# A Review of Avian Mycobacteriosis

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## Characteristics of Mycobacteria

Mycobacteria are aerobic, gram positive acid-fast cells with a thick cell wall rich in mycolic acids. They are hydrophobic and slow growing with a division cycle from a few hours to 20 days. They are widespread in the environment and are typically found in damp shaded soil and air/water interfaces such as the surface of still water. Mycobacteria are able to survive low pH and can pass undamaged through the gastrointestinal tract (GIT) of animals. [5] They are resistant to many disinfectants and survive drying. The cells remain viable for months to years when dried. They are acid-fast staining with the Ziehl-Neelsen method most commonly used.

## Classification and Taxonomy

There are over 70 species of mycobacteria. Some important examples for veterinarians are listed:

| Organism | Classic Disease Caused |
|---|---|
| M. tuberculosis | Human TB |
| *M. bovis* | Bovine TB |
| *M. avium avium* | Avian TB |
| *M. avium paratuberculosis* | Johne's disease |
| *M. leprae* | Human leprosy |
| *M. lepraemurium* | Feline leprosy |
| *M. marinum* | Piscine TB |

In the past, various species of mycobacteria were grouped according to the diseases caused, the species affected and the inability to differentiate them in the laboratory:

### *M. tuberculosis* Complex

- **MAC complex** – Mycobacterium Avium Complex: includes 28 species (formerly referred to as serotypes) including *M. avium avium* and M. avium paratuberculosis.
- **MOTT** – Mycobacteria Other Than Tuberculosis

Mycobacteria have also been classified according to their growth characteristics in culture. Generally, the infectious and highly pathogenic species are slow growing in culture. Environmental species tend to be

faster growing.

### The Immune Response to Mycobacterial Challenge

The immune response to mycobacteria has been described as either TH1 or TH2, based on the T-helper lymphocyte that is predominant. A TH1 response results in activation of macrophages. A TH2 response results in the production of antibodies, while macrophages are not activated. When many mycobacterial cells are initially encountered, or when the host has been previously exposed to mycobacteria, a TH2 response is likely.

Non-activated macrophages are ineffective at killing mycobacteria as the bacteria, once intracellular, are able to switch off the cells killing mechanisms. This is significant due to the high chance of animals being exposed to non-pathogenic environmental mycobacteria prior to infection with pathogenic species – thus a TH2 response is more likely.

While only limited studies have been completed in birds, it is thought that similar immune responses are involved. [3,8]

### Sources of Infection to Birds

In birds, most infections occur via the oral route. The source of the bacteria may be either environmental or from ingested material contaminated with faeces from infected animals. Faecal contaminants can be spread widely by wind after drying, or in water supplies. Vectors such as earthworms and insects may also transmit pathogenic mycobacteria to previously "clean" environments. [5] High concentrations of mycobacteria may be found in wetlands. Carnivorous birds may contract the disease by consuming infected prey.[1]

Less commonly, infection can also occur via skin wounds or inhalation.[16]

### Types of Avian Disease

It is likely that all bird species are susceptible to infection by mycobacteria; however varying levels of susceptibility occur and four levels of susceptibility have been described[9] :

- **Highly susceptible** – including domestic fowl (*Gallus domesticus*), sparrows (*Passer sp*), grey partridges (*Perdix perdix*), wood pigeon (*Columba palumbus*), quail (*Coturnix japonica*), canaries (*Serinus canarius*), and budgerigar (*Melopsittacus undulatus*);

- **Less susceptible** – including guinea fowl (*Numida meleagris f. domestica*), and domestic turkeys (*Meleagris gallopavo f. domestica*);

- **Moderately resistant** – including domestic goose (*Anser anser f. domestica*) and domestic duck (*Anas platyrhynchos f. domestica*); and

- **Highly resistant** – including domestic pigeon (*Columba livia f.domestica*), collared turtle dove (*Streptopelia decaocto*), and rook (*Corvus frugilegus*).

Typically, following ingestion and primary GIT colonization, haematogenous spread results in widespread colonization of body tissues.

In birds three forms of mycobacteriosis occur: classical, paratuberculous and diffuse.[9]

- **Classical disease** takes the form of discrete tubercles in various organs. This form is less common

in birds than in mammals.

- **Paratuberculous** lesions are less defined granulomatous areas often in the liver and GI tract. This is the most common form of disease in birds.
- **Diffuse** infection does not generally result in grossly identifiable lesions, rather a diffuse enlargement of organs. This form is common in some bird groups such as passerine birds.

## Mycobacteriosis for Avian Groups

### Pets

Psittacine and passerine birds are typically infected with either *M. a avium*, *M. genavense* or occasionally other species such as *M. tuberculosis* . While there have been cases documented of human to bird transmission, most infections in this group are contracted from the environment. [16] Disease prevalence has been estimated between 0.5 – 14% and infections tend to be sporadic affecting single individuals. Brotogerid parakeets (*Brotogeris spp*) appear predisposed. [9,16]

### Zoo Collections

Many zoo collections are affected by mycobacteriosis. Susceptible groups include the Galliformes (eg: pheasants, partridges and quail), Gruiformes (eg: Cranes, Rails) and Anseriformes (waterfowl). [4,18] High rates of infection result from a combination of heavy environmental contamination and exposure (in many cases due to cage substrates that are suitable growth media for mycobacteria), overcrowding, stress and poor nutritional health. Eradication of mycobacteria from an infected display is probably impossible. Attention to all of the above factors and the selection of resistant display stock reduces the incidence.

### Endangered species and wild populations

Mycobacteriosis has accounted for 84% of the adult mortalities in the captive breeding program of the white winged duck (*Cairina scutulata*) – an endangered species native to South-East Asia. Thus it represents a significant threat to the survival of this species.[3,19]

Epidemics in wild populations have occurred however most cases are sporadic. Several outbreaks have affected Kenyan lesser flamingos (*Phoenicinaias minor*) – one of which lead to the death of 18,500 birds. In these outbreaks there were other causal factors associated (malnourishment, other disease) so it is likely that immunosuppression was responsible for such high morbidity and mortality.[14]

### Production flocks

The widespread use of closed farming practices and the early slaughter of stock has all but eliminated mycobacteriosis as a cause of poultry production loss in developed countries. However it is a significant cause of losses in developing countries. The move towards free-range farming in developed countries may affect the incidence of disease as stock will be again be exposed to wild birds and fomites suitable to support the growth of mycobacteria.

## Zoonotic Infections

Transmission of mycobacterial infections from human to bird (*M. tuberculosis*) and from bird to human (*M. a. avium*) has been reported. The incidence of MOTT in humans has increased with the incidence of HIV infection and the resulting immunosuppression. Increased use of DNA typing of the mycobacterial species in these cases has shown that most infections are caused by environmental mycobacteria such as *M. genavense*, rather than *M. a avium.* [11]

Caution should be exercised where infected companion birds are found and wherever possible, the species

of mycobacteria identified. The highest risk humans are those with immunosuppressive conditions such as AIDS or chemotherapy treatment, the very young and the very old.

The routine euthanasia of infected pet birds cannot be supported on the basis of zoonotic risk. Euthanasia should however be considered when *M. a avium* is identified.

## Diagnosis of Mycobacteriosis

***Signs***

While there are no pathognomonic signs in avian mycobacteriosis, a history of weight loss while maintaining appetite is suggestive. The presenting sign may however affect the GI, respiratory or other body system. Signs may include:

| | |
|---|---|
| Voluminous faeces | Change in or loss of voice |
| Vomition | Lameness |
| Dysphagia | Limb swelling |
| Dyspnoea | Abdominal distension |
| Cough | Sudden death |

Granulomatous ocular or dermal lesions are sometimes seen. [9,16]

***Faecal ZN Test***

This is one of the simplest investigative methods available however it lacks both sensitivity and specificity. The bacteria may only be present in small numbers and other non-pathogenic acid-fast bacteria may be present. However a finding of acid fast bacteria in serial faecal samples should prompt further testing. [15]

***Serology***

The demonstration of anti-mycobacterial antibodies has been used in several avian species and by various methods. Rapid agglutination, complement fixation, western blot and haemagglutination inhibition can be used. [4,20,21] All these methods are highly species specific and are only available for a limited number of species.

Results are mixed - however, in some species (such as the ring-necked dove *Streptopelia risoria*) the test has proved highly sensitive and specific. [20] In some species, the production of antibodies is variable depending on the stage of disease resulting in false negative serological results – particularly in late stage disease.

In cases where an outbreak is detected within a valuable patient group, development of these tests may be practical and useful for long term monitoring.

***Intradermal Testing***

Intradermal testing similar to that used in mammals has been used extensively by the poultry industry to identify and cull infected stock. An intradermal injection of 0.1ml of avian tuberculin in generally given into one wattle. Swelling observed after 48 hrs is considered a positive result. The test has limited sensitivity in poultry but has been useful as a cost-effective screening test. In other bird species however the results are even less reliable. [21]

***Cytology***

In the case of superficial lesions accessible without surgery, scrapings and aspirates can be examined with Ziehl-Neelsen staining.

***Culture***

Culture of mycobacteria from an infected lesion has always been considered the gold standard test and is still extensively used. Compared with all other test options, culture has been shown in most studies to have the greatest sensitivity for identifying infected individuals. [15,20,21] The slow growth of most pathogenic mycobacteria is the main limiting factor (2 – 24 week culture times are required) and in some cases failure to culture the organism can occur. Culture techniques are specific and the laboratory should be informed that mycobacteria is suspected. Identification of the species is not possible on culture alone.

***Radiography***

As many cases of mycobacteriosis result in organomegally (in particular liver, spleen and intestinal loops), and in some cases discrete granulomas, radiography is a useful tool. Radiographic changes are not specific, however. Avian granulomas do not form a calcified centre as in mammals. Osteomyelitis caused by mycobacteria can be identified by radiography.[10]

***Laparoscopy/Exploratory Surgery***

Granulomatous lesions are often identifiable visually. Laparoscopy allows viewing of the tissues typically affected. However, many of the species seen most commonly in practice often do not develop these lesions. [9,16] Laproscopy does allow biopsy samples to be collected in the least invasive way.

***Haematology***

Results from CBC evaluation vary depending on the stage of the disease. A marked monocytic leucocytosis is consistent with mycobacteriosis but is not always seen. Heterophilia or thrombocytosis can also be seen. [16]

***Serum/Plasma Chemistry***

Elevated liver enzymes would be expected in cases of hepatitis however this is not consistent. Elevated globulin and decreased albumin levels may be present. Protein electrophoresis will sometimes reveal a polyclonal gammaglobulinopathy[16].

***Histology***

The demonstration of acid fast bacilli within granulomatous inflammation on histology from infected tissues is definitive for mycobacteriosis. However, as lesions and bacteria are not evenly distributed, false negative results are possible.

***PCR/DNA Probe***

The development of DNA probe and PCR techniques has revolutionized our understanding of mycobacteriosis in avian species. Many infections that would once have been termed MAC are now being shown to be environmental mycobacteria that pose little or no threat as zoonotic or infectious agents.

Whenever possible, samples from tissues diagnosed by histology should be submitted for PCR typing. Primers specific to various mycobacteria have been developed for human use and these tests are now widely available. PCR can be performed on fresh tissue, formalin fixed tissue, wax blocked tissue or cultured samples. [20] Sensitivity is greatest from cultured samples and false negative results from tissue samples do occur due to low numbers of bacteria in some samples.

## Prevention of Mycobacteriosis

There are two issues relating to disease prevention – preventing exposure to the infectious agent, and ensuring the at risk animal is healthy and immunocompetent.

***Preventing Exposure***

In any environment, other than a closed, pathogen-free establishment, it is not possible to completely avoid exposure to environmental mycobacteria as they are endemic in soil and many water supplies. Levels can be minimized by avoiding heavily contaminated water supplies and soil substrates in bird enclosures. Concrete provides the most practical flooring for cleaning purposes. Mycobacteria can be transported into an enclosure by wild birds or vectors such as earthworms, [5] so prevention of entry by these animals or their faeces is important. One of the main reasons for the eradication of mycobacteria as a cause of poultry production losses in developed countries is the use of such a closed environment.

Other methods by which birds may be exposed include contact with other infected birds or infected humans. Quarantine of incoming birds should include consideration of the risk of mycobacterial carriers. Incoming birds may be screened by any of the methods listed earlier. Routine faecal culture, laparoscopy and liver biopsy are likely to be required to all-but eliminate this risk.

***Immunocompetency***

In the vast majority of cases of avian mycobacteriosis, concurrent factors play a part in the development of disease. Stress, other illness, nutritional or degenerative changes will increase the risk of infection. [14] It is therefore important to focus on these issues as a major part of any mycobacteriosis prevention scheme.

***Vaccination***

Work is ongoing on the production of a mycobacterial vaccine for birds. Trials to date using BCG have failed to produce protective responses. Inoculation with *M. vacciae* has produced limited protective responses in perching ducks (*Cairini spp*) with reduced mortality in a group of vaccinated birds. [3]

One of the limiting factors in vaccine trials to date is the inhibition of a vaccine response seen in birds already exposed to high levels of mycobacteria. [3]

Currently research on the use of DNA vaccines is encouraging in mammals and fish. Plasmid DNA encoding for the Ag85A secreted fibronectin-binding protein of mycobacteria has been injected. The recipient's cells then manufacture this protein. Both antibody and TH1 responses were seen following use of this vaccine in mammalian trials and improved survival rates post-challenge have been seen in fish. [17]

## Treatment Options

Due to their cell wall structure, intra-cellular growth and slow replication cycle, medical therapeutic regimes to treat mycobacteriosis must be specific, aggressive and prolonged. Some antibiotic agents are unable to penetrate the cell wall without the synergistic activity of other agents. [23] It is typical to treat cases for 4-9 months and ideally treatment should be continued for several months past the last positive biopsy or culture result. However, given the sensitivity of culture and the low numbers of bacteria in treated patients, it is questionable whether this is of value.

Mycobacteria rapidly develop resistance to single agent antibiotic treatment so in all cases, combination regimes should be used. [2,6,9,16] In most cases a triple agent regime is used. Many of the drugs are compatible when mixed and can be formulated into combination liquids or pastes that are more practical to administer.

The host immune response to mycobacteria results in an aggressive inflammatory reaction within infected tissues. This is often unproductive and may exacerbate symptoms. In human leprosy, corticosteroids have

been used to reduce this reaction. Given the poor safety of corticosteroids in birds it is unlikely that these drugs would be useful in avian mycobacteriosis. Clofazamine has anti-inflammatory properties which may be useful although assessing the need for this option is challenging. [23]

The following drugs have activity against mycobacteria: [9,13,16,23]

1. Isoniazid – effective against *M. bovis* and M. *tuberculosis* but less so against MAC and MOTT.
2. Rifampin/Rifabutin – bacteriocidal. Inhibits DNA dependant RNA synthesis
3. Ethambutol – interferes with incorporation of mycolic acid into the cell wall so allows other agents to penetrate the cell wall.
4. Azithromycin/Clarithromycin – bacteriostatic.
5. Fluoroquinolones
6. Aminoglycosides – bacteristatic, limited usefulness due to toxicity.
7. Clofazamine – antibiotic with anti-inflammatory properties
8. Doxycycline – limited efficacy against MAC.
9. Cycloserine

Most recommended regimes incorporate rifampin, ethambutol and another agent. Several examples are shown:

- Rifabutin 15mg/kg/Ethambutol 30mg/kg/Ciprofloxacin 80mg/kg – Q 24hrs.

- Rifampin 45mg/kg/Ethambutol 30mg/kg/Clofazimine 6mg/kg – Q 24hrs *Clofazimine has anti-inflammatory properties which may be helpful with severe inflammatory lesions.* [23]

- Ethambutol 20mg/kg/Cycloserine 5mg.kg/Enrofloxacin 15mg/kg – Q 12hrs + Clofazimine 1.5mg/kg – Q 24hrs.
  *Recommended regime for raptors.*[12]

Given the propensity for antibiotic treatment to cause secondary dysbiosis and fungal overgrowth, regular monitoring of faecal samples is needed. Antifungal medication may also be required during treatment.

Surgery should be considered for discrete nodules in the skin or other accessible areas. Complete excision may be curative in these cases.

## Drug Resistance

In human TB treatment, drug resistance is an ever-increasing problem. This is primarily due to the combination of HIV prevalence (and the inherent difficulty of curing mycobacterial infections in these patients) and compliance breakdown with treatment. [2,6]

Detection of resistance can be achieved by either culture methods or DNA screening. [2,6] Phenotypic antimycobacterial susceptibility testing is a slow process due to the delays in culturing these bacteria. DNA screening methods have been developed to detect *M. tuberculosis* gene mutations responsible for resistance to various drugs. The sensitivity of these tests compares favourably with culture methods.

Compliance with long term therapy is also a major limitation in the treatment of avian patients and it should be expected that drug resistance will develop in these patients also. Susceptibility testing has not been described for avian patients but would be possible for infections with *M. tuberculosis*.

## Summary

To date there are very few reports of successful treatment of mycobacteriosis in birds with the major focus

historically on control and prevention. The recommendation to euthanase all avian patients affected has been typical – on the basis of a perceived grave prognosis and high zoonotic and infectious risk. With the advent of new diagnostic and treatment modalities this stance must be reviewed. In an immunocompetent patient, successful treatment is possible. Patient selection is crucial however. Owners who are committed to the high cost and prolonged treatment are required. Compliance breakdown will inevitably lead, not only, to a poor outcome but also to further antibiotic resistance. In many cases euthanasia will still be a sensible recommendation.

Ongoing work in the area of rapid, reliable diagnostic tests and effective vaccination will hopefully improve the situation for captive zoo populations – the worst affected group of patients.

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