MYCOBACTERIOSIS IN A PEREGRINE FALCON

Kathleen G Fearnside
Canley Height Veterinary Clinic
Shop 6, Cnr Harden St & Avoca Rd
Canley Heights NSW 2166

A peregrine falcon (*Falco peregrinus*), owned by a commercial wildlife park, was initially presented to the Canley Heights Veterinary Clinic for lameness of the right leg. The lameness had been apparent for approximately one week. Its age was unknown, although it had been in the care of the wildlife park for over 12 years, and was an adult when received.

On clinical examination the bird was thin, however the initial weight was not recorded. There was gross swelling over the right stifle joint and a reduction in the mobility of the joint. The remainder of the clinical examination was unremarkable.

Conscious radiographs revealed that the swelling was soft tissue in origin with some mild 'moth-eaten' appearance of the distal femur and proximal tibia. The soft tissue swelling extended from the distal third of the femur to the distal tibiotarsus. Differential diagnoses included neoplastic and infectious disease.

A two week course of Enrofloxacin oral solution 15 mg/kg q24h and Metronidazole oral suspension 40 mg/kg q24h was prescribed at this stage as the carer of the peregrine falcon needed permission from authorities for more invasive diagnostic techniques. The antibiotics were administered by injecting them into a day-old chicken carcass which the bird eagerly ate.

The peregrine falcon was re-presented at the end of the course of antibiotics with no improvement. At this time it weighed 504g and was in thin body condition. The right leg was still grossly enlarged with very little change from the previous visit. The bird appeared to be in obvious discomfort on this leg and the prominent keel had developed a large area of pressure necrosis where the bird had been resting on it. Subsequent radiographs revealed little change in the mass of the right leg.

Biopsy of the lesion was offered, however it was decided to euthanase the bird, given its poor quality of life and poor prognosis. Initially the wildlife park did not allow a post-mortem as they planned to send the body for taxidermy but they did allow a few small biopsies of the lesion.

Histopathology of the biopsies revealed extensive areas of caseous necrosis involving degenerative macrophages and heterophils. These were surrounded by plump epithelioid macrophages and multinucleated giant cells. In the surrounding tissue there was extensive fibrosis and infiltrates of plasma cells and multiple aggregates of epithelioid macrophages and scattered giant cells. The macrophages had a large amount of cytoplasm that contained filamentous bacteria. Special stains demonstrated the presence of large numbers of acid-fast bacteria within the areas of necrosis as well as within the macrophages in scattered groups. The changes were consistent with chronic active infection of avian tuberculosis. There was no evidence of neoplasia.

The wildlife park was notified of the results and advised of the potential zoonotic risk, especially if the

peregrine falcon was to be sent for taxidermy. The body was returned after it had been frozen for 5 weeks! A full post-mortem was performed. In addition to the lesion of the right leg there was:

- a 10mm pale raised lesion under the tongue;
- a 12mm cream-coloured lesion of the right lobe of the liver and scattered pale spotting of the liver;
- numerous pale granulomas of various size in the mesentery and caudal air sacs;
- splenomegaly;
- a large nematode in the right lung but no other lesions of the lungs or heart; and
- other viscera were unremarkable.

Multiple samples were sent for histopathology.

The results were as follows:

- The carcase had been rapidly frozen after euthanasia. It was well preserved but fine detail had been lost;
- Leg mass: A series of subcutaneous abscesses in close proximity to each other and containing fibrinopurulent luminal debris. Underlying bone appeared normal. The large subcutaneous abscesses were lined by giant cells and fibrous encapsulation. Close to the abscesses were clusters of macrophage-like cells with fine granular cytoplasm. Ziehl-Neelsen stain showed abundant acid-fast bacilli within macrophages peripheral to the abscesses and also in the actual fibrinopurulent debris of the abscess;
- Liver: Contained multiple granulomatous abscesses, with smaller abscesses adjacent the larger one. The abscesses contained central debris of caseous mass and oedema, peripheral giant cells and an outer rim of macrophage-like cells with H-E blue staining cytoplasm. Haem was prominent and also some bile. There were naked red cells present. Ziehl-Neelsen Stain showed caseous material within abscesses contained clumps of acid-fast bacilli. Acid-fast bacilli were abundant within the slightly granular macrophage-like cells. There were also acid-fast bacilli present in the giant cells surrounding the abscesses;
- Small intestine: Abscess on the mesentery of the duodenal loop. The intestinal villi were well preserved and the epithelium was still attached, but detail lost. Ziehl-Neelsen stain showed abundant acid-fast bacilli within abscesses and macrophages;
- Spleen: Several early granulomas present;
- Pancreas: Nothing untoward;
- Lung: congested. In the lumen of one parabronchus was oedema and probable inflammatory cells, mainly mononuclear cells; and
- Kidney: abscess in pelvic area equivalent. Pigment was present in tubule cells but the detail was unable to be discerned.

The diagnosis was of typical avian mycobacteriosis involving the viscera. The subcutaneous mass on the leg was a collection of mycobacterial abscesses. There was no opportunity to culture or DNA-type the mycobacteriUM, so a diagnosis of avian mycobacteriosis, and not avian tuberculosis, could only be made, since the latter refers to infection with *Mycobacterium avium intracellulare* (MAI) complex.

Avian tuberculosis is a notifiable disease in New South Wales. The relevant authorities were notified and appropriate notification and information was discussed with the wildlife park who owned the

peregrine falcon.

REVIEW OF AVIAN MYCOBACTERIOSIS

Avian mycobacteriosis has been reported in a wide array of birds of all kinds, including domestic fowl, passerines, shorebirds, raptors and waterfowl (Gale, 1981). Avian mycobacteriosis is generally considered a disease of captive populations. Although the incidence of disease is relatively rare, the potential for avian mycobacteriosis to spread to humans makes this subject pertinent for avian veterinarians.

Avian mycobacteriosis may be caused by *Mycobacterium avium intracellulare* (MAI) complex or atypical mycobacterial such as *M. genavense* (Holosboer-Buogo et al., 1997; Hoop-, 1997). Twenty-eight known serotypes of MAI exist (Palik et al., 2000). *Mycobacterium genavense* is a fastidious organism only recently identified and was first isolated from immunosuppressed human patients (Bottger et al., 1992; Wald et al., 1992). Other atypical mycobacteria have been isolated from birds in rare instances, including *M. tuberculosis*.

Pathogenesis

Mycobacteria are ubiquitous environmental saprophytes, most commonly found in soil or water with heavy faecal contamination (Grange et al., 1990; Tell et al., 2002; Pollock , 2006). Although wild birds are a possible source of infection, they are probably not for captive birds (Isaza, 2003; Pollock, 2006). The prevalence of mycobacterial is usually <1% in free-ranging populations (Dorrestein, 1997).

Avian mycobacteriosis is usually transmitted by the ingestion or inhalation of soil or water contaminated by faeces or, less commonly, urine (Gerlach, 1999; Pollock, 2006). Raptors might become infected by ingesting infected prey. After initial colonisation of mycobacteria in the intestine and subsequent subclinical bacteraemia, the organism spreads through the portal circulation to the liver and then by haematogenous spread to distant parenchyma such as the spleen, bone marrow, skin and lungs (Van der Hayden, 1997; Gerlach, 1999).

Avian mycobacteriosis has been reported in virtually all avian taxonomic orders, however susceptibility varies. The greatest incidence of disease has been in captive collections of waterfowl, pigeons, parrots, songbirds, and ground dwelling birds such as farmed ratites and small poultry flocks (Dorrestein, 1997; Van der Hayden, 1997; Tell et al., 2002; Isaza, 2003; Pollock, 2006). Avian mycobacteriosis is considered rare in North American raptors (Redig and Ackerman, 2000) but is frequently found in raptors in the United Kingdom and continental Europe (Heindenreich, 1997; Cooper, 2002).

Clinical Disease

There are three different forms of avian mycobacteriosis recognised, although the pathogenesis has not been clarified:

1. Classical form with tubercles in many organs;
2. Paratuberculosis from with typical lesions in the intestinal tract; and
3. Diffuse form of the disease which may be associated with diffuse enlargement of infected organs and difficult to recognise at necropsy (Dorrestein, 1997; Van der Hayden, 1997; Gerlach, 1999; Pollock, 2006).

Clinical signs associated with mycobacteriosis are highly variable. Clinical disease varies with the species and strain of *Mycobacterium* species, the species of the bird affected and the route of transmission. Classically, however, mycobacteriosis is a disease of the gastrointestinal tract and liver in the bird.

Adult birds usually develop a chronic wasting disease associated with a good appetite, recurrent diarrhoea, polyuria, anaemia and poor feather quality. Abdominal distension due to enlargement of the liver or intestines might also be detected. Ascites is rare (Pollock, 2006). Intermittent switching lameness may occur s a result of painful lesions in bone marrow. Arthritis, mainly of the carpometacarpal and the elbow joints or tubercle formation of the muscle of the thigh or tibiotarsus can be seen occasionally. These clinical changes are particularly common in Falconiformes and Accipitriformes. Skin over the affected joint is often thickened and ulcerated (Gerlach, 1999). Tubercle formation of the skin is rare, but when it is present, pinpoint to pigeon egg-sized nodules filled with yellow fibrinous material may be noted. Granulomas may be seen within the conjunctival sac, at the angle of the beak, around the external auditory canal and in the oropharynx. Clinical signs associated with colonisation of the lungs are rare (Van der Hayden, 1997; Gerlach, 1999). Avian mycobacteriosis might also be associated with neurologic signs due to involvement of the spinal cord, brain or vertebral column (Van der Hayden, 1997; Tell et al., 2002).

Diagnosis

Ante-mortem diagnosis of mycobacteriosis is difficult due to the wide variety of possible clinical signs and physical examination findings. A definitive diagnosis is based on histological identification including characteristic acid-fast bacilli or culture of the organism. The Ziehl-Neelsen stain is the standard method for identification of acid-fast organisms. *Mycobacterium avium* can appear almost coccoid or as long, beaded rods (Tell et al., 2002). A suggestive diagnosis of avian mycobacteriosais may be based on identification of the organism in cytological samples, however, the acid-fast stain is not a specific test, because non-pathogenic mycobacterial can be transient gastrointestinal inhabitants or environmental contaminants. Therefore positive acid-fast cytology should be confirmed by culture, histopathology or DNA probe analysis (Pollock, 2006).

Serologic tests available include haemagglutination (HA, complement fixation (CF) and ELISA. These tests are highly species specific and they are available for only a limited number of species (Pollock, 2006).

Culture has several practical limitations. *Mycobacterium* is only intermittently shed, and careful sample collection is necessary to prevent environmental contamination. Mycobacteria are difficult to culture with least 2-4 weeks are required for visible colonies to appear and up to 8 weeks of incubation is required. Some strains of *M. avium* require up to 6 months before colonies are identifiable (Pollock, 2006).

Molecular techniques may be used to identify organisms grown not only on culture media but also identified within faecal, biopsy or necropsy samples, including formalin-fixed paraffin-embedded sections. Polymerase chain reaction (PCR) methods have been used to identify *M. avium, M. bovis* and *M. genavense* (Palik et al., 2000; Pollock, 2006).

Like the clinical disease the gross post-mortem findings of avian mycobacteriosis also vary widely. Non-specific findings include muscle wasting, loss of subcutaneous and intracoelomic fat and

discoloured, poor-quality feathers. Granulomas are frequently white or yellow in colour and they may range in size from military foci to nodules several centimetres in diameter. Granulomas are most commonly located within the intestinal wall, liver, spleen and bone (Dorrestein, 1997; Tell et al., 2002; Pollock, 2006). Granulomas may also be found in subcutaneous tissue and a variety of other viscera such as the kidney. Tubercle formation within the skin and lungs is rare (Gerlach, 1999; Pollock, 2006). Hepatosplenomegaly is one of the most consistent findings. Distension or thickening of the intestines is also an extremely common finding in granulomatous intestinal disease (Pollock, 2006). Additional lesions that might be identified include proliferative or lytic bony lesions (Tell et al., 2002), dermatitis or diffuse thickening of the skin associated with xanthomatosis (Van der Hayden, 1007), and pulmonary necrosis or ulceration (Dorrestein, 1997). The diffuse form of avian mycobacteriosis is more difficult to recognise at necropsy, because organomegaly may not be observed. There may be no significant gross findings in the diffuse form of mycobacteriosis (Van der Hayden, 1997). In raptors the disease is usually characterised by the presence of tubercles primarily in the liver, spleen, gastrointestinal tract and bones. As described above, subcutaneous tubercles also have been seen in raptors (Heindenreich, 1997; Cooper, 2002).

The most common histological findings reported include granulomatous enteritis, splenitis or hepatitis with variable amounts of acid-fast bacteria. A marked accumulation of macrophages may also be identified within the dermis, mucous membranes, and submucosa of the peritoneum and airsacs Pollock, 2006). Large numbers of acid-fast rods are found in lesions caused by *Mycobacterium avium* (Dorrestein, 1997). Granulomas generally do not possess a region of central calcification (Van der Hayden, 1997; Pollock, 2006). The necrotic regions that are identified in avian mycobacterial granulomas are surrounded by epitheliod cells, multinucleated giant cells and lymphocytes (Holosboer-Buogo et al., 1997; Pollock, 2006). Non-caseous granulomas may contain large macrophages with highly vacuolated cytoplasm and numerous acid-fast bacteria. In the diffuse form of avian mycobacteriosis diffuse infiltrations of histiocytes are seen in various organs, of predominately large foamy macrophages which typically are laden with acid-fast bacilli (Ladds, 2009).

Treatment and Zoonosis

Treatment of tuberculosis is not recommended in raptors and all birds diagnosed with mycobacteriosis should be humanely euthanased, particularly when the birds are in close contact with humans. Birds infected with *Mycobacterium avium* may continuously shed large numbers of organisms into the environment (Tell et al., 2002). The natural hosts of avian mycobacteriosis, both poultry and wild birds, are able to act as hosts for human infection (Meissner and Anz, 1977). Zoo Birds are commonly affected. This potential zoonotic risk is especially important in immunosuppressed individuals, such as those on chemotherapy, the very young, the elderly and human immunodeficiency virus (HIV)-positive. If treatment is deemed appropriate, a number of therapeutic agents have been proposed. The treatment protocol should run parallel to an adequate biosecurity program targeted at containing the disease.

Contact birds should be removed from the contaminated area, quarantined for 2 years and faecal tested every 6 to 12 weeks to determine if they are positive. Birds which remain negative and are in good physical condition following the quarantine procedure can be considered free of the disease (Gerlach, 1999; Tell et al., 2002).

Control and Prevention

Mycobacteria are extremely stable in the environment, are highly resistant to environmental

extremes and remain infectious in soil for up to seven years (Dorrestein, 1997; Gerlach, 1999). Shedding from an infected host occurs primarily in the faeces and urine, causing contamination of soil or water supplies.

There are no absolute means for control of avian mycobacteriosis. Control should focus on identification and elimination of infected birds through quarantine and use of appropriate screening tests. Avoid dirt flooring to reduce exposure to infectious material. Instead, utilise non-porous, easy to clean surfaces, appropriate disinfectants and footbaths. Providing complete, balanced nutrition and utilising good sanitation practices will minimise the impact of disease. Stressors such as overcrowding must also be minimised.

DISCUSSION

This peregrine falcon had a typical classical form of avian mycobacteriosis, with tubercles in the liver, spleen and gastrointestinal tract. This bird also had subcutaneous tubercles which have also have been seen in other raptors. Radiographically there was also evidence of lesions in the proximal tibiotarsi. However, this was not confirmed with the sample that was taken for histopathology.

One important detail of this case is the initial presenting complaint of lameness in the peregrine falcon. In clinical practice lameness secondary to gross soft tissue swelling in not usually one that is associated with mycobacteriosis. Neoplastic disease was assumed the most likely diagnosis in this case. If the biopsy had not been taken the diagnosis would not have been reached. The size of the lesion of the right leg also indicated that this bird was likely to have been infected with mycobacteria for a long period of time before the carer was aware of the problem.

The zoonotic potential is important in this case for two reasons:

- The wildlife park is open to the public and is a common place for school excursions and young families to visit. Birds infected with *Mycobacterium avium* may continuously shed large numbers of organisms into the environment and this bird was likely to have been infected for a long period before diagnosis. This potential zoonotic risk is especially important in immunosuppressed individuals, such as those on chemotherapy, the very young, the elderly and human immunodeficiency virus (HIV)-positive individuals.

- The wildlife park, like many others, often send the less common animals for taxidermy after they die, as was the plan for this peregrine falcon. The risk to humans by inhalation would be much greater once the carcase is open especially if appropriate precautions were not undertaken.

It should also be noted that many other species of animals have the potential to be infected with *Mycobacterium spp.* The wildlife park houses numerous native mammals and reptiles of which there are many reports in the literature of becoming infected, especially in captivity (Ladds, 2009). The potential for infection in other species of animals within the wildlife park and zoonotic risk from such species must also be considered.

Hopefully this case will be singularly occurring since the bird was individually housed. There are no real preventative measures. Avoiding dirt flooring to reduce exposure to infectious material, providing complete, balanced nutrition, utilising good sanitation practices and minimising stressors

on the animals will minimise the impact of disease.

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