FACT SHEET 5

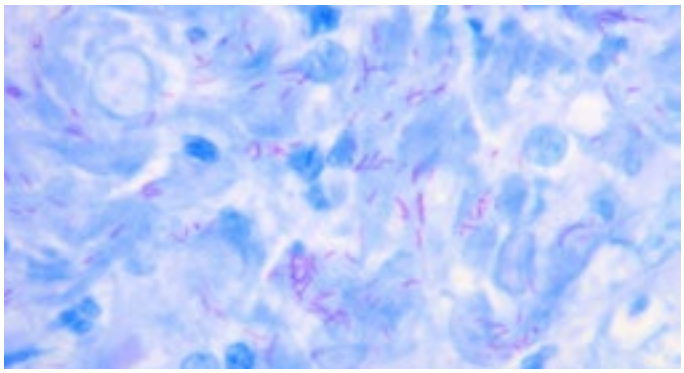
Finn Pathologists *The* Veterinary Pathologists

AUTHORSHIP - The University of Edinburgh

Companion Animal Mycobacterial Disease

THE ROYAL (DICK) SCHOOL OF VETERINARY STUDIES

Mycobacteria are pathogens of global health significance to both human and non-human animals. In recent years the significance of companion animal infections with mycobacteria has become more apparent; it has been demonstrated that approximately 1% of all feline biopsies submitted for histopathological analysis in the UK show changes consistent with mycobacteriosis and a third of these have demonstrable Ziehl-Neelsen (ZN) staining organisms, indicative of mycobacteria. Currently, equivalent data do not exist with regard to dogs, but canine mycobacteriosis is clinically recognised.



Feline Mycobacteriosis

The myriad of mycobacterial species that have been positively identified in cats can be grouped into two main categories, each with its own clinical significance.

1. Tuberculosis (TB) complex

The TB complex consists of nine phylogenetically related species of mycobacteria capable of causing tuberculosis in mammals. Of these nine, only *Mycobacterium bovis* and *M. microti* have been frequently detected in cats; successfully cultured samples in one study confirmed that 19% of mycobacterial infections in the UK were caused by *M. microti* and a further 15% by *M. bovis*.

M. tuberculosis infection, the leading cause of human TB, is very rare in the cat and it has been demonstrated that cats have natural resistance to this pathogen. The majority of companion animal cases reported have occurred in dogs as a result of reverse zoonotic transmission from an infected human.

There is a strong geographical predisposition to feline infection with members of the TB complex. In the UK, *M. bovis* infections are strongly co-incident to areas where there are high levels of endemic *M. bovis* infection in local bovine and wildlife populations, such as the South-West of England. Similarly, *M. microti* infections are most frequent in areas with high prevalence of infection in the wild rodent population, typically to the south and east of London, the North of England and Scotland.

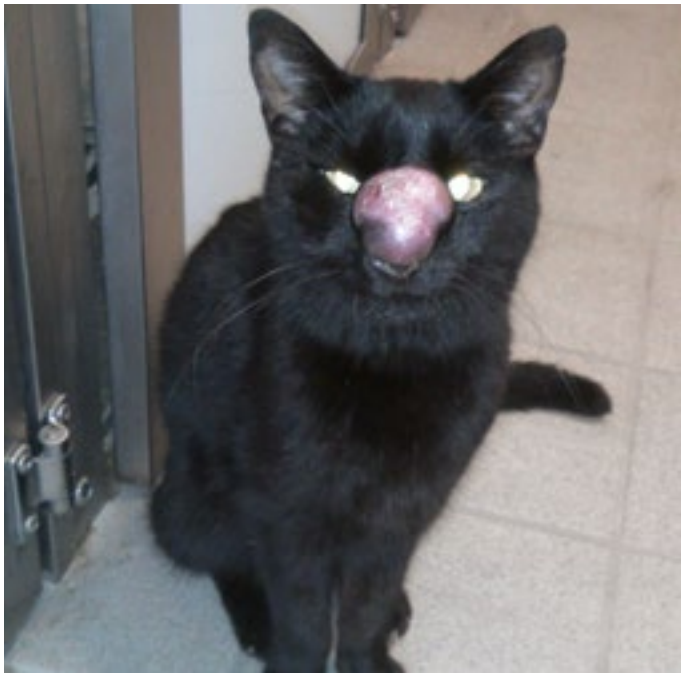
TB is most frequently diagnosed in adult male cats with a history of hunting. The median age of infection is three years for *M. bovis* and eight years for *M. microti*. There is no link between TB complex infection and classical immunosuppression in cats i.e. feline immunodeficiency virus (FIV) and feline leukaemia virus (FeLV) infection.

Clinical Signs:

The majority of TB cases present with localised nodular cutaneous disease, frequently with a degree ulceration and occasionally with a draining sinus tract. The lesions are typically distributed around the face, extremities and tail base – the so-called "fight and bite sites". Skin lesions may be accompanied by a localised or even generalised lymphadenopathy, or lymphadenopathy may be the only presenting sign.

A predominately gastrointestinal (GI) form of the disease exists where granulomas form in the intestines and there is mesenteric lymph node involvement causing weight loss, diarrhoea, vomiting and anaemia. This form of the disease was traditionally associated with cats drinking tuberculous cows' milk and therefore, since the introduction of pasteurisation, this form of TB has declined in incidence.

Pulmonary lesions can occur when bacteria are inhaled, resulting in tubercles and cavitating lesions in the lungs and hilar lymph nodes. Much more common, however, is pulmonary disease secondary to haematogenous spread of bacteria from the site of dermal inoculation. This generates a diffuse interstitial pattern of disease which eventually becomes bronchial and is clinically observable as progressive dyspnoea followed by the development of a soft cough. Radiography can readily discriminate these two forms of disease.



Disseminated disease can cause a range of clinical signs including hepato-splenomegaly, pleural and pericardial effusions, generalised lymphadenopathy, weight loss and pyrexia.

2. Non-tuberculous mycobacteriosis (NTM)

NTM agents are environmental mycobacteria found in numerous biotopes including the soil, water, aerosols, protozoa, deep litter and vegetation. Many species have been identified within this group but the most important clinically are members of the *M. avium-intracellulare* complex (MAC) which are most pathogenic in animals and are also potential zoonoses. This group of opportunistic pathogens typically infect cats via contamination of open wounds.

Historically the condition of **Feline Leprosy Syndrome** (FLS) was described globally. This was presumed to be caused by *M. lepraemurium* but this could not be confirmed due to the difficulties in culturing this organism. More recently, molecular techniques have demonstrated that *M. lepraemurium,* plus a range of other mycobacterial species e.g. *M. visible* can cause this clinical presentation. As a result of these developments there has been a trend in recent years to classify FLS cases as a subset of NTM infections rather than a unique clinical entity.

MAC infections have been seen more frequently in Abyssinian and Siamese cats, but there is no breed predisposition for the other NTM. Adult males that hunt are most frequently diagnosed and some studies have shown that the prevalence is highest in older cats, especially those with chronic kidney disease (CKD) and/or FIV infection.

Clinical Signs:

NTM is more variable in presentation than TB but generally results in;

i) (sub)cutaneous nodules (this group contains infections that can be cultured and others that, as yet, cannot and so were previously called FLS),

ii) granulomatous panniculitis or

iii) disseminated disease.

The cutaneous and disseminated forms can have very similar presentations to TB complex lesions (see above). Granulomatous panniculitis is characterised by multiple punctate drainage tracts and subcutaneous nodules which can coalesce to form large areas of painful, non-healing ulcerated skin. Affected cats are often pyrexic, anorexic and reluctant to move.

Canine Mycobacteriosis

There is little published on the incidence of mycobacterial infections in dogs, and most of the information about disease presentation comes from individual case reports. Anecdotally, the incidence of disease appears to be much lower in dogs than cats but the clinical severity is much greater. Dogs typically present with extensive GI disease (weight loss, vomiting and diarrhoea) and pulmonary pathology. They also seem more likely to be infected with TB complex pathogens

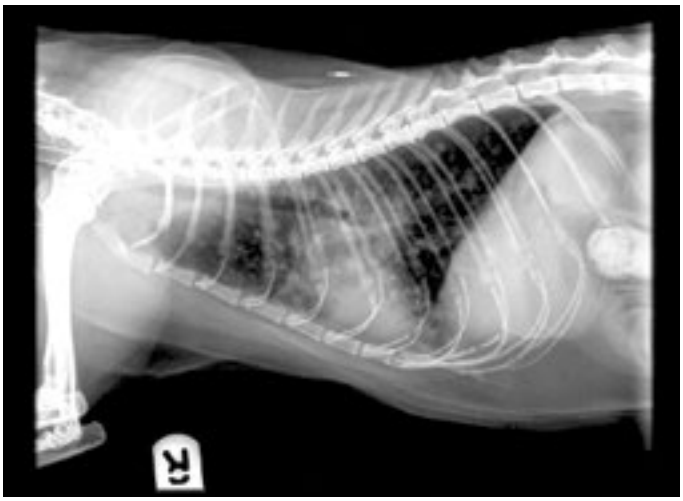
than other mycobacterial species. However, as with cats, the range of clinical signs is wide and has been known to include reticulo-endothelial, musculoskeletal, cutaneous and/or neurological signs.

In 2014, a case of **Canine Leproid Granuloma** (CLG) was diagnosed in Europe (Italy) for the first time. This condition had previously only been seen in Australia, New Zealand, Brazil and the USA. It is characterised by nodular skin lesions, typically seen on the head and dorsal pinnae, which spontaneously resolve over a period of weeks to months. The aetiological agent has yet to be identified but the histological appearance is consistent with granulomatous disease and intracellular ZN-positive bacteria are commonly seen, suggesting a mycobacterial species as the causative agent. Short-coated hunting dogs have been found to be predisposed and active hunting work has been shown to be a risk factor for disease. If CLG is diagnosed, cases require no pharmacological intervention.

Diagnosis of Mycobacterial Disease

A correct diagnosis of mycobacteriosis in companion animals is challenging to reach for a variety of reasons (discussed below), and it is imperative to remember that several aetiological agents can produce overlapping clinical signs but carry very differing prognoses, optimal treatment choice and zoonotic potential.

Cases that should warrant a high index of suspicion for mycobacterial infection would be skin nodules or abscesses that do not heal or are only partially responsive to antibiotic treatment, and/or chronic lymphadenopathy, particularly if the patient resides in the South West, South East or North of England, or Scotland.



Non-specific investigations:

During the clinical examination of any case of suspected mycobacterial infection, it is essential to fully establish the extent of any local disease and detect any cases with disseminated disease or systemic involvement.

Full serum biochemistry and haematology analyses typically only reveal non-specific changes e.g. a stress leucogram, but some changes such as anaemia and elevated serum calcium concentration can indicate more severe disease.

Radiography is useful for detecting systemic involvement, especially pulmonary dissemination, and for monitoring disease progression and treatment response. Whilst radiographic changes are variable, pathology is most frequently seen in the thorax, consisting typically of a diffuse interstitial, alveolar or bronchial pattern with perihilar and sternal lymph node involvement observed with increasing disease severity. It is important to note that no pattern is pathognomonic for mycobacteriosis and that observed lung pathology can be mixed. Similar findings are seen with the use of computed tomography (CT) imaging, though the sensitivity of detection is increased with this modality, and a diffuse structured interstitial lung pattern is most common, being either nodular or reticulonodular in nature.

Abdominal imaging, either using radiography or ultrasonography, can reveal hepatosplenomegaly, abdominal masses, mineralised or granulomatous mesenteric lymph nodes or ascites.

The FIV and FeLV status of cats should be established as a positive status is a poor prognostic indicator for therapy.

Specific investigations for suspected mycobacteriosis cases:

Histopathology is the first line of diagnostic investigation in these cases. The most typical approach taken is to surgically remove or biopsy a non-healing skin lesion, subcutaneous mass or enlarged lymph node. In some cases, if there is only a single cutaneous lesion, then this may prove curative. When specimens are submitted from cases where mycobacteriosis is a differential diagnosis, this should be clearly stated on the submission form, along with a request for ZN staining of tissue sections; this will ensure that the samples are handled safely by the receiving laboratory. Before submitting samples for histopathology it is ESSENTIAL to first section the fresh tissue, ideally in half, and only formalin fix ONE piece whilst keeping the remainder frozen in sterile containers.

Histopathology cannot speciate mycobacteria and this is essential to establish the risk to owners and other animals in the household. Culture can ONLY be attempted on fresh or fresh-frozen tissue samples. The authors recommend that this is the standard approach taken for all cats with skin nodules or lymphadenopathy.

Historically it was thought that histopathological features such as the nature of the granuloma formation and the number of ZN-positive bacteria present could indicate the likely species of agent present. However, ongoing research has shown that this is not the case and further diagnostic investigations must be undertaken to speciate the mycobacterium present.

Bacteriological culture is currently the 'gold standard' diagnostic test, although it fails in ~50% of attempts, even when ZN-positive organisms are present histopathologically. In addition, it can take a long time to culture some species, for example *M. microti* requires a minimum of 12 weeks to culture, during which time treatment is instigated based only on a presumptive diagnosis.

The APHA can undertake mycobacteriological culture although in most cases there will be a charge involved (this does depend somewhat on the region you are based in, and the background to the case, so it may be worth discussing this with your local APHA office). Please contact the laboratory prior to sample submission:

TB Diagnosis Section (SEB2)
Animal & Plant Health Agency
Weybridge
Wood Lane
New Haw
Addlestone
Surrey
KT15 3NB *Tel: 01932 357471*

Culture is also offered by regional Mycobacterial Reference Units and some commercial laboratories, for a fee.

The interferon gamma (IFN-γ) release assay (IGRA) has been adapted for use in cats and is available for use in dogs; however, test sensitivity depends on interpretation and can therefore range from 70-100%. The IGRA can indicate a likely causal species in cases infected with *M. bovis* or *M. microti*, and can be suggestive of MAC infection. Where ZN positive organisms have been identified histopathologically, but the test is negative, this indicates infection with a NTM (but not MAC) organism.

The IGRA test is run by Biobest Laboratories, Edinburgh. Please contact the laboratory prior to submitting samples to check the current sample requirements, price and turnaround times.

Biobest Laboratories Ltd.
6 Charles Darwin House
The Edinburgh Technipole
Milton Bridge
Nr Penicuik
EH26 0PY *Tel: 0131 4402628*

PCR diagnosis can be performed by Leeds Teaching Hospital. An initial test cost can tell whether or not a Mycobacteria is present or not, and identify TB complex from NTM species. Subsequent speciation of NTM is then performed at no additional cost by sequencing. Definitive speciation of a positive result for TB complex requires additional testing. This test is best performed on fresh tissue as it is not very sensitive, especially if there are few mycobacteria present, but can be attempted using fixed sections. Before submitting samples please contact:

Dr Deborah Gascoyne-Binzi
Principal Clinical Scientist
Leeds Teaching Hospital Trust
Department of Microbiology
The General Infirmary
Great George Street
Leeds
LS1 3EX *Tel: 0113 392 3929*

Tuberculosis Order: Identification of *M. bovis* is **NOTIFIABLE** to the AHPA in England, Wales and Scotland.

Management

Treating **canine mycobacteriosis** is challenging, very little evidence is available with regard to optimal treatment strategies and the prognosis is generally poor. For advice on treatment options please contact the University of Edinburgh Companion Animal Mycobacterial Disease team whose details are below.

For **feline mycobacteriosis** the prognosis is generally fair to guarded – depending on the extent of the infection and the species of mycobacteria involved. A study of 184 cases found that 40% reached complete remission (it is unclear whether or not this constitutes bacteriological cure) while the remaining 60% showed variable responses from temporary or partial remission to no response to treatment. However, many of these cases were treated with suboptimal drug regimens e.g. short courses of fluoroquinolone monotherapy or with beta-lactam antibiotics. More appropriate treatment would therefore lead to more favourable outcomes; anecdotally a positive outcome can be achieved in 60-80%

of cases of cutaneous *M. microti* or *M. bovis* infection, with or without pulmonary involvement. The prognosis is best when lesions are limited to the skin, while systemic disease and gastrointestinal signs at the time of diagnosis are negative prognostic indicators.

Treatment is always most successful when speciation of the causative agent has been achieved, so that it is possible to tailor drug regimens to known inherent antibiotic resistance and sensitivity patterns e.g. *M. bovis* is naturally resistant to pyrazinamide.

Zoonotic Risks:

Before beginning treatment, it is important to ensure that clients are fully informed of and understand the potential zoonotic risks associated with being in contact with an infected animal.

The greatest risk is posed by members of the TB complex of mycobacteria:

- ***M. tuberculosis:*** Though rare in the dog and not previously reported in the cat, infection of a companion animal with *M. tuberculosis* would be considered a significant zoonotic risk. Finding an infected companion animal should trigger a search for the infecting human. Infected companion animals should be euthanased.

- ***M. bovis*:** Currently, only ~1% of human TB cases in the UK are caused by *M. bovis* infection. Globally, in the last 150 years, only six cases of human *M. bovis* TB have resulted from exposure to cats, and where infection has occurred the cats have had skin lesions that were draining pus with many ZN- positive bacteria. As a result of this, as of Sept 2015, Public Health England, Public Health Wales and Health Protection Scotland all consider the risks to humans to be "very low". That said, the risk is still present and should be considered seriously in the context of humans with specific risk factors for transmission (see below) and the clinical signs present. Extensive and/or purulent lesions pose the greatest risk to human health and are generally less responsive to treatment. By comparison, single non-ulcerated skin lesions and/or regional lymphadenopathy may be very amenable to treatment.

- ***M. microti:*** The risk to humans of *M. microti* is significantly lower than that of *M. bovis.* A total of 27 human cases of *M. microti* infection have been documented in published literature and 11 (40%) of these had specific risk factors (see below). None were shown to have resulted from exposure to an infected cat.

- **MAC:** Whilst not members of the TB complex, this group of organisms can infect humans in the presence of specific risk factors (see below) – again, none have been shown to have resulted from exposure to an infected cat.

Specific risk factors for zoonotic transmission: This list has been compiled from advice published by all UK public health organisations and WHO guidelines. Humans are considered at heightened risk if they:

- Are under five years old (some sources suggest 12 years)
- Are pregnant
- Are HIV positive
- Suffer from substance abuse
- Have been diagnosed with diabetes mellitus
- Suffer (severe) kidney disease
- Have ever received an organ transplant
- Are a cancer patient receiving chemotherapy or radiation therapy
- Have any medical condition requiring treatment with systemic corticosteroids
- Require specialized treatment for rheumatoid arthritis or Crohn's disease

In any of the above situations, the authors would discourage animal treatment and suggest that the pet should be euthanased. If you would like to discuss any case, please contact the University of Edinburgh team (see below).

Treatment:

If treatment is to be attempted it requires a prolonged course of antibiotic therapy to achieve successful clinical resolution; owner and patient compliance, drug toxicity, and cost can make this difficult to maintain. For *M. bovis* and *M. microti* the recommended treatment consists of "triple antibiosis" including rifampicin, a fluoroquinolone (ideally pradofloxacin) and a macrolide (typically azithromycin) daily for a minimum of three months and for two months beyond the resolution of clinical signs. Where there is secondary pulmonary involvement, as diagnosed by radiography or CT then treatment is extended to a minimum of six months and for two months beyond the resolution of clinical signs. Some cases of NTM infection with extensive cutaneous disease have required up to a year of treatment so owners must be aware of the potential commitment required to achieve a successful outcome.

Please contact the University of Edinburgh Companion Animal Mycobacterial Disease team to discuss treatment of individual cats and dogs; drug protocols, dosages, tips for prolonged administration, and patient monitoring.

University of Edinburgh Companion Animal Mycobacterial Disease Referral and Advice Service:

The Royal (Dick) School of Veterinary Studies, University of Edinburgh, has a growing team of vets and scientists actively treating and researching companion animal mycobacterial diseases. The team is headed by Professor Danièlle Gunn-Moore who has extensive experience and expertise in this field.

The team would actively encourage anyone suspicious of, or actively dealing with, any such case to contact them to discuss any aspects of diagnosis, management and treatment.

Please Help Us to Help You:

The team have unanimously taken the decision not to use experimental animals in their study of these diseases, and therefore rely on the generosity of referring vets and clients to make them aware of cases and provide them with clinical samples at any opportunity. We are always looking for case photographs, blood tube remnants, fixed and fresh frozen tissue samples, fixed tissue blocks from histopathology labs, etc. Only with the generosity of vets and clients can the team continue to develop faster, cheaper, more accurate diagnostics and undertake studies to understand these diseases.*

Contact:

Prof Danièlle Gunn-Moore
Companion Animal Mycobacterial Disease Referral and Advice Service
Royal (Dick) School of Veterinary Science & the Roslin Institute
Easter Bush Campus
Edinburgh
Midlothian
EH25 9RG *Direct Email:* companion.animalTB@ed.ac.uk

This inbox is checked by the team multiple times a day to provide timely replies to enquiries, please provide as much information as possible. Please include a phone number and useful times to call if you wish for us to get back to you by phone.

* When sending any samples (particularly FRESH or FRESH FROZEN tissue) from possible or confirmed cases please be aware that, like all biological samples that potentially contain pathogens, these are Category B UN3373 samples and must be packaged appropriately to ensure safe handling.

Full details can be found at: http://www.docs.csg.ed.ac.uk/Safety/bio/guidance/transport/summary_catB.pdf

Briefly; packaging must be in good condition and samples must be contained within leak-proof primary and secondary receptacles with absorbent material in between (sufficient to absorb the total volume of liquids such as blood in the event of a leak). Outer packaging must be rigid and be labelled *Biological Substances; Category B UN3373*.

It is advisable to contact the team prior to any such samples being sent.