confirmed by the APHA in the past seven years; all of which were due to *M. bovis* (Broughan et al 2013a). By way of comparison 116 cats were diagnosed with *M. bovis* in the same timeframe (Broughan et al 2013a).

Infection with *M. tuberculosis* is exceedingly rare in dogs and cats, where it occurs as an anthropozoonosis (reverse zoonosis), with pets being infected by their owners. There are very few cases in cats (Aranaz et al 1996, Erwin et al 2004, Posthaus et al 2011), in part because they are naturally resistant to this infection (Sollys 1958, Smith 1965). Although dogs are less resistant to it than cats, this disease is now uncommon in the developed world so only occasional case reports have been published; they have come from Switzerland, Germany, Spain, Portugal, South Africa, Brazil, and the US (Aranaz et al 1996, Erwin et al 2004, Hackendahl et al 2004, Turinelli et al 2004, Parsons et al 2008, Posthaus et al 2011, de la Fuente et al 2012, Parsons et al 2012, Martinho et al 2013, Botelho et al 2014, Engelmann et al 2014). However, they are of significant concern as a recent report detailed the case of a dog with disseminated TB which was caused by a multidrug-resistant strain of *M. tuberculosis* (Beijing Strain) (Botelho et al 2014).
Possible routes of infection

Cats and dogs may become infected with *M. bovis* or *M. microti* via a number of different routes:

- **Few cases are believed to result from drinking infected cow's milk** (Greene and Gunn-Moore 2012). This is because infection gained by drinking tuberculous milk tends to result in intestinal disease and this form of TB is now very rare (Gunn-Moore et al 2001a, Greene and Gunn-Moore 2012). That said, the specific strains (spoligotypes) of *M. bovis* identified in cats and dogs in the GB and Ireland are typically the same as those seen in cattle from the same geographic location (Monies et al 2006, Murray et al 2014), so cattle may be responsible for environmental contamination. Since *M. bovis* can survive for extended periods in the environment (Wray 1975, Morris et al 1994) this could lead to infection of domestic cats and dogs, and/or rodents (see below).

- **In some areas of GB and Ireland *M. bovis* is endemic in European badgers (Meles meles)** (Gallagher and Clifton-Hadley 2000). While cats and badgers rarely interact directly, there may be potential for cats to become infected via environmental contamination. In support of this, some owners of the infected cats have commented that badgers have visited their gardens or that a badger sett was located close to their property (Monies et al 2006). Some dogs will fight with badgers or eat dead badgers (van der Burgt et al 2009), or, if small enough, may go down into badger setts, and so may become infected.

- ***M. bovis* can also be endemically present in other species of free-ranging wildlife**, so the risk of feline infection will vary in each country dependent on the likely interaction between these species and domestic cats and dogs (Morris et al 1994, de Lisle 1992) e.g. *M. bovis* is endemic in brushtail possums (*Trichosurus vulpecula*) in NZ and these have been known to infect cats (de Lisle et al 2002). Although an eradication program is well underway, infection is still a possibility.

- **If we look at possible risk factors**, we find that most of the cats that develop TB are keen hunters, reported to be regularly catching small rodents (Gunn-Moore et al 1996). In GB, wild field voles (*Microtus agrestis*), bank voles (*Clethrionomys glareolus*) and wood mice (*Apodemus sylvaticus*) can be infected *M. microti* (Cavanagh et al 2002, Burthe et al 2008), and common shrews, yellow-necked mice, wood mice, field voles, and moles, plus a wide range of other animals e.g. rats, stoats, polecats, feral mink and ferrets, grey squirrels, foxes, deer, pigs, sheep, lama and alpaca can be infected *M. bovis* (Delahay et al 2002, Biet et al 2005, Delahay et al 2007, Une and Mori 2007, van der Burgt et al 2009, Rodríguez et al 2010, Broughan et al 2013a). It is most likely that cats become infected when hunting wild rodents. In the case of *M. bovis* the spoligotypes found in cats are the same as those in the cattle, badger and small rodents found in a particular area (Monies et al 2006, Delahay et al 2007); it is likely that small rodents become infected via environmental contamination, possibly around infected badger setts. The *M. microti* spoligotypes are also the same in the rodents and cats from the same geographic location (Smith et al 2009). Damage sustained by cats when hunting accounts for the disease being mainly cutaneous, with lesions frequently affecting the face and legs, i.e. the areas most likely to be bitten when playing with prey; plus or minus associated lymphadenopathy (most typically affecting the submandibular or popliteal lymph nodes) (Gunn-Moore et al 2011a). A canine case of *M. bovis* was caused by being bitten by a squirrel (van der Burgt et al 2009).

- **Some authors suggest that other than the European badger, there is currently no evidence for a significant self-maintaining reservoir of *M. bovis* in wild mammals in GB and Ireland** (Delahay et al 2002, 2007). That said, whether or not a self-sustaining reservoir is needed is debatable, as new members of these spill-over species are constantly being infected with *M. bovis* from cattle and badger.

- **A number of households have been identified where more than one cat or dog has been infected**.
  - Most cases have involved cats that had little close contact with each other, and the infections appear to result from hunting the same group of infected prey.
  - A small number of cases have occurred in cats that did not go outside, but were living with another cat (or dog) that was known to be infected and/or did go outside and hunt. These cases appear to represent spread via close contact, particularly sleeping with and/or grooming an infected companion (Isaac et al 1983, Posthaus et al 2011, Murray et al 2014, unpublished data).
There have now been a small number of nosocomial cases, where cats naturally-infected with *M. bovis* have infected other cats via contamination within a veterinary practice, particularly during routine neutering (Figure 1; de Lisle et al 1990, Gunn-Moore 2014, Murray et al 2014, Roberts et al 2014). This has resulted most frequently, when a cat has had a large lesion draining significant amounts of ZN-positive pus (Gunn-Moore 2014, Murray et al 2014).

**Pathogenesis**

Infection is classically believed to occur after protracted exposure, e.g. following repeated exposure to infected small mammals, living on a farm housing tuberculous cattle, or living for prolonged periods with infected humans or poultry. It is therefore likely that there are many sub-clinical cases (Snider et al 1971).

However, clinical disease can also occur in cats following a single inoculation and occasionally with, *M. bovis* infection, disease can then progress very rapidly. This has been documented on a number of occasions, always with *M. bovis*, with severe disease being seen in under a month (Francis 1958, Isaac et al 1983, Murray et al 2014). Experimentally, subcutaneous inoculation can result in clinical signs in 28-74 days, and peritoneal inoculation can result in clinical signs in 11-36 days (Francis 1958). This phenomenon has also been seen in naturally occurring nosocomial infections; contamination of a castration site resulted in clinical signs in three weeks (Roberts et al 2014), and 42 days (Murray et al 2014), and contamination of an ovariohysterectomy site resulted in clinical signs in just 16 days (Murray et al 2014). When this occurs, disease can progress very rapidly, resulting in death or the need for euthanasia in as little as 8-23 days (Isaac et al 1983, Murray et al 2014). The reason(s) for this rapid progression are unclear, but probably result from high doses of mycobacteria being inoculated and, possibly, the strain of the mycobacteria being inoculated, and/or whether they have been passaged through an atypical host (Aguilar León et al 2009), perhaps mustelids such as feral mink or ferrets (Murray et al 2014).

**Zoonotic and anthroponotic risks**

Dogs and cats are spill-over hosts for TB and as such are believed to present a low risk of further dissemination, either to humans or other animals (Francis 1961). However, all members of the TB complex pose potential zoonotic risks (Smith 1965, Isaacs et al 1983, Une and Mori 2007).

Until recently, there had been very few published report where cats may have infected humans. In an historical case from 1946, a three year old boy was bitten on the arm by a cat in the advanced stages of *M. bovis* TB, developed generalised infection, and died (Lewis-Jonsson 1949). In the other case the man involved was working in Australia with a colony of five cats and two possums, all of which had clinical *M. bovis* infection, when he became Mantoux test positive (he was never clinically ill) (Isaac et al 1983). However, a recent publication detailed a cluster of nine cats with *M. bovis* infection in GB. Of the 24 owners who took up the offer of tuberculosis testing, two were found to have latent infections, and two were found to have active disease (Roberts et al 2014). The spoligotype of the tuberculosis in both owners with active disease was the same as their kitten: the kitten had a draining scrotal wound that the owners were bathing daily (without wearing gloves or masks) (Roberts et al 2014, T. Roberts personal communication 2014). The author (DGM) also knows of a household of three dogs with *M. bovis* infection, where the young son was found to have latent TB (DGM, unpublished observations). There is also a case where pathology staff were infected with *M. tuberculosis* after they used an electric saw to open the cranium of a dog with tuberculosis in its brain (Posthaus et al 2011).

These cases show that while rare, zoonotic infections can occur from pet cats and dogs to their owners or veterinary surgeons. Since people live in close confines with their pets, often having their pets lick their face, sit on their knees and/or sleep on their bed, it is important that veterinary surgeons are aware of the potential for zoonotic spread and advise accordingly. When dealing with these cases in the veterinary clinic it is important that veterinary surgeons deal with these cases with suitable care.

While it is important that we take care when dealing with tuberculous pets, we should keep the risks in perspective – _the risk of catching tuberculosis from a pet is very low_ (PHE, 2014) – and the greatest risk to humans is spending time with tuberculous humans or, much less frequently, by handling infected cattle.

*M. tuberculosis* and *M. bovis* can both cause anthroponotic (reverse zoonotic) infections. There have been a small number of cases where humans have infected their cats or dogs with *M. bovis* (Hawthorne and Lauder 1962, Parodi et al 1966, Shrikrishna et al 2009) or their dogs with *M.

**Predisposition**

**Age and gender:** Tuberculosis is seen most commonly in adult cats and dogs that have access outdoors: median age for *M. bovis* in cats is three years, for *M. microti* is eight years (Gunn-Moore et al 2011a). Unlike tuberculosis in general, where there is no gender bias in cats or dogs (Jennings 1949), male cats appear to be over-represented with *M. microti* infection (Gunn-Moore et al 1996, Rüfenacht et al 2011).

**Immunosuppression:** No evidence of classical immunosuppression has been found; and cats tested for FIV and FeLV have usually been negative (de Bolla 1994, Gunn-Moore et al 1996, Gunn-Moore et al 2011a). However, cats with TB are deficient in 25 vitamin D and 1,25 vitamin D (Lalor et al 2012, DGM & SL unpublished observations), which may play a role in the effectiveness of their macrophages to fight these infections. Certainly, in humans with TB, hypovitaminosis D can prevent the early clearance of infection (Verrall et al 2014) and the efficacy of treatment (Karczmarewicz et al 2013). In addition, an inherited predisposition to TB in humans has been found to result from defective interferon-gamma (IFN-γ) mediated immunity (Altare et al 1998, Remus et al 2001). While we are currently assessing cats with TB with an IFN-γ release assay (IGRA), as yet, we have not fully correlated these results with the nature of the cat’s immune response (Rhodes et al 2008, & 2011, DGM unpublished observations).

**Clinical signs**

*Depending on the route of infection, affected cats and dogs present with clinical signs related to the alimentary, and/or respiratory tracts, or with localised disease affecting the skin* (Jennings 1949, Gunn-Moore 2010). Historically, cats most commonly developed alimentary lesions (secondary to being infected by drinking tuberculous milk) (Jennings 1949), while dogs developed pulmonary lesions (secondary to inhaling infected droplets of their owner's sputum). In dogs the initial pulmonary lesions tended to spread very quickly, so the animals typically died with widely disseminate disease (Jennings 1949, Francis 1961). Currently, there are really too few cases in dogs to comment; but most appear to present with pulmonary, alimentary and/or systemic signs, although cutaneous or intracranial signs can also be seen. (de Lisle 1992, Bauer et al 2004, Deforges et al 2004, Turinelli et al 2004, Ellis et al 2006, Anon 2007, van der Burgt et al 2009, Jahns et al 2011, Posthaus et al 2011, de la Fuente et al 2012, Broughan et al 2013a, Martinho et al 2013, Engelmann et al 2014), although the author (DGM) recently dealt with a dog with a tongue lesion (unpublished observation).

The most usual presentation for TB in cats is now the cutaneous form, with respiratory and alimentary forms being seen less frequently (Gunn-Moore et al 2010, Gunn-Moore et al 2011a, Rüfenacht et al 2011). In cats, the primary complex is often incomplete, i.e. especially when the infection gains entry via the mouth or intestines; i.e. granuloma form in the local lymph nodes, but there are no obvious lesions at the site of entry (Snider 1971, de Bolla 1994, Gunn-Moore et al 1996, 2011a, Rüfenacht et al 2011, Roberts et al 2014).

Cutaneous disease probably arises from infected bite wounds, local spread, haematogenous dissemination to the skin or occasionally, contaminated (surgical wounds) (Jennings 1949, Smith 1965, Isaac et al 1983, Gunn-Moore et al 2010, Murray et al 2014, Roberts et al 2014, & unpublished data). The lesions often involve the face, extremities, tail base or perineum, i.e. “fight and bite sites”. They generally take the form of firm, raised, dermal nodules, ulceration, or non-healing wounds with draining sinus tracts (Snider 1971, Pedersen 1988, Gunn-Moore et al 2011a, Rüfenacht et al 2011). Extension of granulomatous tissue may involve the subcutaneous structures, muscle and/or bone. Skin lesions are commonly associated with either local or generalised lymphadenopathy. On occasion, submandibular, prescapular or popliteal lymphadenopathy may be the only clinical finding (Smith 1965, Blunden and Smith 1996, Gunn-Moore et al 1996, 2010, 2011a, Rüfenacht et al 2011, Roberts et al 2014).

When the infection spreads to the lungs from other sites, or where it is acquired through inhalation, tubercles arise in the lungs and/or hilar lymph nodes and affected animals present with weight loss, anorexia, dyspnoea, and cough. In cats, most pulmonary cases occur secondary to haematogenous spread from cutaneous lesions so the infection is typically diffuse and interstitial (eventually spreading to bronchial), and may include more generalised small focal lesions, and the cats are dyspnoeic, sometimes with a soft cough (Jennings 1949, Smith 1965, Gunn-Moore et al 1996, Gunn-Moore et al 2010, Bennett et al 2011). Occasional cases may also develop pneumothorax and/or pleurisy with the
accumulation of pleural fluid, and pericardial effusions have also been seen (Snider 1971). Some cases may sneeze and have a nasal discharge (Smith 1965, Jennings 1949, Hultema and van Vloten 1960, van Dorssen 1960, Snider 1971). Only very rare cases develop tubercles which cavitate and break down to communicate with the pleural cavity or bronchii (Jennings 1949). In dogs, pulmonary cases have occasionally presented with hypertrophic pulmonary osteoarthropathy (Snider 1971).

In the alimentary form, tubercles arise in the intestines and/or mesenterial lymph nodes. Affected animals develop intestinal malabsorption and present with weight loss, anaemia, vomiting and diarrhoea (Smith 1965, Jennings 1949, Monies et al 2006). Occasionally tubercles arise in the tonsils, resulting in signs of oropharyngeal disease (Smith 1965, Jennings 1949).

A range of clinical signs may be seen with disseminated disease. These include splenomegaly, hepatomegaly, pleural or pericardial effusions, generalised lymphadenopathy, weight loss and fever (Liu et al 1980, Gunn-Moore 2010). Lameness may result from bone involvement (Jones and Jenkins 1995, Gunn-Moore et al 1996). Ocular involvement can result in granulomatous conjunctivitis, uveitis, retinal detachment, and even signs referable to central nervous system involvement (Formston 1994). Mycobacterial conjunctivitis may be seen on its own (Gunn-Moore et al 1996) or associated with more generalised changes including lymph node and pulmonary involvement (Gow 2006). Signs referable to central nervous system involvement have been seen in some cases (Formston 1994, Gunn-Moore et al 1996).

**Diagnosis**

**Unfortunately, many cases of mycobacteriosis look very similar, regardless of which species of mycobacteria is involved (TB or NTM).** Since different mycobacteria have different zoonotic risks and sources, respond differently to antibiotics and have dissimilar prognoses, further investigation is needed to determine which infection is present. Unfortunately, **diagnosis is always challenging.** This is because many of the mycobacteria do not grow in culture (frequently only ~50% grow) (Gunn-Moore et al 2011a), and even those that do e.g. *M. microti*, can take over three months to be identified (Smith et al 2009). In addition, serological tests have historically proved unhelpful and molecular diagnostics are not always available and can be very expensive (Greene and Gunn-Moore 2012, Broughan et al 2013b).

**Non-specific tests:** a thorough evaluation of the patient is necessary to assess the extent of local infection and the degree of systemic involvement.

- Changes in **serum biochemistry and haematology**, if present, are non-specific and vary with the severity of disease, e.g. hypercalcaemia appears to correlate with disseminated disease (Ellis et al 2006, Gunn-Moore et al 2011a). It is believed to result from vitamin D activation within macrophages during the granulomatous response, and is dependent on the underlying vitamin D status and calcium intake (Chan 1997, Monies et al 2006). However, how this occurs in the face of serum hypovitaminosis D is unclear (Lalor et al 2012).

- **Radiography** can be useful in the appraisal of lung involvement. However, changes are very variable and include tracheo-bronchial lymphadenopathy, interstitial or miliary lung infiltration, localised lung consolidation, or pleural effusion (Bennett et al 2011, Greene and Gunn-Moore 2012). Since pulmonary involvement is usually via haematogenous spread, this leads to diffuse interstitial (later becoming bronchial) changes being seen most commonly (Bennett et al 2011). Bone lesions tend to consist of areas of bony lysis and sclerosis, osteoarthrits, discospondylitis or periostitis (Bennett et al 2011). Similar findings are seen with the use of **computed tomography** (CT) imaging, though the sensitivity of detection is increased, and a diffuse structured interstitial lung pattern is most common, being either nodular or reticulo-nodular in nature (Major et al 2016).

- Abdominal radiography and **ultrasound examination** may reveal hepato- or splenomegaly, abdominal masses, mineralised mesenteric lymph nodes, or ascites.

**Specific tests:** The recently developed interferon-gamma (IFNγ) release assay (IGRA) can detect the hosts immune response to members of the TB complex and *M. avium* (Rhodes et al 2008a,b, 2011, Posthaus et al 2011, Parsons et al 2012). It has sensitivity estimates between 70-100% for *M. bovis* (Rhodes et al 2011). The test is also useful when considering a diagnosis of TB in an animal presenting with only pulmonary changes, but where bronchoalveolar lavage is unrewarding and lung biopsy not possible. Other specific tests have been investigated, but have generally proved unhelpful (Snider 1971, Kaneene et al 2002, Broughan et al 2013b). However, newer tests for **serum antibody responses** are still being developed (Rhodes et al 2011). Unlike other species, cats do not react strongly to intra-dermally administered tuberculin and the results from **intra-dermal skin testing** are

Identification of mycobacteria:

- **Gross pathology** may reveal anything from large solid tumour-like masses to multiple small disseminated masses. Lesions are typically greyish white, sometimes with haemorrhagic edges and/or a soft purulent centre. Pulmonary lesions are often greyish red and may be associated with sero-sanguineous pleural fluid. Renal lesions typically occur in the cortex, in the form of infarcts, while intestinal lesions are typically ulcerated Peyer’s patches with small submucosal tubercles (Jennings 1949).

- **Histopathology** of affected tissue generally reveals granulomatous inflammation, with foamy macrophages containing variable numbers of acid-fast bacteria (AFB, see below), and bacilli may also be seen outside degenerating macrophages that border necrotic areas (Jennings 1949, Snider 1971, Kaneene et al 2002, Malik et al 2002, Kipar et al 2003, Gunn-Moore et al 2011b). Lymphocytes may be numerous, and fibroblasts may be present, but multinucleate giant cells are usually rare or absent (Snider 1971, Kaneene et al 2002, Ellis et al 2006). Necrosis and calcification may occur, particularly in larger tubercles, which may be surrounded by zones of histiocytic cells, and a well-defined fibrous capsule may develop (Snider 1971, Kaneene et al 2002). While pathology can be very suggestive of mycobacterial infection

- **Aspirates and/or biopsy samples should always be ZN-stained.** The number of AFB depends on:
  - the species and strain of mycobacteria involved,
  - the location of the granuloma and
  - the nature of the cat's immune response. (Greene and Gunn-Moore 2012). Where the immune response is poor, lepromatous changes are often seen with large numbers of AFB (e.g. lepromatous FLS), while when the immune response is more robust a tuberculous response is more likely, and AFB will be few (TB or tuberculous FLS due to M. lepraemurium or M. sp. strain Tarwin) (Davies et al 2006, Malik et al 2013).
  
  - The use of **modified Fite's or rapid ZN stains** may improve detection of AFB (Malik et al 1994) and **Romanowsky stains** may sometimes reveal large numbers of macrophages containing negatively-stained bundles of organisms and giant cells in lepromatous samples (Studdert and Hughes 1992, Malik et al 1994, 2002, 2004).

- **Specialist culture** is needed to determine which species of mycobacterium is involved (Gunn-Moore et al 2011a). Unfortunately, many samples that contain AFB fail to culture, including all those with FLS and even some with M. microti, particularly when there are few bacteria present (Smith et al 2009, Gunn-Moore et al 2011a).

- **Molecular PCR and sequencing techniques** can be very useful in identifying mycobacteria (Aranaz et al 1996, Brodin et al 2002, Malik et al 2002, Kipar et al 2003, Davies et al 2006, Malik et al 2006a, Fyfe et al 2008, Malik et al 2013, Reppas et al 2013). However, they are often expensive and currently have limited availability.

Correct handling of biopsy material:

In practice, this usually involves taking a biopsy from a case where mycobacterial disease is only one of a number of possible differential diagnoses. If in-house facilities are available for ZN staining, this can be performed on aspirates or biopsy impression smears. However, in most cases biopsy material must be sent to a veterinary diagnostic laboratory. Collect the biopsy, cut it into two or three pieces, fix one in formalin for histopathology and ZN staining and, pending results, place the others in a sterile container and freeze them. Where other bacterial infections are suspected, a sample should be sent unfixed for routine bacterial culture and ZN staining. If the sample is found to have ZN positive organisms, one of the frozen pieces can be sent for specialist culture (by the APHA and/or a Mycobacterial Reference Laboratory), while the last sample is kept in case further investigation is needed. This is advisable for all enlarged lymph nodes and cutaneous/subcutaneous lesions in cats.

Remember: in GB, ~1% of feline tissue samples submitted to diagnostic laboratories for routine histopathology have changes consistent with mycobacterial infection (Gunn-Moore et al 2013).

Until the organism is identified it should be considered a potential human pathogen.
Whenever handling a potentially tuberculous case, wear gloves and use routine aseptic practices when handling the biopsy and the biopsy site. Under the Tuberculosis Orders in England, Wales, and Scotland, the identification of *M. bovis* in clinical or pathological samples taken from any mammal (except humans) is notifiable to the APHA. The Orders impose a duty on any veterinary surgeon who even suspects tuberculosis in a domestic pet to immediately notify the Divisional Veterinary Manager at the local office of the State Veterinary Service (DEFRA 2013). When a confirmed case is euthanased it is advisable to have the body cremated. Unfortunately, until the sample has been cultured (or identified by PCR) it is not possible to know if the infection is caused by *M. bovis, M. microti* or one of the other mycobacteria that can cause disease in cats and dogs.

Management

A. **Initial decision making before the species of mycobacteria has been identified:**

Many feline cases present with a single skin lesion, which is removed and sent for histopathology. It is only when the pathology report comes back suggesting a mycobacterial infection that this differential is considered and the veterinary surgeon has to discuss treatment options with the owner. The decision to treat a case of mycobacteriosis is difficult for many reasons. Most importantly, until the species has been identified it is not usually possible to tell whether the infection is TB, or NTM (including FLS or CLGS) (Gunn-Moore et al 2011a). In GB, TB is likely in 34% of feline cases, with any particular case having a ~15% chance of being caused by *M. bovis* (Table 1), although this does depend on where the cat lives in GB (Figure 1, Gunn-Moore et al 2011a). Treating a case of suspected feline or canine TB is contentious because of the potential zoonotic risk; the need to use drugs that some people feel should be kept for use only in human TB; and the potential for generating drug-resistant mycobacteria (Masur 1993).

Before undertaking treatment it is important to consider:

- **Potential zoonotic risk** – the disease may be caused by a member of the TB complex. All members of the affected cat's household must be considered. It is important to determine if there is anyone with potential immunosuppression e.g. HIV infection, undergoing chemotherapy, or organ transplantation, etc. (see WHO guidelines, 2016). We strongly advise against treatment where such individuals may be exposed. We also advise against treatment if the affected animal has generalised disease, significant respiratory tract involvement, or extensive draining cutaneous lesions as these may increase the risk of transmission.

- **Treatment is almost always long-term and can be difficult to maintain given patient non-compliance, the inherent toxicity of some of the drugs and the financial costs involved.**

In some cases the drugs may at best suppress disease and indefinite treatment may be required (Sieber-Ruckstuhl et al 2007, Greene and Gunn-Moore 2012). Uncomplicated cutaneous cases carry the most favourable prognosis.

- **Tailoring treatment is difficult:**
  - Sensitivity testing is recommended as drug sensitivities can vary between different isolates of the same organism (Horne and Kunkle 2009); however, in vitro findings do not always correlate with *in vivo* results.
  - Multiples of drugs are recommended to improve the chance of successful treatment and reduce the risk of generating resistant clones. However, when multiples of drugs are used, interaction is likely, and while some combinations are synergistic others are antagonistic. Unfortunately, the effects can be difficult to predict, even when using the same combination of drugs (Choi et al 2012). It can vary i) between the species of mycobacteria involved and even the geographical strain of the species; ii) whether treating intracellular or extracellular bacteria (the location of the bacteria is influenced by the type of pathology present – tuberculous versus lepromatous); and iii) even with the species of host. Unfortunately, as yet, we do not know the best drug combinations to use in cats (or dogs), so treatment advice is likely to change with time.
  - The drugs need to be effective in the face of an inadequate host immune response (i.e. defective innate ± adaptive immunity) (Malik et al 2013)
  - **Drugs used to treat humans with M. tuberculosis may be needed** e.g. rifampicin, and discussion is needed within the veterinary profession as to the appropriateness of using these drugs if there is a risk of producing resistant clones.

- **Surgical excision** of small cutaneous lesions may be considered. However, debulking larger lesions risks wound dehiscence and local recurrence of infection.
### B. Interim Management:

Pending a definitive diagnosis, interim therapy with a fluoroquinolone used to be recommended. However, this should only be considered in cases of localized cutaneous infection (Gunn-Moore et al 2010). Pradofloxacin (or moxifloxacin) are recommended as they are more effective against mycobacteria than the older fluoroquinolones (Govendir et al 2011). With more extensive disease double or triple therapy is advised (Table 2) (Greene and Gunn-Moore 2012, Gunn-Moore 2014). Giving more than one drug generally gives the best chance of clinical resolution, and decreases the potential for resistance to develop. Resistant clones can be a particular problem when treating tuberculosis, especially when using older fluoroquinolones e.g. marbofloxacin or enrofloxacin. This is an important consideration since drug resistance will be detrimental, not only to the individual cat, but may also endanger human patients.

#### Table 2. Potentially useful drugs for the treatment of feline and canine tuberculosis (see text for information on potential drug combinations and treatment duration).

<table>
<thead>
<tr>
<th>Use</th>
<th>Drug</th>
<th>Cat/Dog</th>
<th>Dose (mg/kg)</th>
<th>Interval (hours)</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st line Tx</td>
<td>Enrofloxacin&lt;sup&gt;a&lt;/sup&gt;</td>
<td>D</td>
<td>5 PO</td>
<td>24</td>
<td>Retinal degeneration in cats</td>
</tr>
<tr>
<td></td>
<td>Marbofloxacin</td>
<td>B</td>
<td>2 PO</td>
<td>24</td>
<td>Retinal degeneration?</td>
</tr>
<tr>
<td></td>
<td>Pradofloxacin</td>
<td>B</td>
<td>3-5 PO</td>
<td>24</td>
<td>Vomiting, hypersalivation</td>
</tr>
<tr>
<td></td>
<td>Moxifloxacin</td>
<td>B</td>
<td>10 PO</td>
<td>24</td>
<td>Vomiting</td>
</tr>
<tr>
<td>1st line Tx</td>
<td>Rifamp(ic)in&lt;sup&gt;b&lt;/sup&gt;</td>
<td>B</td>
<td>10-15 PO (Max 600mg/d)</td>
<td>24</td>
<td>Hepatotoxicity, induction of liver enzymes, disoloration of body fluids, generalised erythema + pruritus, poor palatability, nausea, CNS signs, teratogenic</td>
</tr>
<tr>
<td>1st line Tx</td>
<td>Clarithromycin</td>
<td>B</td>
<td>5-15 PO</td>
<td>12</td>
<td>Pinnal or generalised erythema, hepatotoxicity? GI signs? GI signs?</td>
</tr>
<tr>
<td></td>
<td>Azithromycin</td>
<td>B</td>
<td>5-15 PO</td>
<td>24</td>
<td>GI signs?</td>
</tr>
<tr>
<td>2nd line Tx</td>
<td>Isoniazid&lt;sup&gt;c&lt;/sup&gt;</td>
<td>B</td>
<td>10-20 PO (Max 300mg/d)</td>
<td>24</td>
<td>Hepatotoxicity, peripheral neuritis, seizures, acute renal failure</td>
</tr>
<tr>
<td>Prophylaxis</td>
<td></td>
<td>B+C</td>
<td></td>
<td></td>
<td>As above</td>
</tr>
<tr>
<td>2nd line Tx</td>
<td>Dihydro-streptomycin&lt;sup&gt;d&lt;/sup&gt;</td>
<td>B</td>
<td>15 IM</td>
<td>24</td>
<td>Ototoxicity</td>
</tr>
<tr>
<td>2nd line Tx</td>
<td>Pyrazinamide</td>
<td>B</td>
<td>15-40 PO</td>
<td>24</td>
<td>Hepatotoxicity, GI signs</td>
</tr>
<tr>
<td>2nd line Tx</td>
<td>Ethambutol&lt;sup&gt;e&lt;/sup&gt;</td>
<td>B</td>
<td>10-25 PO</td>
<td>24</td>
<td>Optic neuritis</td>
</tr>
<tr>
<td>2nd line Tx</td>
<td>Doxycycline&lt;sup&gt;f&lt;/sup&gt;</td>
<td>B</td>
<td>5-10 PO</td>
<td>12-24</td>
<td>GI signs, oesophagitis</td>
</tr>
<tr>
<td>2nd line Tx</td>
<td>Clofazamine&lt;sup&gt;g&lt;/sup&gt;</td>
<td>C</td>
<td>4-8 (occ. ~10) PO Max 25 total</td>
<td>24</td>
<td>Hepatotoxicity, G-I signs, disoloration of body fluids, photosenstilization.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D</td>
<td>4-12 PO</td>
<td></td>
<td>As above</td>
</tr>
</tbody>
</table>

<sup>a</sup> The authors recommend using a fluoroquinolone that is not enrofloxacin when treating cats as this drug has been associated with retinal degeneration in this species.

<sup>b</sup> These drugs are not licensed for use in pets and may cause potentially serious side effects e.g. hepatotoxicity or nephotoxicity. It is advisable to monitor these animals closely, and check routine haematology and serum biochemistry two weeks after starting treatment and then if there is any change in the cat’s demeanour.

<sup>c</sup> Not effective against M. bovis infection.

<sup>d</sup> Give with food or give water after the medication to avoid oesophageal injury.

<sup>e</sup> Can be difficult to obtain.
Second line treatments for tuberculosis should be reserved for resistant infections. Drugs licensed for human use can be obtained by veterinary prescription from our pharmacy (see below) or larger chemists as long as, in GB, all aspects of cascade prescribing have been considered. In GB, the R(D)SVS Pharmacy can reformulate and supply these drugs for other veterinary practices for use in animals under their care: Liz.Wright@ed.ac.uk; Tel 0131 650 7634.

C. Continuing treatment:
It is strongly inadvisable to continue treating a cat or dog once *M. tuberculosis* has been confirmed or *M. bovis* is disseminated; GB and Scottish law dictates that *M. bovis* infection is notifiable. These cases should be euthanased. *M. microti* is also potentially zoonotic, although very few human cases have been reported, and none due to feline or canine exposure. Unfortunately, in many cases it is not possible to culture the organisms from tissue samples even when AFB are present. Because of this it is essential to counsel owners carefully so that they know that it might not be possible to identify the causal species, making it difficult to predict potential zoonotic risks and treatment complications.

Previously, anti-tuberculosis treatment was given as an initial and then a continuation phase (Greene and Gunn-Moore 2012); however, it is now known that it is better to give all three drugs for four to six months, depending on the extent of disease, and always for at least two months following complete resolution of the lesions. In those cats where triple therapy is not feasible, treatment should still involve two drugs and should be given for a minimum of six to nine months.

A combination of drugs needs to be given. First line treatment for tuberculosis in humans consists of combinations of rifampicin, isoniazid, ethambutol, dihydrostreptomycin and pyrazinamide. However, these drugs are very toxic in cats (their toxicity in dogs is generally unknown), so all but rifampicin are reserved for resistant cases. The newer fluoroquinolones, e.g. pradofloxacin (or moxifloxacin) have good efficacy against most mycobacteria, and clarithromycin (a macrolide) is useful, especially when given in combination with rifampicin and/or another antibiotic as per culture and sensitivity e.g. doxycycline. A useful once daily alternative to clarithromycin is azithromycin. From clinical experience gained over 20 years, we currently recommend an initial phase of rifampicin-pradofloxacin-azithromycin (Table 2).

While these combinations are also used when treating human mycobacterial infections, some experimental studies have shown that with *M. tuberculosis* infection, combining the new fluoroquinolones with clarithromycin (or azithromycin) or rifampicin may be at least mildly antagonistic, and combining fluoroquinolones and rifampicin may be antagonistic against extracellular but not intracellular AFB (Balasubramanian et al 2012). What this means for the treatment of feline and canine cases is not yet clear. Rifampicin can cause significant side effects, including hepatotoxicity. It is therefore sensible to monitor cases closely, and check routine haematology and serum biochemistry two weeks after starting treatment, every few months if possible, and whenever there is any change in the pet’s demeanour and/or appetite.

For ease of administration all three once-daily medications can be given as liquids and placed in a single syringe prior to oral administration, or given as tablets with all three being placed in a single gelatin capsule and administer together using a pill-popper. Alternately, where oral medication proves difficult, an oesophagostomy tube may be placed (through which the liquid medications can be given) and left in place for the duration of the treatment. It is not recommended that owners put their fingers directly in the animal’s mouth when administering medication and they must wash their hands thoroughly after the medication has been given.

In cases where resistance develops, a rifampicin-isoniazid-ethambutol combination may be considered, although toxicity can be severe (Gunn-Moore et al 2010). If necessary, ethambutol can be substituted with dihydrostreptomycin or pyrazinamide. However, *M. bovis* is naturally resistant to pyrazinamide. Rifampicin and isoniazid are more effective and less toxic than ethambutol and dihydrostreptomycin and consequently are more appropriate choices if only two drugs are required.

We know that cats with tuberculosis are deficient in vitamin D (Lalor et al 2012). However, as yet, we do now know if correcting this deficiency will help in their successful treatment.
Prognosis
Prognosis depends on the mycobacteria involved, the extent and severity of the infection and whether treatment involves the appropriate drug(s) for the necessary length of time. In a retrospective study of 184 cases of feline mycobacterial disease, ~40% gained apparent cure or long-term remission while ~60% responded temporarily or poorly to treatment or did not respond at all. However, many cats were treated sub-optimally, with few receiving more than one drug, many receiving inappropriate drugs (e.g. β-lactam drugs) and most (~60%) being treated for less than one month (Gunn-Moore et al 2011b). It is therefore likely that with more appropriate treatment prognosis will be better. Cutaneous and/or pulmonary TB in cats, caused by *M. microti* or *M. bovis*, typically responds well to treatment. That said, the prognosis should be stated as guarded. Interestingly, the author (DGM) has seen a number of cats who, years after being successfully treated for one mycobacterial infection e.g. TB, then contract a different mycobacterial infection e.g. *M. avium*. This may result from an underlying immunosuppression and/or specific susceptibility to mycobacterial infections.

Very few cases of TB in dogs have been treated as most present with disseminated disease and are euthanased. However, one dog with anorexia, weight loss and diarrhoea, associated with abdominal masses due to *M. tuberculosis* infection, was treated with rifampicin, clarithromycin and enrofloxacin for three months, followed by indefinite treatment with rifampicin and clarithromycin, and was still well 31 months later (Engelmann et al 2014).
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• Francis, J. 1961. Tuberculosis in small animals. Modern Veterinary Practice; Sept., 39-42.


