### DIAGNOSIS AND TREATMENT OF FELINE MYCOBACTERIAL INFECTIONS

Professor Séverine Tasker
The Feline Centre, Langford Veterinary Services, University of Bristol
Langford, Bristol, BS40 5DU, United Kingdom

#### SUMMARY OF MAIN FEATURES OF FELINE MYCOBACTERIAL SPECIES (based on Hibbert, 2017)

<table>
<thead>
<tr>
<th>Group</th>
<th>Subgroup</th>
<th>Mycobacterial species</th>
<th>Clinical signs</th>
<th>Transmission</th>
<th>Growth pattern</th>
<th>Zoonotic risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberculosis (TB) complex</td>
<td>No subgroups</td>
<td><em>Mycobacterium tuberculosis</em> (reservoir host = humans), <em>Mycobacterium bovis</em> (reservoir host = cattle, badgers, rodents), <em>Mycobacterium microti</em> (reservoir host = small rodents such as voles)</td>
<td>Cutaneous &amp;/or systemic disease ± regional or generalized lymph node involvement</td>
<td><em>M. tuberculosis</em> – ingestion of infected unpasteurised milk (rare), close prolonged contact infected human. Notifiable in UK. <em>M. bovis</em> – ingestion of infected unpasteurised milk (rare) or raw infected meat; direct/indirect contact infected badgers or rodents. Nosocomial infection recently reported. Notifiable in UK. <em>M. microti</em> – direct contact infected small rodents (e.g. voles, wood mice), ingestion or fight</td>
<td>Slow growing over 2-4 months (≤ 8 weeks for <em>M. bovis</em> &amp; ≤ 14 weeks for <em>M. microti</em>)</td>
<td>M. bovis very low risk of cat to human transmission exists &amp; report exists; <em>M. microti</em> risk also exists but no cat to human transmission reported. Cats very resistant to <em>M. tuberculosis</em> but this would be of high zoonotic risk if it occurred</td>
</tr>
<tr>
<td>Non-tuberculous mycobacteria (NTM)</td>
<td>Mycobacterium avium (MAC) complex</td>
<td><em>Mycobacterium avium</em> subsp. <em>avium</em>, <em>Mycobacterium intracellularare</em></td>
<td></td>
<td></td>
<td>Slow growing</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Slow growing NTM</td>
<td><em>Mycobacterium genavense</em>, <em>Mycobacterium malmense</em> &amp; others</td>
<td>Cutaneous (local or diffuse, can include panniculitis with rapidly growing NTM), very rarely progresses to systemic disease</td>
<td>Opportunistic, environmental (soil, water, decaying vegetation) - transmission of MAC infection may be possible by e.g. ingestion of infected meat or contact with infected soil/fomites contaminated with bird faeces</td>
<td>Slow growing</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Rapidly growing NTM</td>
<td><em>Mycobacterium fortuitum</em>, <em>Mycobacterium smegmatis</em>, <em>Mycobacterium chelonae</em>, <em>Mycobacterium flavescens</em> &amp; others</td>
<td></td>
<td></td>
<td>Rapidly growing (≤ 7 days in culture)</td>
<td>No</td>
</tr>
<tr>
<td>Feline leprosy (FL)</td>
<td>Lepromatous mycobacteria</td>
<td><em>Mycobacterium lepraemurium</em>, <em>Mycobacterium visible</em>, Candidatus ‘<em>Mycobacterium lapraefelis</em>’, Candidatus <em>Mycobacterium tarwinense</em> (in Australia &amp; NZ)</td>
<td>Nodular cutaneous disease; localised, rarely disseminated systemic disease</td>
<td>Direct contact or bites from infected rodents; wound contamination by mycobacteria in soil/on plants</td>
<td>Highly fastidious, often non-culturable</td>
<td>No</td>
</tr>
</tbody>
</table>

### APPROACH TO DIAGNOSIS
Most cats affected by mycobacterial disease are outdoor cats, often with a history of hunting or fighting, from a non-urban area. The clinical presentation is similar with all mycobacterial species; cutaneous lesions (especially around the ‘fight and bite’ sites: face/legs, areas bitten when playing with prey), which may be multiple due to local or haematogenous spread. Local or generalised lymphadenopathy (often submandibular and/or prescapular) is common, and can be the only clinical sign. Systemic signs, typically involving the lungs, are far less common than cutaneous signs, but may occur with M. bovis or M. avium infections (and occasionally M. microti). Systemic signs include generalised (including abdominal) lymphadenopathy, splenomegaly, hepatomegaly, renal abnormalities, ocular signs and bone lesions. Pyrexia is not a consistent feature of feline mycobacterial disease.

Differential diagnoses include neoplasia (lymphoma, mast cell tumours), feline infectious peritonitis, nocardiosis (also acid fast bacilli [AFB], like mycobacterial species), actinomycosis, fungal infections, rhodococcus (also AFB) and toxoplasmosis. If mycobacterial infection is strongly suspected, it is important to determine not only the extent of disease to help direct whether treatment should be given, but also the infecting mycobacterial species due to their varying zoonotic risk.

**Blood testing**
An anaemia of inflammatory disease may be present. Hypercalcaemia can occur with extensive disease or severe panniculitis, and has been reported with M. microti and systemic M. avium infection. The hypercalcaemia (ionised calcium is also elevated) is thought to be due to calcitriol (1,25-OH₂VitD) production by activated macrophages. Mycobacterial cases have low serum levels of calcifiediol (25-OHVitD)², which could be due to elevated calcitriol levels. Mild hyperglobulinaemia and hypoalbuminaemia may occur. Testing for retroviruses may be indicated with NTM but most affected cats are negative.

**Imaging**
Thoracic radiography may show pulmonary involvement; 21/24 cats had abnormalities in one study³ with bronchial, alveolar, nodular structured interstitial or non-structured (absence of miliary pattern, nodules or masses) interstitial patterns visible, with possible perihilar or sternal lymphadenopathy. Osteolytic/proliferative changes may be seen, often with overlying soft tissue changes (and cutaneous lesions). Abdominal radiographic changes were uncommon but included hepatomegaly and/or splenomegaly.

Computed tomography (CT) findings in mycobacterial cases have recently been reported⁴ in 20 cats (6 with M. bovis, 6 with M. microti), and again thoracic abnormalities predominated (19/20) with structured interstitial (15/20), bronchial (9/20), alveolar (8/20) and ground glass (non-structured interstitial) (6/20) patterns visible, which were often mixed. Other changes seen included abdominal or peripheral lymphadenopathy, osteolytic/proliferative lesions and cutaneous or subcutaneous soft tissues masses and nodules. Mild lymphadenopathy was more appreciated in post-contrast studies, so use of contrast should be considered. A recent study of feline TB (primarily M. microti) cases were sequentially monitored by CT, and it was reported that imaging abnormalities only resolved in a minority of cases and that changes in CT findings over time and with treatment were very variable⁵.

Ultrasonography may show abdominal lymphadenopathy, hepatomegaly, splenomegaly or renal changes, and may provide a window for sampling tissues for diagnostic purposes.

**Interferon-gamma (IFN-Ɣ) release assay (IGRA) blood testing**
Feline IFN-Ɣ release assay blood testing (http://www.biobest.co.uk/diagnostics/species/cats.html) shows Promise for the ante-mortem diagnosis of TB. The test is based on research findings⁶ and measures the T-cell response (IFN-Ɣ production) in peripheral blood mononuclear cells (prepared from heparinised blood, which is kept at room temperature) to three different antigens; the pattern of any positive result classifies the case as: ‘Likely to...’

- **be affected with pathogenic TB complex species**: M. bovis, or M. tuberculosis (very rare in cats)
- **be affected with less-pathogenic TB complex species** (M. microti)
- **have been exposed to environmental NTM species**
Sensitivity is 83.3% for *M. microti* and 90% for *M. bovis*, with good specificity for both. A sensitivity of 50% has been reported for detection of *M. avium* infections\(^7\). We have found this test to be most useful when there is a high suspicion of mycobacterial disease and we want to determine whether *M. bovis* or *M. microti* infection is most likely to determine the zoonotic risk to guide treatment. The test may also be useful for monitoring treatment and validation testing for this is underway\(^7\). Current cost (2018) €230+VAT and samples can be submitted from abroad but Biobest should be contacted for advice before submission.

**Confirmation of mycobacterial disease**

Mycobacterial organisms may be visible following Ziehl-Neelsen (ZN) staining for AFB in fine needle aspirates or biopsies collected from affected tissues (e.g. lymph nodes, skin, liver) or bronchoalveolar lavage (BAL) or draining wound/cutaneous lesion samples. Cytology and histopathology from affected cases reveals pyogranulomatous/granulomatous inflammation ± AFB in macrophages. The number of AFB is very variable and can be very sparse; the number was thought to be dependent on the infecting species but the cat's immune response is now thought to be more important\(^8\). However, with NTM infections in particular, AFB are often lost from within the lipid droplets during processing of cytology or biopsy samples, and so alternative staining methods may be needed (rapid ZN or modified Fite’s). If no AFB are present, but cytology or pathology changes are consistent with mycobacterial infection, mycobacterial disease should remain a differential diagnosis and culture should be performed.

Whenever mycobacterial infection is suspected and a tissue biopsy is taken, it should be cut into **FOUR** pieces and distributed as follows:
- **ONE** in formalin for histopathology & ZN staining
- **ONE** for routine bacterial culture and sensitivity
- **TWO** in sterile containers (wrap samples in sterile gauze moistened with sterile saline) which are frozen at -20°C for further investigations that may be required, notably mycobacterial culture and polymerase chain reaction (PCR) if required, pending other investigations

NB. Impression smear made from the cut end of a biopsy sample can be submitted for immediate cytology

NB. Gloves and aseptic practice are required to handle the biopsy (and biopsy site)

**Culture** is done by the National Mycobacterial Reference Laboratories in the UK (Public Health England [PHE], UK), and some species take a long time (2-4 months) to grow, delaying diagnosis. Some species (e.g. FL NTM and some *M. microti*) are impossible to grow. Only ~50% of mycobacteria in cats grow in culture\(^9\). Culture is done on the same samples obtained for cytology or histopathology, although swabs of draining wounds should be avoided as these are usually contaminated with secondary bacteria.

**PCR** may allow diagnosis whilst awaiting culture or with unculturable organisms. However sensitivity can be poor due to problems with DNA extraction and PCR design (mycobacterial genetic sequence variation is difficult to target). PCR is also costly; ~€290 (2018). Ideally PCR is performed on fresh tissue, but fixed tissue can be used if fresh is not available. PCR can also be performed on cytology slides. Current commercial PCR assays do not usually determine the mycobacterial species but can determine if DNA from a TB complex species is present. PCRs exist for differentiating species but, in the UK, these are usually only available from National Mycobacterial Reference Laboratories. Extensive sequencing is required for full speciation but has been performed to determine the epidemiology of infections in outbreaks\(^10\), or occasionally in individual cases\(^11\).

**TREATMENT**

It is important to discuss whether cases should be treated at all, especially cases of disseminated *M. bovis* infection due to zoonotic implications and worries around antimicrobial resistance developing in human TB cases as a result of antimicrobial use in feline cases. The current advice from the UK chief veterinary officer is that cats diagnosed with *M. tuberculosis* and *M. bovis* should be euthanased.

The cat should be kept indoors and the costs and compliance issues of treatment need to be carefully discussed with owners. It is recommended that specialist advice is sought before embarking on treatment. Additionally, owners should be advised to contact their doctor (who may send the owner for TB screening e.g. Mantoux testing and thoracic radiographs).
If treatment of the cat goes ahead, long-term therapy with several antibiotics is required. Although shorter courses of just one antibiotic can result in an improvement, relapses often occur (and the risk of antimicrobial resistance probably increases) so we no longer recommend starting a single antibiotic whilst awaiting a diagnosis, unless dealing with confirmed solitary cutaneous disease, when a fluoroquinolone could be considered. Due to the need for dual or triple antibiotic therapy for months in many cases, compliance must be good. To aid this, medications can be given in a single gelatine capsule, as liquid formulations, or via an oesophagostomy tube. Surgery is sometimes used to treat solitary cutaneous lesions but is difficult for diffuse cutaneous disease due to wound dehiscence and local recurrence.

**TB complex**

Two months of triple therapy with rifampicin (10 mg/kg SID), a fluoroquinolone [marbofloxacin (2 mg/kg SID; different dose for tablets vs liquid) often used], and a macrolide [azithromycin (5-15 mg/kg SID) or clarithromycin (7-15 mg/kg BID)] is recommended, followed by a further 4-7 months of treatment with two of these drugs (usually the fluoroquinolone and macrolide). If triple therapy is not possible then two of the drugs should be given for 6-9 months. Haematology and biochemistry should be monitored 2 weeks after starting treatment and every 2 months thereafter to monitor for possible side effects, especially hepatotoxicity with rifampicin. Rifampicin can also discoulour body fluids orange and cause anorexia, erythema, pruritus and anaphylaxis. Clarithromycin can result in pinnal/generalised erythema. More recently a three month course of triple therapy alone or triple therapy given for 2-3 months beyond resolution of clinical signs or static thoracic imaging abnormalities (typically comprising 4-6 months of treatment) has been described as unpublished observations for the treatment of TB in cats5,7, but follow up on this protocol is not yet available. This treatment protocol is based on recommendations from people where treatment with at least 3 or 4 antibiotics given in combination throughout treatment is said to reduce the development of multi-drug resistant *M. tuberculosis*.

**MAC complex, slow & rapidly growing NTM**

Treatment depends on the infecting species12 and *M. avium* infections are particularly difficult. Clarithromycin and rifampicin or clofamazine should be given for *M. avium* cases, alongside, if possible, doxycycline (10 mg/kg SID or 5 mg/kg BID) or pradofloxacin. Other NTM species show variable responses to antibiotics but generally two or three antibiotics are used. Clarithromycin and pradofloxacin together are good for certain species (e.g. *M. chelonae-abscessus*) whilst a fluoroquinolone and doxycycline are better for others (e.g. *M. smegmatis*). Treatment should continue 1-2 months beyond clinical resolution; up to 12 months of treatment is needed for extensive/systemic infections. Surgical resection may comprise part of the treatment for severe panniculitis cases, with reconstructive surgery sometimes needed to maximise healing.

**FL NTM**

Surgical removal of solitary lesions may be curative; occasionally spontaneous resolution occurs. If further treatment is required, dual or triple antibiotic treatment should include clarithromycin, rifampicin, pradofloxacin &/or clofamazine (4-8 mg/kg SID or EOD), typically for 2-3 months. Recent reviews on the different types of FL NTM have been published13-15.

**PROGNOSIS**

In a recent study of 184 feline mycobacterial cases16, around 40% responded well to treatment long-term (remission or cure), whilst the remainder responded temporarily, poorly or not at all. However, the infecting species was not always known and appropriate treatment was not given in all (e.g. many were given only a fluoroquinolone for <1 month, others were also given steroids). It may be that appropriate triple and dual treatment is associated with a better outcome16 with remission rates of 70-90% for feline TB. Interestingly, a recent imaging study in which feline TB cases (primarily *M. microti* cases) were sequentially monitored by CT reported that imaging abnormalities only resolved in a minority of cases and that changes in CT findings over time and with treatment were very variable5. The prognosis for MAC, slow and rapidly growing and FL NTM is variable, depending on the species involved (*M. avium* infections and those with extensive panniculitis, especially due to *M. fortuitum*, are particularly poor).
ZOONOTIC CONSIDERATIONS

Although cat-to-human transmission of *M. bovis* is said to be of ‘very low risk’\(^1\), the zoonotic potential must be discussed with the owner, especially for those in close contact with the affected cat. Some people are considered to be at heightened risk of mycobacterial infection e.g. < 5 years old, pregnant, HIV infected, cancer patients on chemotherapy or radiotherapy. *M. avium* is zoonotic and can infect immunosuppressed people but there are no records of cat-to-human transmission of NTM. The zoonotic risk may be greater if the cat is coughing or has draining skin lesions. However, owners in close contact with the cat have probably already been exposed to infection before the cat visits the veterinarian. As soon as effective treatment is started in the cat, the risk of cat-to-human transmission goes down. It is generally thought that protracted exposure is required to transmit infection from cats to humans, but a single inoculation with high doses could result in infection transmission, so care is always required.

Zoonotic potential must also be considered when collecting samples from cats; inhalation of organisms from cats with respiratory signs, or contact with organisms in draining wounds via open sores/wounds in personnel could allow transmission to humans. A fitted face mask (FP3) (fitting done by someone trained to be able to do this) should be worn when in close proximity to the face of coughing cats (e.g. during intubation, collecting BALs) to prevent inhalation of organisms. Gloves and protective clothing should be used when handling cases with draining skin lesions to avoid spread to other cats, as well as covering of any open sores/wounds in personnel to prevent zoonotic transmission. Strict asepsis is very important as nosocomial spread of mycobacterial infections has been reported when cats undergoing neutering were infected with *M. bovis* via contact with contaminated staff uniform and/or hands\(^1\). Hand washes and disinfectants must be mycobacteriocidal e.g. high (60-90) percentage alcohols and halogenated tertiary amines and 3% iodine preparations are mycobacteriocidal but 4% chlorhexidine is not. Breathing systems and endotracheal tubes used for suspected cases should be disposed of after use in clinical waste (double bagged) and investigations on suspected cases should be performed at the end of the day in well ventilated rooms. If open surgery is required on a suspected TB patient, in addition to the protective clothing mentioned above, an appropriately filtered airflow helmet is required e.g. Dustmaster Powered Respiration kit. Following euthanasia of a TB patient, cremation rather than burial should be recommended to reduce the risk of environmental contamination.

REFERENCES


