Mycobacteriosis in birds

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Summary

Avian mycobacteriosis is an important disease which affects companion, captive exotic, wild and domestic birds. The disease is most commonly caused by Mycobacterium avium and Mycobacterium genavense. Lesions are typically found in the liver and gastrointestinal tract, although many other organ systems can potentially be affected. The authors review those species of Mycobacterium reported to affect birds, the epidemiology of avian mycobacteriosis, immunological responses to mycobacterial infection, ante- and post-mortem diagnosis, treatment and prevention or control of the disease.

Keywords


Mycobacterial species affecting birds

Mycobacteriosis is a world-wide disease which has been reported widely in pet birds, free-living and captive wild birds and poultry. Since the late 1800s, this disease has been called avian tuberculosis (42). However, since classical tuberculous lesions are only one of the possible manifestations of mycobacterial infection in birds, mycobacteriosis is a more appropriate term for this disease.

A further reason for using the more inclusive term, mycobacteriosis, is that several species of mycobacteria can cause the disease in birds, principally Mycobacterium avium, M. intracellulare and M. genavense. As M. avium and M. intracellulare share several growth characteristics and some species-specific antigens, these species are often grouped together and termed M. avium-intracellulare (MAI) complex (56). Mycobacterium avium subsp. paratuberculosis (the causal organism of hypertrophic enteritis, or Johne’s disease, in cattle) and M. lepraeum (which causes a leprosy-like disease in rodents) are also closely related to M. avium and are usually included by bacteriologists in the MAI complex. Mycobacterium scrofulaceum is another organism often included in the MAI complex. However, these species rarely cause mycobacteriosis in birds. Mycobacterium genavense is a recently identified and genetically distinct species which causes a disease in birds that is clinically and histopathologically indistinguishable from that caused by MAI (1, 116, 122). Mycobacterium fortuitum is occasionally cultured from lesions in avian species (74, 78, 138). Infection of birds with M. tuberculosis and M. bovis has been reported, although these organisms are less common causes of mycobacteriosis in birds (151, 156).

Until a decade ago, most cases of mycobacteriosis in birds were assumed to be caused by MAI. In fact, the majority of identifiable mycobacteria cultured from birds prior to 1990 were M. avium (Table I). However, in a number of studies published before this date, a significant percentage of samples yielding acid-fast bacilli on microscopic examination resulted in either no growth on mycobacterial culture or culture of an unidentifiable mycobacterium (49, 86, 89). It is tempting to speculate that many of these cases involved M. genavense, a fastidious organism with special growth requirements that is currently identified with deoxyribonucleic acid (DNA) probe technology, since culture is so difficult. This mycobacterium was first identified in 1990, in a human patient with acquired immunodeficiency syndrome (AIDS) (12). The species has since been proven to be pathogenic for birds.

In a recent survey of necropsy findings in pet birds in Switzerland, M. genavense was the predominant mycobacterial species isolated (78). Of a total of 5,345 necropsies performed on pet birds, mycobacteriosis was diagnosed in 204 birds (3.8%). Of the samples in which the mycobacteria were identified, M. genavense was isolated in...
Biochemical testing (56). However, several of the isolates included M. fortuitum (4%), M. tuberculosis (4%), M. gordane (2%) and M. nonchromogenicum (2%).

A smaller necropsy survey of 253 pet birds in Switzerland found a similar distribution of mycobacterial species (74). Mycobacterium genavense accounted for 73% of isolates and M. avium for only 11.5%; M. fortuitum was identified in one bird. This predominance of M. genavense, a known human pathogen, in pet birds may simply be a reflection of the synanthropic circumstances of this particular population of birds. However, this organism has also been reported in several birds at the Antwerp Zoo in Belgium (116). Twenty-seven cases of M. genavense infection were reported in five different orders: twelve Passeriformes, seven Psitaciformes, four Galliformes, three Piciformes and one Condoriforme.

Three serotypes of M. avium (serotypes 1-3) and over twenty serotypes of M. intracellulare (serotype 4, onward) have been described on the basis of culture characteristics and biochemical testing (56). However, several of the isolates formerly classified as M. intracellulare, including serotypes 4-6 and 8, are now considered to be serovars of M. avium (2). The classical M. avium serotypes 1-3 are far more pathogenic for birds than are those of M. intracellulare. In fact, M. intracellulare appears to be minimally pathogenic for birds (25, 109). In the study by Collins et al., eleven of twelve tested strains of M. intracellulare were non-pathogenic in chickens (25). In contrast, nineteen of twenty-one tested strains of M. avium (serotypes 1-3) caused infection in chickens; seventeen of these strains proved lethal within 115 days of inoculation. Ferguson et al. reported a case of disseminated mycobacteriosis in a wood duck (Aix sponsa) from which M. intracellulare was cultured (45). However, the organism proved to be non-pathogenic for chickens.

Virulence differs slightly among the three classical serotypes of M. avium. In the study by Collins et al., only one of the three tested strains of M. avium serotype 3 was lethal in chickens, leading the authors to conclude that serotype 3 is of low virulence in chickens (25). In contrast, five of six strains of serotype 2 were lethal; and three of four serotype 1 tested were lethal in chickens. Virulence may also vary among bird species. Serotypes 1 and 3 have been reported as causes of high mortality in some waterfowl collections in the United Kingdom (UK) (29, 107, 134).

**Susceptibility of various birds**

Mycobacteriosis has been reported in virtually all orders of birds, although susceptibility varies. As illustrated in Table II, findings differ somewhat among studies. The orders that appear to be most susceptible in zoological collections are Anseriformes, Gruiformes and Galliformes (41, 86, 131). One reason for the disparity among studies may be that the reported incidence of mycobacteriosis in particular orders is more a reflection of the prevalence of those birds in the avian collection than an indication of order susceptibility.

Hejlicek and Treml studied the epidemiology and pathogenesis of M. avium in several synanthropic species of wild and domestic bird (67). The authors concluded that domestic fowl, sparrows, pheasants, partridges and laughing gulls (Larus atricilla) are highly susceptible to infection with M. avium. Turkeys and guinea fowl were considered to be moderately susceptible; geese and ducks moderately resistant; and pigeons, turtle-doves and rooks (Corvus frugilegus) highly resistant to M. avium infection. Pavlas et al. concluded that geese are highly resistant to oral challenge with M. avium (serotype 2) (110). In contrast, ducks, geese and swans in waterfowl collections in the UK are clearly susceptible to M. avium serotypes 1 and 3 (15, 29, 107). Factors that could increase the susceptibility of waterfowl to mycobacteriosis are discussed below.

A report by Quaranta et al., detailing a serious outbreak of mycobacteriosis on a pheasant farm housing 426 birds, confirmed that pheasants are highly susceptible to M. avium infection (118). Over a two-month period, clinical disease was found in 25% of birds, 50% of which died. Following the diagnosis of mycobacteriosis, all birds were destroyed. At necropsy, forty of fifty birds sampled at random had typical granulomatous lesions from which M. avium was cultured.

Quail also appear to be highly susceptible to M. avium (23, 102). Clark and Collins tested a single-dose oral challenge model of disseminated M. avium infection in Japanese quail (Coturnix japonica) and White Leghorn chickens (23). The quail developed disseminated mycobacteriosis after both oral and intravenous challenge, whereas the chickens developed disease only after intravenous challenge. Following oral challenge, M. avium was recovered from the faeces on at least one sampling in 75% of quails but in only 13% of chickens. Mycobacterium avium was cultured from 25% of quail but from none of the chickens challenged orally. The authors commented that preliminary results of enzyme-linked immunosorbert assays (ELISA) suggest that humoral responses to M. avium differ among birds, a finding

**Epizootiology of avian mycobacteriosis**

Avian mycobacteriosis has a world-wide distribution, although the disease is reportedly uncommon in certain countries, such as Japan (101, 133). The disease is now rare in large commercial poultry flocks, owing to the success of measures implemented to control the disease (53, 104, 121, 140). Over the past two decades, most reports of avian mycobacteriosis have involved pet birds, large collections of birds (e.g. zoos and wildlife reserves), farmed ratites and small poultry flocks (29, 74, 78, 86, 104, 107, 116, 118, 122, 131, 132, 136, 138, 139, 141).
<table>
<thead>
<tr>
<th>Avian species</th>
<th>Clinical signs</th>
<th>Mycobacterial isolates</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anseriformes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chinese goose</td>
<td>Anorexia (concurrent duck plague)</td>
<td>M. avium</td>
<td>82</td>
</tr>
<tr>
<td>Northern pintail</td>
<td>Found dead</td>
<td>M. avium serotype 3</td>
<td>107</td>
</tr>
<tr>
<td>Mandarin duck</td>
<td>Progressive debilitation, ulcerative caseous lesions on tibiotarsal joints, enlarged metatarsus and digits, ulcerative caseous nodules on head, throat and palateum</td>
<td>M. avium complex</td>
<td>51</td>
</tr>
<tr>
<td>Mandarin duck</td>
<td>Abdominal distention</td>
<td>M. avium complex (not types 1 or 3)</td>
<td>128</td>
</tr>
<tr>
<td>Mandarin duck</td>
<td>Sudden death (severe amiation)</td>
<td>M. avium serotype 1</td>
<td>133</td>
</tr>
<tr>
<td><strong>Columbiformes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pigeon</td>
<td>Clinically normal birds</td>
<td>Mycobacteria</td>
<td>96</td>
</tr>
<tr>
<td>White carneaux pigeon</td>
<td>Anorexia, lameness, torticollis, cutaneous nodules</td>
<td>M. avium</td>
<td>115</td>
</tr>
<tr>
<td>Carrier pigeon</td>
<td>Weight loss, lethargy, tubercle on skin surrounding cloaca</td>
<td>M. avium serotype 2</td>
<td>101</td>
</tr>
<tr>
<td><strong>Galliformes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken</td>
<td>Clinically normal birds</td>
<td>M. avium, M. fortuitum</td>
<td>138</td>
</tr>
<tr>
<td>Chicken</td>
<td>Chronic diarrhoea, progressive emaciation, decreased egg production, paralysis</td>
<td>M. avium</td>
<td>106</td>
</tr>
<tr>
<td>Ring-necked pheasant</td>
<td>Listlessness, death</td>
<td>M. avium</td>
<td>133</td>
</tr>
<tr>
<td>Peacock</td>
<td>Subcutaneous masses in distal part of neck and sternal region</td>
<td>M. avium</td>
<td>153</td>
</tr>
<tr>
<td>Turkey</td>
<td></td>
<td>Acid-fast organisms</td>
<td>22</td>
</tr>
<tr>
<td><strong>Gruiformes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whooping crane</td>
<td>Lethargy, lameness, swollen legs</td>
<td>M. avium serotype 1</td>
<td>146</td>
</tr>
<tr>
<td>Sandhill crane</td>
<td>Clinically normal birds</td>
<td>M. avium serotype 1</td>
<td>150</td>
</tr>
<tr>
<td><strong>Passeriformes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>European starling</td>
<td>Clinically normal birds</td>
<td>M. avium</td>
<td>9</td>
</tr>
<tr>
<td>Blue-and-white flycatcher</td>
<td>Respiratory and nervous signs for two weeks, emaciation</td>
<td>M. genavense</td>
<td>77</td>
</tr>
<tr>
<td>Zebra finch</td>
<td>Weakness, inco-ordination, emaciation</td>
<td>M. genavense</td>
<td>77</td>
</tr>
<tr>
<td>Island canary</td>
<td>Diarrhoea, slight dyspnoea; somnolence, lethargy, ruffled plumage</td>
<td>M. genavense</td>
<td>122</td>
</tr>
<tr>
<td><strong>Psittaciformes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green-winged macaw</td>
<td>Progressive eyelid edema, intermittent diarrhea, occasional stertorous respiration, listlessness, inappetence, weight loss, multiple nodules on eyelids, conjunctivae, unfeathered areas of face and head, tongue, choana and glottis, heart murmur</td>
<td>M. tuberculosis</td>
<td>73, 162</td>
</tr>
<tr>
<td>Maximilian's parrot</td>
<td>Bilateral keratitis (Maximilian's parrot); weight loss, lethargy, mass protruding from left ear (blue-headed parrot)</td>
<td>Acid-fast bacilli (isolation attempts unsuccessful)</td>
<td>145</td>
</tr>
<tr>
<td>Yellow-naped parrot</td>
<td>Acute dyspnoea, marked inspiratory stridor</td>
<td>M. genavense</td>
<td>1</td>
</tr>
<tr>
<td>Orange-winged parrot</td>
<td>Chronic wasting, emaciation</td>
<td>M. genavense</td>
<td>77</td>
</tr>
<tr>
<td>Budgerigar</td>
<td>Sudden death (emaciated); sudden death (in good condition); acute dyspnoea (emaciated)</td>
<td>M. genavense</td>
<td>77</td>
</tr>
<tr>
<td>Budgerigar</td>
<td>Found dead (budgerigar); weight loss despite good appetite, weakness</td>
<td>Acid-fast organisms</td>
<td>125</td>
</tr>
<tr>
<td>Parakeet, parrot</td>
<td>Sinusitis and ocular infection (parakeet); feather loss, chronic malaise (parrot)</td>
<td>Acid-fast bacilli</td>
<td>14</td>
</tr>
<tr>
<td>Blue-headed parrot</td>
<td>Marked muscle wasting, anaemia, recurrent diarrhoea, dulling or loss of feathers</td>
<td>Acid-fast bacilli</td>
<td>108</td>
</tr>
</tbody>
</table>
Table I (contd)

<table>
<thead>
<tr>
<th>Avian species</th>
<th>Clinical signs</th>
<th>Mycobacterial isolates</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raptors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Northern goshawk (Accipiter gentilis)</td>
<td>Weakness, central nervous system signs</td>
<td>Acid-fast organisms</td>
<td>96</td>
</tr>
<tr>
<td>Lanner falcon (Falco biarmicus)</td>
<td>Chronic lameness, swollen knees</td>
<td>M. avium serotype 2</td>
<td>96</td>
</tr>
<tr>
<td>Red-tailed hawk (Buteo jamaicensis)</td>
<td>Depressed, cachectic, unable to fly</td>
<td>M. avium serotype 2</td>
<td>147</td>
</tr>
<tr>
<td>Great horned owl (Bubo virginianus), red-tailed hawk (Buteo jamaicensis)</td>
<td>Inability to fly, anorexia, emaciation, focal feather loss (owl), ataxia, hyperreflexia, hyperextension of both legs, yet alert and able to eat (hawk)</td>
<td>Acid-fast bacilli</td>
<td>94</td>
</tr>
<tr>
<td>Ratites</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Greater rhea (Rhea americana)</td>
<td>Lethargy, progressive weight loss, death</td>
<td>M. avium</td>
<td>148</td>
</tr>
<tr>
<td>Greater rhea (Rhea americana)</td>
<td>Large subcutaneous mass on lower neck</td>
<td>M. avium complex</td>
<td>132</td>
</tr>
<tr>
<td>Ostrich (Struthio camelus)</td>
<td>Prolapse of terminal intestine, abdominal lesions noted during colopexy</td>
<td>Few acid-fast bacilli</td>
<td>13</td>
</tr>
<tr>
<td>Ena (Dromaius novaehollandiae)</td>
<td>Granulomatous conjunctivitis (good body condition)</td>
<td>Mycobacteria characteristic of M. avium</td>
<td>136</td>
</tr>
<tr>
<td>Ena (Dromaius novaehollandiae)</td>
<td>Mass on nictitating membrane, recurring despite resection</td>
<td>M. avium complex</td>
<td>114</td>
</tr>
<tr>
<td>Kiel (Apteryx australis mantelli)</td>
<td>Change of feeding behaviour, weight loss</td>
<td>M. avium isolated in faeces of mate</td>
<td>35</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lesser flamingo (Phoenicopterus minor)</td>
<td>Epidemic of deaths</td>
<td>M. avium serotype 1</td>
<td>27, 88</td>
</tr>
<tr>
<td>Micronesian kingfisher (Halcyon cinnamomina)</td>
<td>Lethargy, abdominal distention, progressive weakness</td>
<td>M. avium serotype 1</td>
<td>. 124</td>
</tr>
<tr>
<td>Tauraco (Musophaga sp. and Tauraco sp.)</td>
<td>Soft tissue masses in infraciliary sinuses, nasal turbinates, oral mucosa, cloaca and abdominal muscles</td>
<td>M. avium-intracellulare complex</td>
<td>141</td>
</tr>
<tr>
<td>Common buzzard (Buteo buteo)</td>
<td>Bumblefoot</td>
<td>M. avium serotype 2</td>
<td>160</td>
</tr>
</tbody>
</table>

corroborated by Cromie et al. (33). This suggestion is consistent with the findings of Gross et al., who demonstrated a difference in susceptibility to M. avium between chickens selectively bred for either high-antibody or low-antibody response to sheep erythrocytes (60).

Anseriformes are particularly susceptible to mycobacterial infections. Cromie identified approximately sixty isolates of M. avium serotype 1 from various Anseriformes in a captive waterfowl collection in the UK (28). In addition, Cromie et al. published the results of an epidemiological survey of deaths due to avian mycobacteriosis from the same collection, concluding that both genetic and environmental factors affect the susceptibility to M. avium in waterfowl (29). The incidence of mycobacteriosis within taxonomic tribes varied widely from zero in steamerducks (Tachyeres spp.) to 52% in perching ducks (tribe: Cairinini). The authors suggested that one of the reasons why the incidence was high in perching ducks was because in the wild these birds spend much of their time in arboreal habitats, where immunity to mycobacteria may be of less importance than in ground-dwelling birds. Within the captive collection, these ducks are pinioned, therefore in far greater contact with the ground, and hence potential sources of mycobacteria. However, for perching ducks, such as the highly susceptible white-winged wood duck (Cairina scutulata), the susceptibility is likely to be in part genetically determined (30). Sea ducks (tribe: Mergini) had the second-highest incidence of mycobacteriosis (48.7%); yet within this group, the incidence in eiders was 23%, but in mergansers, scoters and goldeneyes, almost 68%. These ducks share similar habitats and feeding patterns, so the difference in prevalence may indicate genetic differences in susceptibility to this disease. Cromie et al. also reported that birds adapted to either hot or cold climates were more

Table II

Studies reporting the incidence of mycobacteriosis in various avian groups

<table>
<thead>
<tr>
<th>Avian species</th>
<th>Clinical signs</th>
<th>Mycobacterial isolates</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>National Zoological Park (United States of America)</td>
<td>Charadriiformes, Galliformes, Passeriformes, Gruiformes and Anseriformes</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Regent’s Park Zoological Gardens, other zoological collections, samples from practising veterinarians and wild birds (Great Britain)</td>
<td>Anseriformes, Gruiformes and Columbiformes (are in Poitaciformes, Passeriformes, Sphenisciformes and Strigiformes)</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>Antwerp Zoo (Belgium)</td>
<td>Puffins, Poitaciformes *</td>
<td>115</td>
<td></td>
</tr>
<tr>
<td>Alipore Zoological Garden (India)</td>
<td>Anseriformes, Galliformes and Gruiformes</td>
<td>131</td>
<td></td>
</tr>
<tr>
<td>Wild birds (Netherlands)</td>
<td>Buzzards and falcons</td>
<td>140</td>
<td></td>
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</tbody>
</table>

* this study reported only on cases of mycobacteriosis caused by Mycobacterium genavense
susceptible than those from temperate climates. However, this finding may reflect environmental stressors as much as genetic susceptibility. Recent unpublished studies from the same collection of birds have indicated clear differences in species susceptibility, with the percentage mortality ranging from 0% to 94%.

In any avian species, susceptibility to mycobacterial infection probably increases as the intensity or number of stressors increases. Possible stressors include malnutrition, overcrowding, adverse environmental conditions (e.g. drought, extreme ambient temperatures), pinioning and concurrent disease (29, 60, 82, 88). Valente et al. reported on an avian experimental model for paratuberculosis enteritis (Johnne’s disease) in cattle (155). A group of chicks was immunodepressed with cyclophosphamide and concurrent inoculation with infectious bursal disease virus, then the birds were orally infected with M. paratuberculosis of bovine origin (two separate doses of 5 mg dry weight per dose). The immunocompromised chicks passed mycobacteria in the faeces from one to three months after inoculation and developed intestinal lesions typical of mycobacterial infection. Given that M. paratuberculosis (strains 22, 23, 24 and 27; intravenous inoculum; dose: 1.0-2.5 x 10^9 viable units) is reportedly of low pathogenicity in chickens (25), this study highlights the impact of immunosuppression on the susceptibility of birds to mycobacterial infection.

In pet birds, Van der Heyden believes a clear species predilection exists (156). Mycobacteriosis appears to be far more common in Amazona, Pionus and Brotogeris spp., budgerigars (Melopsittacus undulatus), canaries, siskins and toucans than in other pet birds commonly seen in veterinary practice. Van der Heyden (156) proposes three possible reasons, as follows:

a) genuine species susceptibility

b) common sources of exposure during importation of parrots and parakeets caught in the wild

c) husbandry practices, such as communal flights with solid floors, which increase environmental contamination with mycobacteria.

Once again, both genetic and exposure factors are implicated in the susceptibility of certain birds to mycobacterial infection.

**Gender and age**

Males and females appear to be equally susceptible to mycobacterial infection (29, 86). However, in one report, turkey hens were clearly more susceptible than turkey cocks to infection after inoculation with M. avium (68). Moreover, in two reports involving psittacine and passerine pet birds, mycobacteriosis was slightly more common in females than in males (77, 108).

Mycobacteriosis is diagnosed far more frequently in adult birds than in young birds (29, 104). In a retrospective survey of diseases in swans in waterfowl collections in the UK, mycobacteriosis was the cause of death in 33% of adult swans, 4% of juvenile swans, but no downy cygnets (15). However, the findings of this and other studies probably reflect the chronic and insidious nature of the disease rather than any difference in susceptibility between older and younger birds. In challenge studies using chickens as young as eight weeks of age, young birds are clearly highly susceptible to mycobacterial infection (4, 5). Feldman summed up the situation thus: ‘Under natural conditions… the infection begins while the bird is immature and requires many months or even years before the infection induces a significant influence upon the well-being of the animal’ (43). This pattern of disease progression is common to mycobacterial infections in both avian and mammalian species.

**Sources of infection**

Most mycobacteria, including those species that are pathogenic for birds, are ubiquitous environmental saprophytes (56). Mycobacteria are commonly found in surface water, such as marshes, ponds, lakes, streams and rivers. These organisms may also be found in aerosols formed by the disturbance of surface water. Grange whimsically referred to mycobacteria as ‘the ducks of the microbial world: found at the interface of air and water’ (55). Potentially pathogenic mycobacteria have even been cultured from water pipes and municipal water supplies (117).

Mycobacteria can also be cultured from soil, especially acidic soils and areas which are marshy or prone to flooding. Particularly high numbers of mycobacteria may be found in soils rich in organic matter or heavily contaminated with animal faeces (56). Species of the M. avium complex have been cultured from sawdust, potted plants, house dust, bedding material and cigarettes (81, 117). Chronic exposure to environmental and/or pathogenic mycobacteria could possibly change immune responses over time and hence the response to disease, but no data have been reported to date, as artificial challenge experiments are not a good reflection of natural infections.

As the mycobacteria pathogenic for birds are opportunistic saprophytes, the primary source of infection is a contaminated environment. Faeces from infected birds which are shedding the organisms via the intestinal tract are a principal source of infection for other birds (15, 35, 44, 67, 107). Mycobacteria can survive in soil for years (44). Hence, infected soil and other organic material is a potential source of infection for successive generations or new groups of birds (44, 53, 104).

Infected wild birds are a possible source of infection for pet birds, captive exotic birds and poultry (9, 26, 67, 107, 116). Hejíček and Balat cultured mycobacteria from 3.6% of wild birds in areas surrounding poultry farms on which avian tuberculosis was endemic (65). None of the wild birds had
lesions consistent with mycobacteriosis. The authors noted an association between the intensity of infection in tuberculous poultry flocks and the occurrence of mycobacteria in wild birds in the surrounding area. This finding raises the question of whether the wild birds became infected through contact with tuberculous poultry, or whether mycobacteria were spread to the poultry by the wild birds. In a seven-year survey performed in the United States of America (USA) by the National Wildlife Health Research Center, M. avium was diagnosed in sixty-four birds (Anseriformes, Podicipediformes, Gruiformes and Falconiformes) submitted from sixteen states (26). Mycobacteriosis was the primary diagnosis in 81% of the cases and was an incidental finding in the remaining 19%.

Mycobacteriosis has become endemic in a captive collection of birds in the UK (107). The centre was initially stocked entirely with birds hatched on-site or with very young birds from other related centres; no adult birds were introduced into the collection. All wildfowl at the centre are routinely vaccinated with an experimental vaccine against avian tuberculosis. However, five years after the collection was begun, eight birds died from avian mycobacteriosis; M. avium serotype 3 was isolated from a number of these birds and from birds which died subsequently. Since that time, the disease has become endemic and accounts for approximately a third of adult mortalities. No cases of mycobacteriosis involving this serotype have been reported in waterfowl at other related centres, therefore the author concluded that the organism was probably introduced by wild birds. Another possibility is that the organism was already present in the grounds before stocking.

The feeding habits of certain species may increase exposure to pathogenic mycobacteria. In the previously-mentioned survey of mycobacteriosis in a waterfowl collection in the UK (29), the feeding habits of birds affected the incidence of mycobacteriosis, although the diet had no effect. The lowest incidence was found in the grazing birds (birds which feed on vegetable matter by grazing the land), and the highest incidence was found in the diving birds, especially sea ducks. The authors proposed that the low incidence in the grazing birds may occur because these birds are able to selectively avoid heavily contaminated vegetation (e.g. grass soiled with faecal material); in addition, the food source is exposed to the 'sterilising' effects of solar ultraviolet radiation. This latter suggestion stemmed from the finding that the shaded pens had the highest incidence of mycobacteriosis and the highest mortality rate (55%). Alternatively, these findings may be due to the mycobacteria inhabiting aquatic environments more than terrestrial environments.

Another interesting finding by Cromie et al. was that the few birds with primary pulmonary mycobacteriosis were ducks that fed by dabbling in shallow water (29). Dabbling may aerosolise mycobacteria, which can then be inhaled. In a survey of cases of M. genavense mycobacteriosis at Antwerp Zoo in Belgium, extensive invasion of the lungs was reported in most birds (116). Given that the lung is an uncommon site for mycobacterial lesions in birds, this presentation was an unexpected finding and suggested an airborne source of infection, either in dust or as aerosols.

Feldman presented the opinion that mycobacteria are unlikely to be spread via eggs (44). Embryos produced by infected hens seem to not be naturally infected with the organism. Similarly, Feldman also stated that the surface of the egg is unlikely to be important in the transmission of mycobacteria to the hatching chick or to other birds. In contrast, Kyari found that M. avium could be transmitted to duck embryos by vertical transmission and faecal contamination of the egg shell (93). Other unlikely, but possible sources or modes of mycobacterial transmission to birds are ticks, rodents, fly larvae and coitus (44, 90).

**Zoonotic potential**

Although rare, MAI and M. genavense can cause disease in humans (12, 59, 117). Immunocompromised patients, such as those with AIDS, and young children appear to be more susceptible than immunocompetent adults (11, 39, 105).

Some concerns have been raised that synanthropic birds (e.g. pet birds, pigeons, ducks and other species that commonly coexist with, or live in close proximity to humans) are a potential source of mycobacteriosis in humans. However, as mycobacteria are environmental saprophytes and humans and synanthropic birds share the same environment, a common environmental source is the most likely explanation. According to Grange et al., 'for practical purposes all MAI infections in humans are of environmental origin' (56). At the 1985 National Consensus Conference on Tuberculosis, in Denver, Colorado, USA, the MAI committee stated that serotypic divergence of human and animal MAI isolates indicates that infected animals are not an important source of human infection (98). Rather, environmental sources are considered the principal cause of mycobacteriosis in humans (80). Boian et al. and Bottger consider M. genavense to be also an opportunistic pathogen which, in both humans and birds, is acquired from the environment (10, 11). Nevertheless, removal of birds with confirmed mycobacteriosis from households containing young children or immunocompromised individuals seems an appropriate precaution.

Infections with M. tuberculosis are fairly common in pet psittacines (50, 72) and should always be considered a differential for masses involving the face and head of a bird. These infections are most likely due to contact with tuberculous owners. In one case, M. tuberculosis was cultured from a pet parrot four years after active tuberculosis had been diagnosed in two of the human occupants of the house. The owners admitted to occasionally feeding the parrot from their...
own mouths, so the parrot is likely to have contracted mycobacteriosis from the owners (73, 162).

**Economic impact**

Avian mycobacteriosis can result in both direct and indirect economic losses. Direct losses include reduced production as a result of mortality or decreased egg production (44, 104, 118), condemnation at slaughter in poultry (44, 121), and the costs of treatment and/or control programmes (e.g., hygiene measures, renovation of facilities, culling and restocking, surveillance programmes). Direct economic losses to the small producer can be severe. Mutalib and Riddell discussed outbreaks of avian tuberculosis in small flocks of poultry in western Canada, and commented that flock mortality rates were as high as 80% on some farms (104).

Indirect losses may be less obvious, but can be far-reaching. These include loss of valuable, rare, or endangered species from collections or breeding programmes, such as the white-winged wood duck captive breeding programme (30). The disease may even cause a negative impact on tourism in wildlife reserves. For example, an epidemic of mycobacteriosis in lesser flamingos (Phoenicopterus minor) along the shores of Lake Bogoria and Lake Nakuru in Kenya contributed to the deaths of more than 18,500 flamingos over a three-month period in 1993 (88). Another cause of indirect economic loss is transmission of mycobacteria from infected birds to swine and cattle (44, 59). In swine, MAI organisms can cause clinical disease. In cattle, such organisms can cause non-specific positive reactions to tuberculin, thus compounding problems in eradication and surveillance programmes for bovine tuberculosis.

**Immunological responses to mycobacterial infection**

The chronic nature of avian mycobacteriosis is testament to the prolonged immunological battle between host and pathogen. Mycobacterial immunity depends on primarily cell-mediated immune responses, with humoral immunity being of apparently either limited or dubious value. Initially, the invading pathogens are engulfed by macrophages and the ability of the bacteria to survive and multiply intracellularly determines the disease outcome. Classically, the sequence of events following phagocytosis by the macrophage includes processing and presentation of antigens by the major histocompatibility complex I and II molecules to specific T lymphocyte subsets. These cells proliferate with subsequent activation of macrophages via various cytokines. These molecules facilitate bacteriostasis and ultimately killing of the mycobacteria within the macrophage (8, 19, 54, 72, 79, 84). In mammals, gamma interferon, interleukin-2, granulocyte macrophage colony stimulating factor and tumour necrosis factor play key roles in this cell-mediated immune response.

The identity and function of the avian cytokines involved in mycobacterial immunity remain undetermined (33, 69, 70).

The cell-mediated immune response to invading mycobacteria or mycobacterial antigens is exploited in the diagnostic intradermal tuberculin test. In some birds, such as chickens, this causes a typical swelling at the skin test site 48 h-72 h post injection. However, this test gives highly variable results with non-domestic birds. Moreover, negative tuberculin test reactions may be a result of birds with advanced disease reaching a state of anergy.

The precise mechanism of survival of mycobacteria within avian macrophages is as yet unknown (119). As in mammals, mechanisms are likely to include inhibition of phagosome-lysosome fusion, possible disruption of cytokine production and action, and inhibition of enzymatic action (72, 123). Mycobacterial virulence appears to reside in the lipids of the cell wall (72). The lipid content of a mycobacterium may be as great as 40% of the total dry weight, most of which is found in the cell wall. These lipids include mycolic acids, glycolipids, phospholipids and mycosides, which play a role in virulence of the infecting bacteria and influence the immunological response to infection. Mycosides, phospholipids and sulphotides are thought to protect the bacterium from phagocytosis. Glycolipids cause a granulomatous response and enhance the survival of the bacterium once phagocytosed.

In mammals, the responses to the common or shared mycobacterial antigens are thought to confer protection against mycobacterial infection by promoting mycobacteriocidal responses (143). Responses to the antigens specific to the slowly growing mycobacteria, such as *M. avium*, are thought to be detrimental to the host, resulting in tissue necrosis as a result of killing of cells expressing mycobacterial antigens on their surfaces rather than killing of the pathogens themselves (142). A similar scenario is likely in avian species, given that T cell subsets of chickens have been shown to respond to one of the major common mycobacterial components (65 kDa heat shock protein) (3). Immunological studies in waterfowl during progressive *M. avium* infection have shown a loss of cellular immune reactivity to the common antigens and an increased cellular immune reactivity to non-common antigens, i.e. the antigens specific to slowly growing mycobacteria and/or the species-specific antigens of *M. avium* (33). In this study, changes in cellular responses were coupled with a dramatic increase in humoral responses directed primarily at the common antigens, as occurs in mammals (46). However, this humoral response does not appear to confer protection. The response may facilitate the progression of the disease by interfering with appropriate T cell function; alternatively, high levels of serum antibody or circulating immune complexes may suppress lysosomal enzymes in the phagocytic cells.
Although the role and function of antibodies in avian mycobacteriosis remains speculative, the increase in detectable antibody levels during infection has been successfully exploited as a diagnostic indicator of disease (31, 149). However, not all birds appear to produce this obvious antibody response (24, 33), and as mycobacteria are common environmental organisms, current antibody detection assays continue to be hindered by the problems of cross-reactivity.

Antibodies of some of the more ‘primitive’ birds, such as waterfowl, are structurally distinct from those of chickens. As such, these antibodies are relatively inept at secondary sensitisation and complement fixation (70). Such functional differences may be responsible for some of the problems in ante-mortem diagnostic tests of mycobacteriosis.

Numerous host factors affect immune responses to mycobacteria, including species, age, nutritional status and stress. A state of general malnutrition clearly affects immune capability (85, 119, 154). However, a number of specific nutritional deficiencies, such as zinc and vitamin A deficiency, particularly affect the production and function of T cells. Attention to specific dietary requirements must therefore be paramount in any animal management situation (154). Stress is thought to have a particular impact on cell-mediated immune responses (57), and hence may readily affect mycobacterial immunity. This appears to be borne out in both the pathology (60) and the epidemiology (29) of the disease.

Overall, the development of efficacious vaccines, therapies and diagnostic technologies for domestic and wild birds may be principally hindered by the paucity of information about avian immune responses to mycobacteria.

Ante-mortem diagnosis of avian mycobacteriosis

Ante-mortem diagnosis of mycobacteriosis in birds is problematic and typically unrewarding. The clinical presentation is highly variable and practical diagnostic tools which are highly sensitive and specific in all bird species are not available. Newer techniques involving amplification and identification of species-specific mycobacterial DNA fragments hold much promise for ante-mortem diagnosis and for epidemiological investigations. The problem with utilising this technology is the intermittent shedding of mycobacterial organisms and the difficulty in targeting tissues to test.

Clinical findings

Table I illustrates the remarkable diversity of clinical signs of avian mycobacteriosis. The classical presentation is of a chronic wasting disease which results in death after several months (42, 104, 131). Affected birds are emaciated, lethargic and weak. The feathers are in poor condition, and in chickens the comb becomes pale or in some cases cyanotic (104, 108). The liver may be readily palpable (both as a consequence of atrophy of the abdominal musculature and of hepatic enlargement) and loops of thickened intestine may also be appreciated in emaciated birds with severe intestinal lesions (156). In waterfowl, a distended abdomen due to hepatic enlargement and build-up of ascitic fluid is the most specific clinical sign of this disease.

At the other extreme, some infected birds are simply found dead, having presented a normal appearance and behaviour during the period leading up to sudden death (77, 104, 116, 125). In the report by Mutalib and Riddell, sudden death in apparently healthy chickens with mycobacteriosis is stated to often be the result of hepatic rupture and exsanguination into the abdominal cavity (104). However, as Hoop et al. observed, some birds that die suddenly (as reported by the owner) are found on necropsy examination to be emaciated, indicating a chronic process (77). In other cases, the bird appears to be ill for only a few days or weeks before death from mycobacteriosis (77, 122, 124, 125, 139). Van der Heyden commented that many infected pet birds are able to maintain good body condition until the disease becomes disseminated (156).

Diarrhoea, whether chronic or intermittent, is fairly common in birds with mycobacteriosis (73, 104, 106, 108, 122, 156). This finding is readily explained; the oral-faecal route is the most common mode of transmission in naturally-occurring cases of mycobacteriosis in birds, and the intestinal tract is a common site for gross or microscopic lesions. Van der Heyden noted that undigested food, mucus and fat globules may be observed on faecal examination (156); haemoglobin, erythrocytes or leucocytes may also be present in the faeces. Another relatively common presentation is lameness, usually as a consequence of bone or joint involvement (45, 104, 115, 146). This also reflects the distribution of lesions in birds following mycobacterial infection.

Less common, but widely-reported manifestations are cutaneous, subcutaneous or ocular granulomatous lesions (13, 14, 45, 73, 75, 101, 114, 115, 141, 145, 153, 162). Several authors caution that mycobacteriosis should be included on the differential list for any bird with such lesions. In birds of prey, cutaneous mycobacterial lesions are more often found on the limbs, breast or throat; in buzzards, abscesses on the feet are commonly infected with mycobacteria (96, 140, 160).

Dyspnoea and other respiratory signs are less common, presumably because pulmonary involvement is not very common in birds (77, 116). Dyspnoea with stridor has been reported in a few cases; on clinical or necropsy examination, granulomatous masses were found in the infraorbital sinuses, oropharynx or trachea (1, 73, 141). Neurological signs, a
consequence of vertebral or central nervous system involvement, are occasionally reported (94, 96, 106).

Mycobacteriosis can be an insidious problem in a flock or aviary because many infected birds are clinically normal (35, 82, 107, 115, 122, 132, 136, 138, 139). Although severe outbreaks have been reported (118), a history of prolonged illness and/or periodic death of one or two birds in a flock is more common (104, 122, 139).

**Complete blood count and serum biochemistry**

In individual birds, haematology can be a useful screening tool as systemic mycobacteriosis often causes a marked leucocytosis (the result of heterophilia, monocytosis, or both) and mild to moderate anaemia (17, 64, 91, 128). Bush et al. compared the total white blood cell (WBC) count in healthy quail, quail experimentally-infected with *M. avium*, and birds with naturally-occurring mycobacteriosis (17). The average WBC count in uninfected quails was 4,750 cells/mm\(^3\). Following experimental infection, the average WBC count increased to 8,650 cells/mm\(^3\) in mildly affected birds and 26,250 cells/mm\(^3\) in severely affected birds. In clinical cases, the average WBC count was over 62,000 cells/mm\(^3\).

Hawkey et al. reported WBC values of a similar magnitude in domestic fowl and various species of cranes with mycobacteriosis (64). The mean WBC count in infected domestic fowl was 52.5 x 10\(^9\)/l (normal count for this population: 6.6 ± 1.9 x 10\(^9\)/l), with values ranging from 10.3 x 10\(^9\)/l to 230 x 10\(^9\)/l. Values were similar in the cranes. Leucocytosis was found in 92% of infected fowl and 100% of infected cranes. Monocytosis was detected in all infected birds. Heterophilia was found in 81% and 100% of infected fowl and cranes, respectively. The authors noted that the magnitude of the haematological response was related to the severity of the infection. Also of note were the prevalence of hyperfibrinogenaemia (62% of infected fowl and 100% of infected cranes tested) and thrombocytosis (50% of infected fowl and 6% of infected cranes tested).

The mycobacteriosis control programme described by Bush et al. used an upper limit of 18,000 WBC/mm\(^3\) (17). Although leucocytosis is not specific for mycobacteriosis, and not all birds with mycobacteriosis develop leucocytosis, these authors felt that the combination of physical examination and haematological testing was useful for identifying birds with moderate or advanced mycobacteriosis.

As performing haematological tests on a large group of birds is labour-intensive, Cromie et al. used a visual assessment of buffy coat thickness and estimate of packed cell volume as a preliminary screen for mycobacteriosis (31). Any sample with an increased buffy coat was subjected to a full haematological profile. In this study which compared diagnostic techniques in a group of waterfowl, haematological tests were not very reliable, identifying only 58% of diseased birds. False-negative and inconclusive results were common.

Mild to moderate elevations in serum aspartate aminotransferase have been reported in pet birds with mycobacteriosis (1, 73), but as Van der Heyden observed, serum biochemistry values are often unremarkable in pet birds with mycobacterial infections (156). Bush et al. reported a moderate increase in total plasma protein in quail mildly affected following inoculation with *M. avium* (17). In contrast, the increase in severely affected birds was not as great, and the average total protein in naturally-infected birds was no different from that in healthy birds, despite other haematological changes (marked leucocytosis and moderate anaemia). Van der Heyden commented that serum albumin levels are often reduced in birds with chronic disseminated disease, probably as a result of intestinal loss and decreased hepatic production (156). In some cases, serum globulin values may be elevated at some stage during the course of the disease (73, 156).

**Radiography and ultrasonography**

Whole-body radiography can be useful in identifying the bony lesions sometimes present in birds with disseminated mycobacteriosis (17, 115, 156). Findings of significance include bone lysis and/or sclerosis consistent with osteomyelitis, osteophytosis surrounding arthritic joints, and pathological fractures. These lesions are most often found in the mid-shaft of the long bones.

In comparison, radiography is unreliable for identifying lesions in the organs most commonly involved, such as the liver and spleen (17). Mycobacterial lesions in birds generally do not calcify, even when the lesions are grossly tuberculous. Thus, radiography may miss the majority of infected birds if used as a sole screening tool. An exception is in severe disseminated cases, where radiography may be useful. In a green-winged macaw with disseminated *M. tuberculosis* infection, enlargement of the cardiac-hepatic silhouette was evident radiographically (73).

Similar to radiography, ultrasonography has limitations, but can be utilised to assess coelomic masses and changes in organ size and parenchymal architecture.

**Laparoscopy**

Laparoscopy is a useful technique for identifying mycobacterial lesions on the serosal surfaces of the liver, spleen, intestine, lung or air sacs in valuable or pet birds (17, 156). Not only does laparoscopy allow visualisation of the organs in the coelomic cavity, the technique also allows biopsy of tissues (e.g., liver and spleen) or discrete lesions. The granulomas have been described as white or tan in colour, and are rounded, in comparison to the disk-like lesions typical of aspergillosis (156). Mycobacterial lesions can be mistaken for neoplastic masses, so biopsy should be performed on any masses identified during laparoscopy.
A drawback of this technique is that laparoscopy requires sophisticated and expensive equipment. The technique also necessitates general anaesthesia, which has inherent risks in a systemically ill bird, although Bush et al. considered the risk to be minimal (17).

Microscopic evaluation

Mycobacteria are non-spore-forming, non-motile bacilli. The complex lipid-rich cell wall and thick mycolic acid layer renders the organisms acid-fast, meaning that they retain the colour of arylmethane dyes such as fuschin after being rinsed with a dilute acid (55). The usual method of identifying these bacilli in biopsy or faecal specimens is the Ziehl-Neelsen (carbol-fuschin) staining technique. Mycobacteria are rod-shaped bacteria; M. avium tends to be somewhat pleomorphic, so can appear almost coccal or as long beaded rods (2). Depending on the chronicity and severity of the infection, these acid-fast organisms may be found either intracellularly (within tissue macrophages or epithelioid cells) or extracellularly in clinical samples. Using an acid-fast stain such as Ziehl-Neelsen, the rods are pink-red in colour. If routine stains are used, non-staining ('ghost') rods may be seen intracellularly (156). Forster recommended using the modified Ziehl-Neelsen staining technique (the Fite-Faraco procedure), in which peanut oil is used with xylol at the beginning of the staining process (49). The addition of peanut oil limits damage to the lipid layer of the mycobacterial cell wall by the xylol. Aranaz et al. suggest that fluorescent dyes, such as auramine or acridine orange, are superior to the conventional fuschin stains because the mycobacteria are rendered more visible and a larger area of the smear can be examined in a shorter time (2).

Cytological examination is rapid, inexpensive and provides immediate results, but organisms can be easily missed if numbers of mycobacteria are low. The limit of detection for cytological specimens is estimated to be 10,000 bacilli/ml; by comparison, culture has a detection limit of 100 bacilli/ml (18). Debris in the sample can further hamper cytological identification of these organisms, therefore faecal samples, whether from clinically normal birds shedding mycobacteria or from diarrhoeic birds, may be negative on cytological examination. Ensly et al. found that acid-fast staining of tracheal and cloacal swabs was unsatisfactory for screening birds in a zoological collection for mycobacterial infections (41). Mycobacteriosis should not be ruled out on the strength of a negative faecal cytological examination. Conversely, diagnosis of mycobacteriosis should not be based on the presence of acid-fast organisms in the faeces, as non-pathogenic mycobacteria may pass through the gastrointestinal tract or, if the faecal sample was harvested from the enclosure, the organisms may be environmental contaminants. Therefore, positive faecal examination should be confirmed by either culture or DNA probe analysis of faeces which have been collected directly from the bird, and not from the environment.

Intradermal tuberculin test

The intradermal tuberculin test has been used widely in poultry for decades (44, 111). In addition to management changes, this has been a valuable tool in the virtual eradication of mycobacteriosis from commercial flocks in most countries. The test uses 0.1 ml of avian tuberculin, injected intradermally into the wattle or comb of the chicken. The injection site is then assessed 48 h-72 h later. Thickening at the injection site is considered a positive reaction.

The test is much less useful in other avian species. Most species of birds do not have featherless wattles or combs, so the operator must find or create a featherless area in which to inject the tuberculin. Sites in which this test has been attempted include the vent, wing web, eyelid, the skin over the hock, and in ratites, the skin just caudal to the auditory opening (17, 136). A further drawback to the test is that the birds must be handled twice: once to inject the tuberculin and once, two to three days later, to assess the reaction. This requirement renders the tuberculin test impractical for groups of free-ranging birds, such as those in wildlife reserves (64). The potential for handling trauma in wild birds is also increased. However, more importantly, the test has proven unreliable in several species of birds, including pigeons, geese, quail, raptors and various exotic species (17, 41, 96, 110, 115), false-negative results being the usual finding.

False-negative results can even be a problem in poultry. Following oral challenge with a virulent strain of M. avium serotype 2, only 48% of pullets reacted positively to avian tuberculin (111). In part, due to cross-reactivity, false-positive results are also possible. Pavlas et al. reported reactions to avian tuberculin on some tuberculosis-free poultry farms (111). Contamination of the sawdust litter with M. intracellulare (serotype 8) was determined as a frequent cause of such reactions. A rapid agglutination test (see below) proved more sensitive and specific, and the authors recommended parallel screening of flocks with both intradermal tuberculin testing and rapid haemagglutination for elimination programmes, or use of rapid haemagglutination alone for screening of poultry flocks.

Haemagglutination

Haemagglutination tests on samples of whole blood or serum have been used for decades to identify birds with mycobacteriosis (31, 64, 71, 92, 111, 129). The test involves combining a drop of prepared antigen with a drop of blood or serum on a white tile. The presence and degree of agglutination is subjectively scored within the first thirty to sixty seconds, while gently agitating the tile (31, 64).

This test has the advantages of requiring only one blood sample (and thus, single handling of the birds), being rapid and simple to perform, requiring no sophisticated or expensive equipment, and providing an immediate result. The test has been demonstrated to be of some use in ducks,
birds naturally infected with *M. avium* were examined for the presence of complement fixing antibodies to *M. avium*. Phalen *et al.* conducted a study in which sera from a variety of avian species (24, 31, 33, 47, 149), including domestic fowl (64, 92, 96, 110, 111), were tested at higher dilutions. All birds with serum antibody titres of 1:20 or greater can be placed in one of the following categories:

- **a)** confirmed *M. avium* infection
- **b)** clinical disease consistent with *M. avium* infection
- **c)** exposure to birds with *M. avium* infection
- **d)** grey-cheeked parakeet (*Brotogeris pyrrhopterus*).

In most cases, a titre of 1:10 did not correlate with either clinical disease or exposure. Serum from an ostrich (*Struthio camelus*) with localised pericocular *M. avium* infection was negative for antibodies to the clinical isolate. Serum from a turtle dove (*Streptopelia turtur*) with radiographic evidence of *M. avium* infection and acid-fast bacilli present in the faeces was negative for antibodies to all isolates. Phalen *et al.* suggested that failure to detect infection in these birds could indicate a failure of antibody production or infection with a non-cross-reactive serotype (113). Use of multiple antigens may be necessary to improve the sensitivity of the assay.

**Enzyme-linked immunosorbent assay**

Enzyme-linked immunosorbent assays have been developed for identification of mycobacterial infection in a few avian species (24, 31, 33, 47, 149). While these tests are sensitive, specificity relies on the use of highly specific antigens (34).

Cromie *et al.* developed an ELISA which was both highly sensitive and specific in feral barnacle geese (31). The antigens used were from *M. fortuitum*, *M. vaccae*, two local clinical isolates of *M. avium*, and a secreted antigen of *M. avium*, with the type of sample used for haemagglutination tests may also affect the sensitivity of the test (111). This is corroborated by the study of Cromie *et al.*, in which a haemagglutination test was evaluated in a flock of feral barnacle geese (*Branta leucopsis*) (31). The antigen used for the haemagglutination test was prepared from a field isolate of *M. avium* serotype 1. The test was performed in triplicate, using fresh whole blood, whole blood in ethylenediaminetetraacetic acid (EDTA), and serum. Of the three sample types, fresh whole blood had the highest sensitivity (100%, compared with 29% for whole blood in EDTA and 77% for serum). However, testing fresh whole blood in the field was abandoned because the antigen underwent autoagglutination when particles of skin or feathers fell into the sample. Serum had the highest specificity (86%, compared with 50% for fresh whole blood and 57% for whole blood in EDTA). The high proportion of false-negative results for samples collected into EDTA indicates that this anticoagulant should not be used when performing this test.

Pavlas *et al.* demonstrated that the haemagglutination test could also be used to differentiate between non-specific (para-allergic) and specific reactions to avian tuberculin (111). As mentioned in the preceding section, a number of positive tuberculin reactors were found on tuberculosis-free poultry farms. The haemagglutination test using the *M. avium* antigen was useful in determining that many of these were non-specific reactions, subsequently discovered to be due to a non-virulent mycobacteria isolated from the litter.

Various authors have compared haemagglutination tests with other screening procedures, such as haematology, intradermal tuberculin testing, and ELISA, in a variety of avian species (15, 31, 64, 110, 111). The haemagglutination test is not 100% reliable in identifying infected birds, although this test is possibly more sensitive than haematology and intradermal tuberculin testing (15, 31, 64, 110, 111). Both false-positive and false-negative results can occur, the former at such a high rate that the test is no longer used in certain captive waterfowl collections in the UK.

**Complement fixing antibody titres**

Phalen *et al.* conducted a study in which sera from a variety of birds naturally infected with *M. avium* were examined for the presence of complement fixing antibodies to *M. avium* antigens (113). The antigens included three serotypes (1, 2 and 8) and one untyped clinical isolate of *M. avium*. Sera were initially tested at a dilution of 1:10; positive samples were then tested at higher dilutions. All birds with serum antibody titres of 1:20 or greater can be placed in one of the following categories:

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- **c)** exposure to birds with *M. avium* infection
- **d)** grey-cheeked parakeet (*Brotogeris pyrrhopterus*).

In most cases, a titre of 1:10 did not correlate with either clinical disease or exposure. Serum from an ostrich (*Struthio camelus*) with localised pericocular *M. avium* infection was negative for antibodies to the clinical isolate. Serum from a turtle dove (*Streptopelia turtur*) with radiographic evidence of *M. avium* infection and acid-fast bacilli present in the faeces was negative for antibodies to all isolates. Phalen *et al.* suggested that failure to detect infection in these birds could indicate a failure of antibody production or infection with a non-cross-reactive serotype (113). Use of multiple antigens may be necessary to improve the sensitivity of the assay.

Although this ELISA proved to be useful in controlling mycobacteriosis in this flock of feral geese, the authors noted that the technique is labour-intensive, requires at least 24 h to generate results, and uses anti-duck antibody, and is therefore unsuitable for use in other avian genera (47). Further use of this assay with a broad range of waterfowl species demonstrated that the test was capable of detecting 70% of the tuberculous birds that would die from mycobacteriosis during the following year, although the rate of false positive results was high (27%) (33). The assay was also noted to be poor for detection of antibodies from some taxonomic tribes of waterfowl.

Clark *et al.* developed an ELISA for detection of antibodies against *M. avium* in chickens and quail, although at the time
of reporting, the assay was still primarily a research tool (24). As noted by these authors, the extent of cross reactivity among immunoglobulins of diverse avian species will determine the usefulness of this and other ELISAs for diagnosis of mycobacteriosis in birds.

**Culture**

Culture is a definitive means of confirming mycobacterial infection in birds, although the technique has several practical limitations. The mycobacteria which are pathogenic for birds are slowly growing organisms, having a replication time of at least 15 h. Two to four weeks can be required for visible colonies to appear on culture media, and some strains of *M. avium* require up to six months before colonies are identifiable (2, 18). Mycobacteria require special culture media and have particular growth requirements in terms of substrate, nutrients, temperature and oxygenation which may vary according to the species (2, 56). The sample must therefore be submitted to a laboratory with experience in culture of these organisms. Often no growth of cultured mycobacteria occurs, even when appropriate protocols and growth media are used. A bird may have lesions typical of mycobacteriosis with confirmed acid-fast bacilli, but cultured samples fail to grow, producing a false-negative result. *Mycobacterium genavense* is particularly fastidious, and special culture techniques and DNA probes are required to identify this organism (21, 77, 78).

The length of time required for culture has been reduced by a radiometric culture technique, namely, the BACTEC system. This process involves culture using a liquid medium which contains a radiopharmaceutical agent (14C-palmitic acid). Mycobacterial growth is detected by monitoring the production of 14CO2 in the culture medium. This system can halve the detection times of conventional mycobacterial culture. The sensitivity of culture is also improved, the culture rate is often higher than using conventional methods (2). Using this technique, results may be obtained in as little as two weeks. Isolation of *M. avium* from faecal and tissue samples in birds infected either experimentally or naturally has been successfully performed using this technique (24). The main limitation is that the radiometric technique requires specialised and expensive equipment, hence only certain laboratories offer this culture method.

Hoep et al. found the BACTEC system particularly valuable for isolating *M. genavense* from pet birds (78). Only three of thirty-four *M. genavense* isolates grew on conventional solid media. However, the radiometric culture technique failed to detect only eleven of the thirty-four isolates. The authors concluded that amplification by polymerase chain reaction (PCR) and subsequent sequencing of the 16S ribosomal ribonucleic acid (rRNA) gene is not only the method of choice for species identification, but is very often the only possible means of detecting *M. genavense* in tissue samples; Chevrier et al. concurred (21).

Once growth is evident on culture, identification to the species level has traditionally been based on physical characteristics of the colonies, such as growth rate and pigment production, and on drug sensitivity patterns and other biochemical tests. Biochemical testing for species identification can add two to three weeks to the time lag between sample submission and reporting of results (18). Recent development of rapid assays, such as high-performance liquid chromatography and DNA probes which are species-specific for mycobacterial rRNA, now allow accurate species identification in a few hours (2, 18). This is an advantage, because proper identification of the species (and the serotype in the case of *M. avium*) can be important for epidemiological investigations.

**Molecular techniques**

Molecular techniques to identify specific antigens or gene sequences have revolutionised the detection and species identification of pathogenic mycobacteria in clinical samples. Many variations exist, but the basic process involves release of nucleic acids from the mycobacterial cells, amplification using PCR or another technique, and identification of target antigens or gene sequences. Butcher et al. present a comprehensive overview of the molecular techniques used for diagnosis of mycobacterial diseases (18). These techniques can be applied to organisms grown on culture media, clinical materials (faeces, tissue samples taken at biopsy or necropsy), and even to formalin-fixed, paraffin-embedded sections (2, 6, 62, 98). Further advantages over other diagnostic techniques include rapid results (in one to three days), the ability to detect mycobacteria when very few organisms are present in the sample, and accurate identification of the mycobacterial species (2, 97).

Although these techniques were developed for detection and species identification of pathogenic mycobacteria in human patients, numerous reports exist of experimental and clinical use in birds (1, 10, 40, 48, 73, 74, 77, 78, 116, 122; 133, 139, 141, 152, 162). Thornton et al. compared the sensitivities and specificities of smear, culture and PCR on a variety of specimens (blood, bone marrow, bursa and faeces) from ducks with confirmed mycobacteriosis (152). In this study, the specimens were processed with the zwitterionic detergent C18-carboxypropylbetaine (CB-18), a substance which improves both smear and culture sensitivity for detection of mycobacteria in human respiratory specimens. The authors proposed a model for initial screening of flocks by processing faecal samples with CB-18 and analysing sediments using PCR. Independent of specimen type, the sensitivities relative to gross and histological post-mortem findings were 44% for smear, 89% for culture, and 100% for PCR. The specificities were 84% for smear, 58% for culture, and 16% for PCR. The lower specificities for culture and PCR were primarily due to results obtained from faecal specimens. In several birds, the positive faecal PCR results for birds that tested negative at post-mortem were confirmed by smear and culture. The authors suggested that the number of PCR-positive faecal
samples among post-mortem-negative birds was indicative of the sensitivity of the test in identifying birds in the early stages of disease (i.e. before post-mortem abnormalities are evident), rather than a high incidence of false-positive results.

Ford and Eriks described a method of removing extraneous substances from faecal samples to improve the sensitivity of the PCR/restriction enzyme assay for faecal samples in birds (48). The method involves the use of magnetic beads coated with a monoclonal antibody which is specific to a cell wall protein found in mycobacteria.

Post-mortem diagnosis of avian mycobacteriosis

In avian species, lesions of avian mycobacteriosis have distinct characteristic features which are most frequently seen in the liver, spleen and intestines, although any tissue may be affected. Tissue predilection, the nature of the lesion, and species resistance may be associated with the avian species infected, the species of the mycobacteria, and the immune status of the infected bird, in addition to numerous other factors.

Retrospective studies of avian mycobacteriosis in large numbers of single avian species have helped to map out common organ predilection in specific species. In a 1914 study, necropsy findings at the North Dakota Agricultural Experimental Station and four previous studies on tuberculosis in chickens were summarised (159). In this study, the liver was the most commonly affected organ followed by the spleen and intestines. The next most commonly affected tissues, although affected dramatically less often than the aforementioned organs, were lungs, kidneys, bones, joints, ovaries, gizzard, skin and heart. Liver, spleen, intestines and bone marrow were the tissues most frequently affected in chickens in two other studies (43, 63).

Liver and spleen are also most frequently affected in other species. Infected geese and ducks have lesions primarily in the liver and spleen, with occasional infection of the intestines and lungs (120). A para-tuberculosis-like lesion with primary infection of the intestine can occur in species of Amazona, Pionus, Brotogeris and Psittacula, and the horned parakeet (Eunymphicus cornutus) (52).

The frequency of lesions in the bone marrow and bones is extremely variable, reports in the literature ranging from 2% to 93% (43). Reports of bony lesions due to mycobacteria are rare in geese (87). In a retrospective study of necropsy findings in mixed species of birds from the National Zoological Park of the USA, 10% had some type of osseous lesion due to mycobacteria (100). Reports of *M. avium* infection in the bones appear to be more numerous in raptors than in other avian species. In one report, a falcon had swelling and osteolysis of the proximal tibia, fibula, patella and femur, and granulomas in the liver (96). Lesions in the breast, throat, limbs and feet were described in buzzards. The same report suggested that bumblefoot lesions infected with mycobacteria are common in buzzards and may be a result of contamination and infection of wounds (160).

Numerous case reports in the literature describe atypical distribution of mycobacterial lesions in single birds. Periocular and ocular infections in a rattle, a red-tailed hawk (*Buteo jamaicensis*) and a parrot have been described (75, 145, 147). Dermatitis is occasionally associated with systemic *M. avium* infection in psittacine birds (37). Skin and lungs are the common sites of infection with *M. tuberculosis*. Nodules on the eyelids, conjunctiva, unfeathered areas of the face and head, and in the oral cavity were reported in a green-winged macaw (*Ara chloropterus*) infected with *M. tuberculosis* (162). The soft tissue of the legs of an Amazona parrot with *M. avium* infection was affected in the absence of skeletal lesions (95). Granulomatous aortitis and cardiopulmonary arteritis was reported in fairy-bluebirds (*Irena puella*) with mycobacteriosis (103). Cerebral tuberculosis has been described in a chicken (106), a red-tailed hawk (147) and a goshawk (*Accipiter gentilis*) (161).

The nature of the lesions caused by mycobacterial infections may vary according to the infected avian species. Granuloma formation in the intestine and reticuloendothelial organs is the typical pathology of mycobacterial infections in Falconiformes, Strigiformes, Galliformes, Ciconiiformes, Cuculiformes, Piciformes and Gruiformes. In contrast, granulomas are generally absent in Columbiformes, Anseriformes, Passeriformes and Coraciiformes and has also been reported in a kingfisher (58, 124). Canada geese (*Branta canadensis*), mute swans (*Cygnus olor*), tundra swans (*Cygnus columbianus*) and some parrots develop asciites and diffuse enlargement of the liver and spleen due to generalised amyloidosis (163). In some species, lesions in the lungs are more necrotising, particularly in geese and weaver finches (52). Polycystic livers have been reported to be associated with *M. avium* infection in waterfowl (126).

The immune status of the infected bird may also affect the nature of the lesion formed. Stress has been demonstrated to induce earlier lesions in experimentally-infected birds (20). Another study demonstrated that the numbers and nature of the lesions were influenced by both genetic and environmental factors. The numbers of granulomas with necrotic centres increased over the numbers of granulomas with central diffuse histiocytic inflammation in stressed birds (60).
**Gross pathological findings**

The overall body condition of birds with clinical mycobacteriosis is poor. Muscular wasting and loss of subcutaneous and cavity fat are common, as well as other signs of health deterioration. Feathers may be discoloured, worn with damaged edges and fail to exsheath (156).

Affected organs may have nodular contours with randomly distributed tan or yellow granulomas of variable size, or the organs may be uniformly enlarged with diffuse miliary foci (granulomas) or a uniform pale colour (63). Enlarged, uniformly coloured parenchymatous organs may have non-tuberculoid histiocytic inflammation, or histiocytosis and diffuse amyloidosis. Hepatic rupture and haemorrhage is sometimes seen in birds with enlarged, pale, firm livers, due to amyloid deposition (43).

Granulomas may vary in size from pinpoint foci to several centimetres in diameter. Granulomas at the surface of organs can sometimes be enucleated from the surface of the involved organ. In other cases, protruding tan nodules are firmly attached and may appear as multinodular tumorous growths. Some nodules may have central caseous material; some may have homogeneous tan/white centres. Nodules may be distinctly delineated from, and compress the surrounding parenchyma of the affected organ, or tan firm nodules may blend into the affected tissue, particularly with nodules in the intestinal wall.

Paratuberculosis-like lesions can occur in *Amazona*, *Pionus*, *Brotogeris* and *Psittacula* species, and in the homed parakeet (52). This lesion is characterised by tube-like thickening of the intestine. The mucosa of the affected intestine has very prominent thickened or clubbed villi, giving the mucosa a 'shaggy carpet' appearance. The intestine may be the only organ in which lesions are seen grossly, but often scattered accumulations of macrophages are also present in the liver and spleen. In birds with disseminated classic granulomas, tubercles may be present in the wall of the intestine and, less frequently, in the serosa.

Lesions of non-parenchymatous tissue may be ulcerative and caseating. Ulcerative caseating lesions have been described in the nasal- and oral-pharynx in birds, mimicking trichomoniasis or pox. Additionally, abscesses can occur in the liver of Columbiformes, thereby mimicking trichomoniasis (52).

Peri-ocular and ocular infections have been reported in birds. Peri-ocular tissues found to be infected have included palpebral and retrobulbar tissue. Infected ocular tissue has included pectin and cornea (14, 145, 147, 164). Keratitis accompanying systemic dissemination of *M. avium* in a Maximilian's parrot (*Pionus maximiliani*) was thought to be predisposed by corneal trauma (145). A papillary mass on the conjunctiva of an ostrich had multiple surface nodules of granulomatous inflammation with numerous intranuclear acid-fast bacilli (135).

Bony lesions may be proliferative or lytic with subsequent pathological fractures. In a report of mycobacteriosis in a wood duck, ulcerative caseating lesions over the tibiotarsal joint extended into the bones (45). One study suggested that the ribs and breast bone were the bones more commonly infected (63).

Polycystic livers associated with *M. avium* infection have been reported in waterfowl. The enlarged livers were mostly comprised of serosanguinous fluid-filled, thin-walled cysts, varying in size from 2 mm to 3 cm (126). A single fluid-filled cyst was reported in the lung of a cockatoo (112).

**Microscopic findings**

Three different types of lesions are recognised in avian species with tuberculosis, as follows:

a) dissemination of tubercles in various tissues and organs

b) a paratuberculosis-like form with primary lesions in the intestinal tract

c) a non-tuberculoid form in which gross enlargement of organs may or may not be observed (52).

A few classic descriptions of mycobacterial tubercles are presented in the literature. Two forms of mycobacterial lesions are described in domestic animals, namely: a proliferative form in which central necrosis is surrounded by granulomatous inflammation and fibroplasias, and an exudative form in which the lesion is comprised of caseated fibrin, heterophils and mononuclear inflammatory cells (38). Other authors describe three types of nodules (63). The first type of nodule having round, oval to flattened epithelioid cells surrounded by mononuclear cells distinctly delineated from surrounding tissue. This first nodule has minimal caseation. In the second form, degenerating cells stain less clearly and the cell structures are broken down into colourless debris surrounded by epithelioid cells, mixed mononuclear cells and sometimes heterophils. The third type of nodule is typically larger and has considerable central caseous necrosis which may be homogeneous and hyalinised. This nodule may show concentric stratification surrounded by epithelioid cells, maybe flattened by compression, surrounded by a connective tissue capsule. Calcification is rarely seen in birds. Sometimes granulomas in infected birds have a monocellular population of histiocytes lacking pleocellular inflammation, suggesting a less than complete immune response, possibly due to immunosuppression.

Enlargement of the liver and spleen is common and may be due to widely distributed granulomas of variable size, diffuse
histiocytic infiltration or to deposition of amyloid. Amyloid occurs mainly in the liver, but is also seen in the spleen and in blood vessels and parenchyma of many organs (41). Granulomas vary in size, may be randomly distributed, and multifocal to confluent in heavily infected organs. Several types or forms of granulomas may be present in an organ, some with diffuse sheets of histiocytes and epithelioid cells and some with necrotic centres.

In birds with the paratuberculosis-like form, fusion of the villi of the intestinal mucosa may occur, together with villous expansion by diffuse granulomatous infiltrates composed of sheets of macrophages, epithelioid cells or sometimes multinucleated giant cells. A report of a Johnes's disease-like lesion in wood pigeons (Columba palumbus) and psittacine birds in the Netherlands probably describes the paratuberculosis-like form (158).

Non-tuberculoid lesions may cause enlargement of the affected organ through diffuse infiltration by large foamy histiocytes. In a kingfisher, foamy histiocytes with intracytoplasmic acid-fast bacilli diffusely infiltrated the liver causing marked displacement and atrophy of hepatic plates (124). Similar non-tuberculoid lesions have also been reported in Passeriformes and Coraciiformes (58).

Acid-fast staining of granulomatous tissues typically reveals large numbers of acid-fast bacilli in contrast to other Mycobacterium spp. such as M. bovis and M. tuberculosis, in which organisms are rare within tubercles (156).

Polycystic livers associated with tuberculosis have been reported in ducks (126). Cystic granulomas may efface large portions of the hepatic architecture in polycystic livers. Cystic walls comprised of folding bands of connective tissue with macrophages and rare multinucleated cells may be collapsed and cystic spaces may contain exfoliated cells. Acid-fast bacilli may be present within macrophages in the wall or in exfoliated cells in cystic spaces.

Periocular and ocular lesions reported have been palpebral and retrobulbar. Keratitis which was probably predisposed by corneal trauma is described in one case report as subcorneal granulomatous infiltrates between the epithelium and the anterior limiting membrane (145). The epithelium was hyperplastic.

In the report on the wood duck, chondritis, osteitis and osteomyelitis with lysis of bony trabeculae were observed in the nasal bone. Nasal passages had caseating and non-caseating necrotic areas surrounded by a granulomatous inflammatory reaction with multinucleated giant cells and numerous macrophages, heterophils, lymphocytes and plasma cells (45). Indirect microscopic lesions include serous atrophy of fat and lymphoid atrophy.

Treatment of mycobacteriosis in birds

Treatment of avian mycobacteriosis remains controversial and essentially unfeasible. Owing to the infectious nature of this disease to other birds and mammals, the debilitating effects to the diseased individual bird and the zoonotic potential (although slight), a case could be made for the humane euthanasia of all infected birds. Another consideration is the potential for poor compliance by the owner, leading to the selection for multi-drug resistant mycobacteria. In combination with the potential zoonotic risk, this should discourage the use of anti-mycobacterial drug treatment regimes in birds.

Despite these problems, treatment is sometimes undertaken in pet birds. Before treatment is initiated, the owner should be advised that therapy may need to continue for several months, and even then may not effect a cure. Owner commitment is essential for a successful outcome, and lack of compliance is a common source of treatment failure. In addition, the owner should be encouraged to consult his or her physician for examination and screening.

Antimicrobial therapy

*Mycobacterium avium* is notoriously resistant to anti-mycobacterial drugs. With certain discrete cutaneous, subcutaneous, or ocular masses, surgical excision is possible and may be curative. However, with more disseminated forms of mycobacteriosis, systemic antimicrobial therapy may be indicated. Initial treatment of tuberculosis (M. tuberculosis infection) in humans typically involves a combination of three or four different antimicrobials. The three key drugs are isoniazid, rifampicin and pyrazinamide (99). Other drugs which may be added to the regimen include ethambutol, rifabutin, aminoglycosides (streptomycin, kanamycin, amikacin, aminosidine), semisynthetic erythromycin derivatives (azithromycin, clarithromycin), fluoroquinolones (ciprofloxacin, enrofloxacin) and clofazimine. Van der Heyden presents an excellent review of the use of antimycobacterial drugs in birds (157).

*Mycobacterium avium* complex organisms isolated from human patients are reportedly sensitive in *vivo* to rifampicin, rifabutin, ethambutol, amikacin, azithromycin, clarithromycin, clofazimine and ciprofloxacin, but resistant to isoniazid and pyrazinamide (157). The pharmacokinetics of most of these drugs in birds are unknown. Although case reports have described successful treatment of mycobacteriosis in birds, no controlled studies in birds have been reported to date.

Van der Heyden recommends commencing treatment of mycobacteriosis in birds with a combination of rifabutin (56 mg/kg/day), ethambutol (56-85 mg/kg/day), isoniazid (5-10 mg/kg/day) and either rifampicin or clarithromycin (50 mg/kg/day).

Van der Heyden recommends commencing treatment of mycobacteriosis in birds with a combination of rifabutin (56 mg/kg/day), ethambutol (56-85 mg/kg/day), isoniazid (5-10 mg/kg/day) and either rifampicin or clarithromycin (50 mg/kg/day).
and either azithromycin (43 mg/kg/day) or clarithromycin (85 mg/kg/day) (157). Successful treatment has been reported using lower doses than these (128, 156). In birds which respond poorly or relapse, a fluoroquinolone or an aminoglycoside (15 mg/kg twice a day for either drug class) should be added to the regimen. Each of these antimycobacterial drugs can be administered orally, in a small amount of soft food or palatable liquid. Repeated handling of birds, particularly captive wild birds during treatment, is not advised due to the possibility of stress exacerbating the disease.

Immunotherapy

Immunotherapy with a killed preparation of M. vaccae has been used in the treatment of tuberculosis in humans (143). This preparation appears to have some immunomodulatory effects in tuberculous patients by altering the pattern of cytokine release and reducing sensitivity to the toxic effects of tumour necrosis factor. In efficacy studies, the addition of immunotherapy to conventional chemotherapy (combinations of anti-mycobacterial drugs) improved one-year survival rates and decreased the percentage of patients with acid-fast bacilli in the sputum, particularly in situations in which few patients took more than one month of adequate chemotherapy. Clinical effects (weight gain, improved comfort) and a decrease in the percentage of patients with acid-fast bacilli in the sputum were observed after only ten days (144).

Immunotherapy with M. vaccae may be particularly useful for treatment of multidrug-resistant tuberculosis when compliance to chemotherapy is poor, and when concurrent immunological problems exist. At least two of these scenarios (drug resistance and poor compliance) are common obstacles in the successful treatment of mycobacteriosis in birds. This treatment was used in a small trial performed in a captive waterfowl collection in the UK (16), although results were inconclusive.

Control/prevention of mycobacteriosis in avian collections

The widespread adoption by the poultry industry of management practices aimed at controlling mycobacteriosis has demonstrated amply that this disease can be virtually eliminated when the environment of the bird can be controlled. These management practices are based on the following two principles:

a) identification and either elimination or permanent segregation of infected birds
b) strict hygiene practices to minimise contact with faeces, soil and other potentially contaminated materials.

These principles can be adapted for effective control of mycobacteriosis in pet birds, private and commercial aviaries, and zoological collections (7, 36, 137). Control of mycobacteriosis presents unique challenges in wildlife reserves in which the population comprises or includes free-ranging birds (32).

Husbandry

The generally accepted methods for controlling avian tuberculosis in poultry include identification and eradication of infected birds using intradermal tuberculin testing, replacement of contaminated housing and equipment, and isolation of new stock from the contaminated environment by rearing and maintaining new stock on wire or concrete floors (42, 53, 83, 104). Avoiding overcrowding and other stressors, and ensuring that the birds are fed a nutritionally complete and balanced diet are also important in minimising the incidence and impact of this disease (76).

If a decision has been made not to euthanise, birds with confirmed mycobacteriosis should be kept permanently separated from other birds, regardless of whether the bird is undergoing or has completed anti-mycobacterial therapy (76). Pet or captive birds should be housed in cages or aviaries in which contact is prevented between birds in different enclosures and contact with soil, surface water, faeces, feathers, discarded food, or other potentially contaminated material is minimised (76). Any in-contact birds should be segregated for at least twelve months and periodically tested. Birds demonstrating clinical or bacteriological evidence of infection should be separated permanently from the rest of the group. Good sanitation practices are also essential for the effective control of mycobacteriosis (41). Cages or pens and all water and food containers should be thoroughly cleaned and disinfected daily. Faecal matter, litter, any plant material and organic debris that may have been contaminated should be removed from the cage or pen and incinerated or disinfected. Infected aviaries should have the topsoil removed and this soil and the soil beneath it should be limed, as a high pH appears to reduce M. avium load. Personnel attending infected birds should take appropriate precautions to avoid spreading potentially contaminated material from infected birds and enclosures. Strategically placed disinfectant foot baths may help to limit the spread of contaminated material from one area to another (17). Attention should be paid to other potential fomites such as crates and wheels of feed barrels. Those caring for the birds should also be advised to practice good personal hygiene, such as washing hands thoroughly after handling a bird or cleaning an enclosure. Use of a face mask which prevents inhalation of dust, dander, or aerosolised material may be a worthwhile precaution in some circumstances (76).

To reduce the incidence of mycobacteriosis, several husbandry practices can be instituted. Ideally, dirt floors should be avoided and substrate materials which are less likely to support mycobacterial growth encouraged (36).
Where possible, enclosures should be constructed or situated to prevent contact with free-living wild birds (83) and pest control should also be considered (36). Any new birds should be quarantined for three to six months and screened by appropriate methods (e.g. physical examination, faecal smears for acid-fast organisms, faecal culture, haematological tests, serological tests) before being introduced into the existing group (76, 141). Hatching of chicks in an incubator or by carefully screened broody hens should also be considered for restocking (64).

Gill and Blandy described successful efforts to control avian tuberculosis in a free-range commercial poultry flock of over 2,000 hens simply by segregating old infected birds from new stock and thoroughly cleaning the pens down to bare earth between batches (53). The ‘flooring’ in the outdoor pens consisted of clay which was brought in from another farm, soaked, and allowed to harden. Birds which had been kept under the former system in which all batches were mixed together were gradually culled over a twelve-month period. Subsequent batches were managed from day-old under the implemented control system. After four and a half years under this system, no reactors to intradermal tuberculin tests were present. However, the absence of macroscopic lesions at slaughter indicated that mycobacteriosis had been eliminated from this flock after only two years. While this approach may have several shortcomings, it was effective in this flock and limited the financial burden on the producer.

A number of management strategies are being tested in captive waterfowl collections in the UK, including reduction in stocking density and general improvements in water quality. The use of reed bed bio-filtration systems for removing contamination from water courses is currently being investigated.

**Biocides**

Mycobacteria are more resistant to biocides (disinfectants) than other non-spore-forming bacteria. The following compounds have mycobactericidal activity:

- alcohols
- aldehydes (formaldehyde, glutaraldehyde, succinaldehyde and glyoxal)
- halogens (chlorine- or iodine-releasing agents)
- peroxygens
- phenolics
- chemosterilising gases (ethylene oxide and betapropiolactone) (130).

However, even at high concentrations, chlorhexidine and the quaternary ammonium compounds are merely mycobacteriostatic. With any biocide, the antimycobacterial activity increases as the concentration and temperature increase. However, long periods of contact may be required for good effect. Chlorhexidine and the quaternary ammonium compounds are less effective in the presence of organic matter (130).

**Vaccination**

Poultry have been the focus of most previous vaccination attempts. The vaccines most commonly tested have been BCG (the human vaccine for *M. tuberculosis* infection) and killed or inactivated strains of *M. avium* (61, 127). None have proved to be highly efficacious. A vaccination trial in captive waterfowl in the UK used killed *M. vaccae*. Despite promising *in vitro* results, the vaccine gave protection to only one of the taxonomic groups of birds in trials. Trials using new doses and timing of vaccination are currently being performed in an attempt to improve efficacy in other groups of birds (33). At present, no commercial vaccines are routinely used for mycobacteriosis in birds.
Mycobactériose aviaire

L.A. Tell, L. Woods & R.L. Cromie

Résumé
La mycobactériose aviaire est une maladie importante qui affecte aussi bien les oiseaux de compagnie ou exotiques en captivité que les oiseaux sauvages et domestiques. La maladie est due le plus souvent à Mycobacterium avium et à Mycobacterium genavense. Les lésions sont surtout observées dans le foie et l’appareil gastro-intestinal, mais de nombreux autres organes peuvent être atteints. Les auteurs étudient les sérotypes de Mycobacterium observés chez les oiseaux, l’épidémiologie de la mycobactériose aviaire, les réponses immunitaires à l’infection mycobactérienne, le diagnostic ante et post-mortem, le traitement, la prévention et la prophylaxie de la maladie.

Mots-clés

Micobacteriosis en aves

L.A. Tell, L. Woods & R.L. Cromie

Resumen
La micobacteriosis aviar es una importante enfermedad que afecta a todo tipo de aves (de compañía, exóticas en cautividad, salvajes o domésticas). Sus agentes etiológicos más comunes son Mycobacterium avium y Mycobacterium genavense. En general la enfermedad causa lesiones en el hígado y el tracto intestinal, aunque también pueden verse afectados otros muchos órganos. Los autores pasan revista a las especies de Mycobacterium que afectan a las aves, y repasan la epidemiología de la micobacteriosis aviar, las respuestas inmunitarias a la infección por micobacterias, el diagnóstico antemortem y postmortem, el tratamiento y la prevención o control de la enfermedad.

Palabras clave
References


