

A Review of the Management of Avian Mycobacteriosis in Zoological Collections



Jaclyn Gatt BVSc MVS (Conservation Medicine)
Bird and Exotic Animal Clinic
19 Ponting St, Williamstown VIC 3016
jaclyn.gatt@gmail.com

Introduction

Mycobacteriosis is the term used to describe the classic granulomatous disease seen in a number of species including birds, mammals and fish caused by a variety of mycobacterial infections. Avian mycobacteriosis (AM) is commonly associated with *Mycobacterium avium* subspecies. *avium* or *Mycobacterium genavense*, with the organism varying amongst avian species (reviews by Manarolla et al., 2009; Shivaprasad and Palmieri, 2012). The well known causative agent behind bovine Johne's Disease, *Mycobacterium avium paratuberculosis*, is very closely related to *M. avium* subsp. *avium*, allowing some extrapolation of data regarding its environmental stability where specific *M. avium avium* data are lacking (Collins, 2003). A number of papers exist that discuss the diagnosis, treatment, pathological findings and, more recently, the risk factors involved with this disease in a zoological setting (Witte et al., 2008; Witte et al., 2010; Lécú et al., 2011). This paper aims to provide a discussion on the diagnosis of avian mycobacteriosis and the necessary features of a management plan for the disease.

Physical Findings

A standard diagnostic approach should begin by reviewing the history of the animal and completing a thorough physical examination. History may reveal that the bird has come from a source with a history of mycobacteriosis or enclosure where mycobacteriosis has been detected in other birds. Signs of mycobacteriosis are generally not specific. Birds generally have a moderate to marked loss of pectoral muscle mass. Bodyweight alone is unreliable as an indicator of chronic disease, as in some patients an accumulation of coelomic fluid or granuloma formation may increase mass despite an overall loss in muscle mass and body condition, masking true weight loss (Lennox, 2007; Saggese et al., 2008). Masses around

the face or subcutaneous masses elsewhere are occasionally seen. In some instances coelomic fluid can be palpated and in other cases hepatomegaly can be detected on palpation. Bone lesions occur commonly in birds infected with mycobacteria and in a moderate number of cases affected birds will present with lameness.

Blood and Serum

Haematology and biochemistry results can range from unremarkable to markedly altered, depending on the organs affected by mycobacterial granulomas. Mild anaemia and a leukocytosis with a heterophilia and monocytosis may be seen reflecting chronic, non-specific disease with mild generalised inflammation (Tell et al., 2001; Saggese et al., 2010). Changes on biochemistry may include an elevated AST, bile acids or high CK. These are also non-specific changes though may be associated with hepatic disease in some cases (Saggese et al., 2010).

Serological assays can be used to identify infected birds, however the interpretation of these results will vary depending on the avian species. Cromie et al., (2000) attempted a vaccine trial using killed *M. vaccae* given to the endangered white-winged duck (*Cairnia scutulata*), in an effort to confer immunity against *M. avium* subsp. *avium*. The vaccine provided no protection, and they suggest that chronic exposure to non-pathogenic, environmental mycobacteria may have reduced the ducks' ability to mount an effective cell-mediated response when faced with a pathogenic variety later in life. Cellular-based assays such as intradermal tuberculin tests have only been validated in domestic poultry, and despite numerous trials being undertaken in other avian species this remains an unreliable method of diagnosis (Tell et al., 2001).

Faecal Analysis

Faecal cytology can be easily and cheaply utilised to detect acid-fast bacteria with a Z-N stain (Tell et al., 2003; reviewed in Brandão and Beaufrère, 2013). Faecal samples are typically collected during enclosure cleaning so caution must be taken to ensure environmental *Mycobacterium* do not contaminate samples, and risk giving false positive results regarding the actual birds health status. This method relies on the bird to be actively shedding organisms into their gastrointestinal tract (Lennox, 2007); it risks not detecting disease from birds that shed very low levels of bacteria and do so only intermittently. Perhaps the greatest limitation of faecal screening is that it would fail to detect birds with lesions located outside of the intestinal tract, such as in the spleen or liver. Faecal cytology thus provides very poor sensitivity, with one study of experimentally infected Japanese Quail having less than 53% positive results even with end stage mycobacteriosis (Tell et al., 2003). Tell et al. (2001) also put forward that the use of the Truant stain for evaluation of acid-fast bacteria may be more sensitive when compared to Z-N stains, as the Truant stain causes bacteria to fluoresce against a dark background, enabling easier identification of lower numbers of bacteria. It must be noted however that due to a small sample size (n=8) further comparative studies are needed. Despite the convenience of using faecal samples for testing, a stand alone result is highly unreliable. There may be some value in utilising serial faecal samples as a component of an ongoing screening program however, as the sensitivity may improve slightly with repeated sampling.

Imaging

Survey radiographs (whole body VD and lateral views) should be taken to allow thorough evaluation of the musculoskeletal system and relative size and position of the viscera. If present, mycobacterial granulomas in the long bones may appear as osteolytic lesions (reviewed in Dahlhausen et al., 2012). The cardio-hepatic silhouette should be evaluated for size, with hepatomegaly being a common but again non-specific finding for many birds with mycobacteriosis (Lennox, 2007). Bloods and radiographs have the advantage of being relatively non-invasive procedures, however the radiographs will require a brief general anaesthetic.

Endoscopy is an excellent screening and diagnostic tool that allows visualisation of multiple organ sys-

tems and simultaneous biopsy collection (reviewed in Dahlhausen et al., 2012). Disadvantages include limitations due to small patient size and the invasive nature of the procedure. The issue of mycobacterial lesions in the liver, spleen or lungs presents a major challenge for antemortem diagnosis, some of which may be overcome by use of endoscopy. Viscera are viewed in situ and lesions may be visualised. Tissue biopsy of lesions and grossly normal tissue is desirable, though even this result may present false negatives if haematogenous spread has not reached the particular organ being sampled (Saggese et al., 2008).

To maximise the value of endoscopically acquired biopsies, Saggese et al. (2008) suggest collection of multiple samples for histological and cytological evaluation, PCR and culture. A study by Saggese et al (2008) compared a number of diagnostic techniques and concluded that multiple hepatic biopsies are likely to be most useful as an antemortem diagnostic tool. This study examined naturally infected Ring-necked Doves (*Streptopelia risoria*) and found a predilection for the spleen in this species. These are just two examples of different clinical expressions of disease caused by *M. avium* in different avian species, and highlights the need to understand the unique biology of the species of bird before undertaking a diagnostic investigation.

In larger birds, ultrasound may provide additional information as a follow up to screening radiographs, as it offers a method of assessing soft tissue architecture and characterising lesions. This technique has major skill and technical limitations and requires high quality equipment, making it one of the less practical options. Other advanced imaging techniques such as CT or MRI may be extremely useful for lesion localisation, however many institutions and veterinary practices would not have these on-site and transport of birds to them would be impractical for general screening, not to mention prohibitively expensive.

Postmortem Findings

On gross necropsy birds are generally thin to emaciated. A fibrinous exudate may be present in the coelom of some species, particularly doves. Most birds have both hepatomegaly and splenomegaly. The liver can be diffusely enlarged and discoloured or may contain granulomas. Granulomas are the most common finding in birds with mycobacteriosis and, in addition to the liver, are commonly found in the spleen. They can also be found in consistently

in a number of other organs (Palmieri, 2013). If the intestine is diffusely infected then the mucosa will be thickened and may have a cobble stone or “shaggy carpet” appearance as the result of histiocyte infiltration into the lamina propria (Dahlhausen et al., 2012; Palmieri, 2013). Acid-fast organisms can be seen on histological sections or impression smears of diseased organs stained with Ziel-Neelsen (Z-N) to identify acid-fast bacteria (Shivaprasad, 2012). In some instances organisms will be abundant and in others they will be rare and difficult to find.

A Screening Program

Careful daily observation and monitoring by keeping staff should form an important part of any animal health management program, as in a zoological setting these professionals take on the role of the pet owner and are responsible for noticing subtle changes in the bird’s behaviour, eating habits or movement patterns. Other methods to maintain optimal health in the collection animals are not always so structured, but can be implemented based on the needs of the species or group of animals under consideration.

To enable screening for AM to occur in the collection birds at the zoo, one option is to consider introducing routine scheduled health checks. Using a standard set of guidelines a brief physical exam, weight and body condition score can be recorded with relatively low stress to the bird. This information can be logged to provide baseline data on the individuals normal values. When sick birds are identified, they should be rapidly isolated and placed away from the main flock. A minimum database should be collected for sick birds following examination under anaesthesia, including a physical exam, radiographs, haematology and biochemistry, faeces for PCR and cytology using a Z-N and/or Truant stains (Lennox, 2007; Tell et al., 2003; Dahlhausen et al., 2012). Given the limitations of individual diagnostic tests, obtaining samples from various sites and using multiple testing modalities should maximise the probability of an accurate antemortem diagnosis of mycobacteriosis (Saggese et al., 2008).

There is likely to be little to no advantage to carrying out the tests listed in the ‘sick bird’ database on all healthy birds caught up for routine health checks. The majority of diagnostic tests available are of low sensitivity and specificity, even in animals known to be experimentally infected with *Mycobacterium* (Tell et al., 2003). Secondly, there is going to be a degree of increased stress associated with capturing,

restraining and anaesthetising any bird, including a healthy one (Schrenzel et al., 2008).

Quarantine

Based on our knowledge of the epidemiology of *Mycobacterium spp.*, a minimum of 3-6 months in quarantine on arrival at the zoo is recommended. During this time the birds should be closely observed for dyspnoea, weight loss, abdominal distension, diarrhoea, polyuria, anorexia, poor feathering or general ill thrift (reviewed in Lennox, 2007; Palmieri, 2013). Sick birds should be isolated if housed with an incoming flock, subject to the sick bird screen discussed previously, and the remainder of the flock scrutinised closely. For birds in quarantine who continue to look healthy, faecal samples should be collected fortnightly for PCR and cytological testing. By utilising a test with typically low sensitivity in series, it is hoped that the sensitivity will be increased, thus increasing the chances of detecting any shedding or mycobacterial organisms (Tell et al., 2003).

A dilemma arises when a positive sample is detected on faecal examination. Typically pooled faecal samples are used making identification of the shedding individual bird or birds difficult. The group should be closely observed to attempt to identify subtle signs of illness and weight and body condition scores measured. If no clear culprit can be identified then a management decision on whether to cull the group and source new stock, or persist with identifying unwell individuals, must be made based on the conservation status and breeding value of the species.

Following release from quarantine into their enclosure, increased vigilance and monitoring is advised for at least the first three months in case any animals were incubating mycobacteriosis but were non-clinical for the duration of their quarantine. This second move from quarantine to a new exhibit with new cage mates may be a stressful time and could trigger a disease event.

Formulating a Management Plan

In a zoological setting where many of the birds housed are endangered or part of species conservation breeding programs, effective disease management tools that aim to preserve individual birds and their genetic value are of the utmost priority. For a disease such as mycobacteriosis where the control has typically focused on bird to bird transmission, and thus eliminating the positive birds and those

around them to reduce spread of disease, this poses a very real and practical dilemma. Although it is recognised that both aerosol and faeco-oral routes are important for disease transmission (reviewed in Lennox, 2007; Brandão and Beaufrère, 2013). Schrenzel et al. (2008) proposed that indigenous environmental mycobacteria are an even more important source of infection a zoo setting. It is also acknowledged that wild birds may become infected with mycobacteria, however numerous studies have concluded that these birds are unlikely to be a significant source of new infection in birds housed in zoological collections (Tell et al., 2001; Witte et al., 2008).

To best screen for, manage and control this disease we will go back to the key principles of epidemiology and approach it from three main aspects; the host, the environment and the pathogen.

The Avian Host

The ability to closely monitor an individual animal will depend on the housing arrangements of the bird, in particular the enclosure size or type (e.g. open flight aviary vs smaller aviary). The enclosure design may limit the ability to collect samples from a specific individual, making follow up on positive test results problematic. In order to reduce stress on the birds it is essential to avoid overcrowding and ideally reduce the number of species being mixed in each aviary (Witte et al., 2008). The latter recommendation is not always compatible with curatorial decisions however as mixed species exhibits are popular with the public, provide interesting interactions and are generally aesthetically pleasing. Once birds have become established in their aviaries it is preferable to limit unnecessary handling and reduce movements between enclosures to help reduce stress. (Witte et al., 2008)

Care of each bird should include a comprehensive health screen on arrival as part of quarantine procedure, an established parasite prevention program and providing tailored nutrition according to the species. Diet reviews should be conducted to ensure the most current literature is being accessed and a balance of fresh and commercial food plus supplements (such as Wombaroo®) are utilised as required. It is hoped that by keeping stress to a minimum and providing high levels of appropriate nutrition that individuals will be equipped to maintain their immune system at an optimal level (Griffin, 1989).

The role of genotype in determining how well a

hosts immune system is able to evade and defend against mycobacterial pathogens has been proposed as one reason for inter-species differences in disease susceptibility. The study of Saggese et al. (2008) looked at a captive population of ring-neck doves (*Streptopelia risoria*) and compared phenotypic traits (feather colour) with necropsy lesions to determine if a difference in lesion distribution existed. They go on to propose a potential link between the genes determining colour and those that modulate the immune response to mycobacterial infection. This concept needs far greater research to be understood, however does provide yet another reason to preserve genetic diversity in captive populations.

While monitoring and quarantine procedures are crucial to disease management, care should also be taken to ensure that any newly acquired birds are being sourced from reputable source. Witte *et al* (2008) looked at retrospective data from avian mycobacteriosis cases at the San Diego Zoo facilities and found that the majority of newly diagnosed cases were in birds imported from external sources. It is worth noting that not much was known about the previous housing or health conditions of these birds prior to their arrival at the zoo, so it was not possible to differentiate between pre-existing, chronic disease, those who acquired infections from shedding cage mates due to the stress of transport or other unknown exposure factors. As such, every effort should be made to attempt to source birds from facilities with equal or higher health status and protocols for this disease, reputable breeders with known medical information on their flocks or other zoos (provided their disease status is known). The acquisition of birds from pet stores or lay persons, situations where birds of multiple sources could be mixed together, should be avoided due to the vastly increased risk of pathogen transmission.

The Aviary Environment

Captive birds have a distinct disadvantage to their wild counterparts in terms of the space that they occupy and the number of individuals with whom they share this space. As a result, the onus falls on the staff of the zoo to ensure that we maintain their environment in a pristine condition to limit buildup of waste products, depletion and competition for resources and allow them to thrive within this micro-habitat. A daily cleaning schedule with clearly defined tasks is essential to ensure aviary hygiene is maintained and allow keeping staff to more effectively achieve this goal. Some chronically infected birds that are

yet to show signs will often begin shedding mycobacterial organisms from their intestinal tract in faeces, providing a source of mycobacteria to contaminate the aviary environment (reviewed in Lennox, 2007). Regular removal of faecal material from the ground, paths, cage furniture, feed stations and nesting areas will reduce how readily this occurs.

Aviary substrates should be easy to spot-clean and disinfect, simple and cost-effective to change out, suitable for the birds being housed and add aesthetic value in the enclosure. A solid concrete floor for example may be easy to clean and hose down, but is likely to cause pododermatitis in most water-bird species long term (Blair, 2013) and is ultimately a welfare issue. Where soil or sand substrates are used, the top layers should be changed regularly and a lime layer could be utilised under the base to aid acidification and reduce bacterial growth (Collins, 2003). It is important to note that *M. avium* is capable of surviving and replicating in soil and water, due to its ability to produce mycobactin, enabling it to acquire environmental iron (Collins, 2003). For this reason water-soil interfaces should be avoided and large open ponds cleaned thoroughly, with water treated with UV sterilisation. To date, ultraviolet disinfection is the most reliable method for killing mycobacterial organisms as the bacteria's unique structure make chemical disinfection difficult and often incomplete (Collins, 2003).

Careful planning and design of aviaries should ensure perches and roosting sites are not located above feeding stations, with food and water dishes being covered. As these areas are likely to be the most frequented by birds, and subsequently the most heavily soiled, disposable floor covers under these areas to allow quick and complete removal of all faecal material may be an option. Feed and water bowls should be removed daily for cleaning and soaking in a tuberculocidal disinfectant, then thoroughly dried before the next use. As with all disinfection procedures, organic debris should be removed first to avoid deactivating chemical products and the manufacturer's directions followed to ensure adequate concentration and contact time.

Simple measures such as removing caked on mud or hosing boots between aviaries may limit spread of infected materials between aviaries. While this practice is unlikely to change the bacterial soil burdens significantly, there may be some indirect benefit to reducing the opportunity for mycobacteria from different aviaries to be combined.

All of these measures have the same goal, and that is to prevent buildup of mycobacteria in the aviary over time. Studies of *M. avium paratuberculosis* have shown it to be stable in soil for up to 7 years in some studies, (Collins, 2003), so environmental persistence is likely to be a potential source of new infection to all birds housed in these enclosures in the future (Palmieri, 2013). It is impractical and almost impossible to eliminate the bacteria making those aspects of the management protocol that can be controlled of even greater importance.

The Mycobacterial Pathogen

Historically *Mycobacterium avium* subsp *avium* had been the most commonly diagnosed pathogen behind this disease (Tell et al., 2001). As molecular diagnostic capabilities have improved so too has our ability to detect additional mycobacterial species. Palmieri et al., (2013) propose that *M. genavense* be considered the leading cause of mycobacteriosis in psittacine birds, but point out that *M. avium* and *M. intracellulare* are still frequently isolated from birds housed in zoos, or found in the wild.

There is frequently discussion on the topic of why some avian groups appear more susceptible to clinical mycobacteriosis, however a lack of credible data exists to support this. Anecdotally, it was believed that anseriformes were over represented following necropsy of a number of birds at the San Diego Zoo (Witte et al., 2010). Analysis of the data showed there to be no statistically significant increase in numbers of these birds dying from mycobacteriosis compared to any other species. The higher numbers of these birds housed at the zoo, their large size making them more likely to be found soon after death and lesions easier to see on post mortem, plus their preference for living with water may make them more likely to be exposed to the pathogen, however these things do not conclusively contribute to an increased susceptibility risk.

Interestingly, *M. avium* subsp. *avium* is considered a 'mycobacterial opportunist', able to live and reproduce independently of the host (Schrenzel, 2012). He stated that some species "exhibit wide ranges of behaviour, including extracellular replication in environmental biome communities, intracellular survival, replication, and dispersal in water-born *Acanthamoeba*". It is this variety of adaptive mechanisms that have allowed Mycobacteria to become such successful pathogens, even undergoing genotype changes and strain variation whilst outside of a

host. In real terms, this translates to a need to minimise movement of soil, particulates, equipment and cage furniture between enclosures in order to reduce mixing of the bacteria that likely reside in a particular area. By attempting to reduce bacterial cross-contamination we may in theory limit the introduction of additional bacterial genomic material for use by the pathogens.

Conclusions

There are a number of challenges associated with the diagnosis and control of avian mycobacteriosis. The lack of any definitive diagnostic testing means that a number of testing modalities need to be combined in order to increase the chances of obtaining a positive diagnosis, but even then the result may lack sensitivity and specificity. Obtaining the samples needed to undertake a thorough work up often requires that the bird be anaesthetised, carrying with it another set of risks, and a high standard of clinical skill and technical ability is needed to pursue the more advanced diagnostic techniques such as endoscopy. Following a positive diagnosis decisions must be made regarding the individual genetic value and conservation importance of the bird, as there may be the possibility of breeding from selected rare individuals should they not be clinically unwell. In such a case it is recommended that any offspring be hand reared to avoid exposure to bacteria being shed by the parent birds.

Early identification of at risk birds and those with clinical signs to allow appropriate work up to take place is essential. It is important to optimise bird health through good husbandry and diet, to support immune system function and give them the best possible chance to mount a defence when they do encounter environmental mycobacteria. The main goal in managing aviaries is to reduce buildup of faecal material and mycobacterial contamination over time, which in turn reduces the exposure risk to birds that live in the space.

Avian mycobacteriosis is an endemic disease and is unlikely to ever be eliminated from the zoo grounds, and would be exceptionally challenging to eliminate from the existing and future avian population even with the most prudent quarantine measures. It is with this mindset of endemicity and disease control, rather than elimination, that a sensible and practical approach to mycobacterial management should be implemented across the zoo. Only then can we ensure the best possible outcome for individual birds,

high conservation-value species and the wider zoological avian collection.

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