Mycobacteriosis in naturally infected ring-neck doves (Streptopelia risoria): investigation of the association between feather colour and susceptibility to infection, disease and lesions type

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Prevalence of infection and disease. the degree of organ involvement and the nature of the lesions were investigated in 11 white and 18 non-white ring-neck doves coming from a flock naturally infected with Mycobacterium avium subsp. avium. Lesions were common in the liver, spleen, lung, kidney, intestines, ovary and bone marrow. Overall, 18 out of 29 (62%) birds were considered infected with a sequenc of M. avium subsp. avium that contains serotypes 2, 3, 4 and 9. The prevalence of infection in the white doves (36.4%) was significantly lower than in the non-white morphs (77.7%). White doves had on average fewer organs affected (mean = 3.1) than the non-white doves (mean = 5.9). A diffuse pattern of inflammation in the liver and spleen was observed mainly in non-white doves. Focal or multifocal granulomatous inflammation of the liver and spleen was predominant in white doves. Genetic mechanisms of immunity to mycobacteriosis may be contributing or determining these differences. There are three basic colour morphs in ring-neck doves—dark or wild type, blond and white—and the alleles coding for colour are sex-linked and located on the sex (Z) chromosome. Female’s single sexual chromosome (ZW) and homozygous males (ZZ) can be white if they carry the white alleles. It is very probable that the gene or genes modulating the immune response to M. avium subsp. avium infection in these doves could be associated to these loci or at least located in the same (Z) chromosome, as the association with white colour suggests.

Introduction

Mycobacteriosis is a relatively common cause of disease in pet, avicultural, zoo and free-ranging birds (Montali et al., 1976; Tell et al., 2001; Pollock, 2006; Converse, 2007). Most cases of mycobacteriosis are caused by Mycobacterium avium subsp. avium and Mycobacterium genavense, although other species of mycobacteria such as Mycobacterium tuberculosis, Mycobacterium fortuitum, Mycobacterium gordonae, Mycobacterium nonchromogenicum, Mycobacterium smegmatis and Mycobacterium celatum may also infect birds (Hoop et al., 1996; Tell et al., 2001; Bertelsen et al., 2006; Steinmetz et al., 2006; Travis et al., 2007). The pathogenesis of natural avian mycobacteriosis has been minimally investigated (Hejl Bee & Treml, 1995; Cromie et al., 2000; Tell et al., 2001, 2003) and many aspects of the disease remain unclear.

Although reports of mycobacteriosis exist for most orders of birds, susceptibility, prevalence of infection and diseases, the degree of organ involvement and the nature of the lesions vary widely (Montali et al., 1976; Hejl Bee & Treml, 1995; Cromie et al., 2000; Friend, 2001; Tell et al., 2001; Schmidt et al., 2003; Pollock, 2006; Converse, 2007). Environmental factors have been proposed to explain different susceptibility (Montali et al., 1976; Tell et al., 2001; Pollock, 2006). Malnutrition, overcrowding, concurrent diseases, and poor hygiene alone or combined are potential stressors that may predispose birds to mycobacteriosis. However, environmental factors do not explain completely the different susceptibility of birds to mycobacteriosis. Not all birds housed in the same facilities and under the same management practices become infected and develop disease (Cromie et al., 1991, 1992; M. D. Saggese, unpublished data) and their response to these different stressors may vary.

Variations in the pathology of natural and experimental infection, the degree of organ involvement and the nature of the lesions of mycobacteriosis has been attributed either to characteristics of the agent, to the stage of the disease or to the bird’s immune response (Montali et al., 1976; Cromie et al., 2000; Schmidt et al., 2003). Limited studies suggest that host genetic factors

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play a major role in determining susceptibility to avian mycobacteriosis (Cromie et al., 1991, 1992; Hejlicek & Treml, 1995). A differential susceptibility to mycobacteriosis, reflected by the number of affected birds and the nature of their lesions, has been demonstrated in two chicken lines challenged with M. avium (Gross et al., 1989). Recently, genetic susceptibility has also been suggested as a predisposing cause of disease and the nature of lesions in captive white-winged-ducks and two species of doves (Cromie et al., 1991, 1992; Saggese & Phalen, 2005; Saggese et al., 2007).

Pigeons and doves (Order Columbiformes) are considered particularly susceptible to avian mycobacteriosis (Feldman, 1938; Francis, 1958; Van der Schaaf et al., 1976; Montali et al., 1976; Pond & Rush, 1981; Bougiouklis et al., 2005). Other authors, however, consider some species of pigeon and dove to be highly resistant to experimental and natural infection (Hejlicek & Treml, 1993, 1995). These contrasting opinions may be explained both by genetic factors as well as by local environmental and other risk factors. In one study, differences in species susceptibility to natural and experimental infection with M. avium were observed in collared doves (Streptopelia decaocto) and turtle doves (Streptopelia turtur) (Hejlicek & Treml, 1993). The former were found to be highly susceptible while the related turtle doves were resistant (Hejlicek & Treml, 1993, 1995).

Other evidence for a possible genetic component for this different susceptibility comes from a natural outbreak of mycobacteriosis in Texas in 1998. A flock containing diamond doves (Geopelia cunea) as well as two different colour morphs (white and wild-type) of ring-neck doves (Streptopelia risoria), showed significant differences in organ involvement, distribution and histopathologic lesions and serologic responses (Saggese & Phalen, 2005). While all three groups of doves presented with microscopic lesions in the liver and the spleen, lesions in the intestines, bone marrow and lungs were found only in wild-type ring-neck doves. Multifocal granulomatous hepatitis was observed in all of the birds, but a diffuse granulomatous hepatitis was only observed in the wild-type doves (Saggese & Phalen, 2005). The finding, by polymerase chain reaction (PCR) and sequencing of the dnaJ gene, of the same M. avium subsp. avium in these doves and in the shared environment in which these birds were housed suggested that these variations were not due to different organisms or to differences in exposure. Furthermore, the absence of antibodies (as detected by complement fixation) in the white ring-neck doves compared with seropositivity in wild-type and diamond doves suggested that a different humoral immune response occurred in these birds. This also supports the suggestion that differences in the pathogenesis and immune response were associated with colour. Although the number of birds in this study was small, and several variables were not formally controlled, precluding a statistical analysis, these data together with previous studies (Hejlicek & Treml 1993, 1995) suggested the possibility that differences in susceptibility and pathology of mycobacterial infections may occur in ring-neck doves with different colour morphs, and points to a possible association between genes coding for feather colour and susceptibility to infection.

Ring-neck doves present in several colour morphs, obtained by natural or artificial selection. The genetics of these colour mutations in these species have been analysed (Miller, 2007). Identifying and understanding differences in pathogenesis and susceptibility to mycobacteriosis in closely related species or strains of birds could assist identification of the genes involved in differential susceptibility and pathogenesis. The occurrence in Texas of a captive population of ring-neck doves, including individuals of different colour morphs, suffering from avian mycobacteriosis offered an opportunity to investigate the presentation of this disease in a large number of birds and to look for evidence of differential susceptibility. We hypothesized that lesion type and distribution and susceptibility to infection and disease of naturally infected ring-neck doves would be different in white doves compared with other colour morphs. Therefore, the main goals of this study were to investigate the prevalence of mycobacterial infection and disease in the different colour morphs found in this flock; to describe and characterize the gross and microscopic lesions in affected birds; to identify the species and sequevar of mycobacteria involved; and to determine whether an association exists between susceptibility to this infection and disease, lesion characteristics and feather colour morphs of ring-neck doves.

Materials and Methods

Specimens. Seventy adult ring-neck doves were obtained from an aviary near Hillsboro, Texas, USA in July 2005. These birds came from a flock where more than 60 doves had died during the previous 6 months. Mycobacterial infection had been previously confirmed in these birds at the Texas Veterinary Medical Diagnostic Laboratory (College Station, Texas, USA) and the condition of some of the surviving birds (decreased productivity, weight loss, depression) was consistent with mycobacteriosis. These birds were all housed in a small shed that was open to and surrounded on two sides by an outdoor flight. There was heavy faecal contamination of the floor and perching surfaces of the shed. Food and water were contaminated with faeces. The surviving birds were donated and transferred to an isolation building at the College of Veterinary Medicine and Biomedical Sciences, Texas A&M University (College Station, Texas, USA). During the following 2 weeks, 29 birds (11 white, eight blond, six pied, one orrigin ge, two wild-type and one white-silky coloured) (Oliver, 2005; Miller, 2007) were randomly selected, anaesthetized by intramuscular injection of ketamine and xylazine, and euthanized by exsanguination through cardiac centesis.

Doves were necropsied and samples collected within 24 h of euthanasia. Tissues were collected for PCR using cleaned, autoclaved instruments that had been treated with bleach and formalin. A different set of instruments was used to collect tissues from each bird and organ to prevent DNA carryover. Samples of the liver, spleen, lung, bone marrow and of other organs with gross lesions were collected and frozen for PCR and culture. The liver, spleen, lung, trachea, heart, kidney, oesophagus, crop, proventriculus, ventriculus, intestines, gonads, pancreas and skeletal muscle were examined for lesions consistent with mycobacteriosis. Specimens from these organs were formalin-fixed and paraffin-embedded and stained with haematoxylin and eosin and Ziel-Neelsen.

Microscopically, inflammatory lesions were scored semiquantiatively as mild, moderate and severe based on the number of inflammatory cells within the lesions and the area of tissue affected. Histopathological changes compatible with mycobacteriosis were described and classified as focal/multifocal granulomatous, consisting of well-defined foci of inflammatory cells, sometimes surrounded by a ring of fibrous tissue and with (sometimes without) a variable amount of central caseous necrosis, or classified as diffuse granulomatous inflammation, with a variable degree of diffuse inflammatory cell infiltration without formation of discrete foci or nodules, absence of a fibrous capsule and little or no...
caseous necrosis. The numbers of acid-fast bacilli were subjectively graded as none, few, many or massive. Congo red staining was employed to identify caseous necrosis. The numbers of acid-fast bacilli were subjectively assessed, and the remaining sample was stained with acid-fast bacillus stain or with Ziehl-Neelsen stain.

Bone marrow aspirates, using a 20 G needle and 2 ml syringe, were obtained from the distal ulna. A bone marrow smear was made, heat-fixed and the remaining sample saved at 80°C. The cellular cavity was opened aseptically through a midline ventral approach and a piece of liver approximately (4 mm x 4 mm x 4 mm) was excised from the caudal border of the right lobe. This is the location and size of tissue that would be collected from a live bird of this size during a routine liver biopsy. A small portion was saved for PCR and culture, and the remaining tissue was aspirated material was smeared onto a glass slide, heat-fixed and Ziehl–Neelsen stained. Bone marrow aspirates and “biopsied” liver were collected from a live bird of this size during a routine liver biopsy. A small portion was saved for PCR and culture, and the remaining tissue was aspirated material was smeared onto a glass slide, heat-fixed and Ziehl–Neelsen stained. Bone marrow aspirates and “biopsied” liver were collected from a live bird of this size during a routine liver biopsy.

Detection of mycobacteria in tissues. Swabs from the investigated tissues from all of the doves were inoculated into 5 ml Middlebrook 7H9 broth (Becton Dickinson, Franklin Lakes, New Jersey, USA) containing 0.5% (v/v) glycerol and 10% (v/v) oleic acid-albumin, and incubated at 39°C following mycobacterial culture standards (Mahon et al., 2007). Cultures were inspected weekly for microbial growth and examined for the presence of mycobacteria by Ziehl–Neelsen staining.

DNA was extracted from all of the investigated tissues using the Puregene® Genomic DNA Purification Kit (Gentra Systems, Minneapolis, Minnesota, USA) following the instructions of the manufacturer. PCR screening for mycobacterial DNA was performed using primers T1 (5′-GGGTTACCCG/CTACGCAATGGGCCCAG-3′) and T2 (5′-CGGTTGTCGTATACTTCCTT-3′) for amplification of the 23S-rRNA gene as described by Morita et al. (2004). The PCR reaction parameters were as follows: one initial cycle of 94°C for 5 min; 40 cycles at 94°C for 30 sec, 60°C for 45 sec and 72°C for 1 min; and an additional elongation step at 72°C for 5 min. Positive (M. avium subsp. avium) and negative reaction control (DNA-RNA-free sterile water) were utilized in each reaction. Amplified DNA was visualized after electrophoresis on a 1.5% ethidium bromide-stained agarose gel. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen Inc., Valencia, California, USA). Sequencing reactions were performed using an ABI Prism® Big Dye Terminator v3.0 Cycle Sequencing Kit (Applied Biosystems Inc., Foster City, California, USA). Nucleotide sequences were determined with an ABI3100 automated DNA sequencer (Applied Biosystems Inc.). All sequences were aligned using Clustal X 1.81 (Thompson et al., 1997) and were compared with sequences retrieved from GenBank (www.ncbi.nlm.nih.gov/Genbank/index.html).

Detailed information about the sensitivity and specificity of these diagnostic tests and hamoral response in these doves has been described elsewhere (M. D. Saggese, unpublished data; P. Gray, unpublished data).

Infection and health status. Criteria for the classification of the diseased status of the birds (diseased or healthy) were based upon the presence of gross and microscopic lesions. A positive identification of mycobacteria by culture and/or PCR or by the identification of acid-fast organisms in stained tissues or smears defined infection status (infected or uninfected). Based on these criteria, four subcategories were identified: diseased infected, birds with moderate to severe inflammation in one or more organs and positive for mycobacteria; diseased uninfected, birds with mild lesions compatible with mycobacteriosis and negative for mycobacteria; healthy uninfected, birds without lesions and negative for acid-fast bacilli in tissues but positive PCR and/or culture results. Statistical analysis. The association between white and non-white coloured doves with the type of inflammation in the spleen and liver was analysed using contingency tables and Fisher’s exact test after demonstration of normality using Kolmogorov–Smirnov, Shapiro–Wilk and D’Agostino–Pearson tests. Test statistics were considered significant at P < 0.05. All statistical analysis was conducted using the formula package in Prism 5 for Windows® (GraphPad Software, Inc.; available online at www.graphpad.com). This research was approved by the ULAC/IAACUC at Texas A&M University (Animal Use Protocol 2005-56).

Results

Macroscopic findings. Twenty-nine doves were examined by necropsy. The gender, colour, health and infection status of white and non-white colour morphs are presented in Table 1. Twelve doves were male and 17 doves were female. Nineteen birds (65.5%) presented with gross lesions compatible with mycobacteriosis. The spleen and liver were most commonly affected, followed by the intestines and the lungs. The heart, kidney, air sacs and trachea were rarely grossly affected. Most affected birds had multiple organ involvement. No significant gross lesions were observed in the oesophagus, proventriculus, ventriculus, gonads, pancreas, central nervous system and skeletal muscle. Gross lesions in the liver of most birds consisted of severe, diffuse, pale orange–tan discoulouration and enlargement (Figure 1a). In two birds, the liver was moderately enlarged and contained single or multiple white–yellow nodules of variable size (range 1 to 10 mm) embedded in the parenchyma (Figure 1b). Gross lesions in the spleens consisted of one or more, caseous, firm yellowish foci, of variable size. The largest foci usually had a caseous central core. Splenomegaly was observed in 13 birds. Focal or multifocal caseous nodules of variable size and number were found in the lungs and kidneys. Diffuse thickening of the duodenum and a variable number of yellow, round or oval-shaped foci 2 to 5 mm in diameter were observed in the

<table>
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<th>Colour</th>
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<th>Gender</th>
<th>Mean number of organs affected*</th>
<th>Infected*</th>
<th>Diseased</th>
<th>Liver</th>
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<th>Liver</th>
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<tr>
<td>White</td>
<td>2</td>
<td>2 male; 9 female</td>
<td>3.1</td>
<td>4/11 (36.4%)</td>
<td>7/11 (63.6%)</td>
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<td>2</td>
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<td>Non-white</td>
<td>18</td>
<td>10 male; 8 female</td>
<td>5.9</td>
<td>14/18 (77.7%)</td>
<td>14/18 (77.7%)</td>
<td>12</td>
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n, sample size. Diseased: doves with gross and/or microscopic lesions with disregard of infection status. Infected: doves with a positive identification of mycobacteria by culture and/or PCR or by the identification of acid-fast organisms in stained tissues or smears.

*Differences statistically significant.
intestinal wall or protruding on the serosal surface in 11 birds. These foci were usually more numerous and evident in the duodenum but they were also observed along the jejunum, ileum and colon. Five birds presented with ascites. Diffuse air sacculitis and pericarditis were additional findings in three birds. Gross lesions were not observed in the remaining organs.

**Microscopic findings.** Twenty-one of 29 (72.4%) birds had microscopic lesions consistent with mycobacteriosis. Lesions were common in the liver, spleen, lung, kidney, intestines, ovary and bone marrow (Figure 2).

Microscopic lesions of the liver could be divided into two forms that corresponded to the observed gross lesions. The first was a severe granulomatous inflammation characterized by nodules of variable size. They were composed mainly of histiocytes, lymphocytes and multinucleated giant cells, sometimes with small numbers of plasmacytes (Figure 1c). These granulomas rarely contained a central zone of necrosis, except for two birds and, in some cases, a thin fibrous capsule surrounded the largest nodules. These nodules tended to be perportal, but also widely scattered in some birds. Many to massive numbers of acid-fast organisms were observed. They were restricted to the central necrotic core of the granulomas. The second form of microscopic inflammatory pattern in the liver was characterized by a severe diffuse infiltration of the parenchyma with histiocytes, scattered multinucleated giant cells and, to a lesser degree, lymphocytes and plasmacytes (Figure 1d). Thick layers of amyloid were observed between the hepatocytes and the space of Disse. No or few acid-fast organisms were associated with this inflammation pattern.

Two similar patterns of inflammation were also observed in the spleen. The diffuse form was characterized by infiltration of this organ with large numbers of histiocytes and multinucleated giant cells, with lymphocytic and erythrocyte depletion. Caseous necrosis was observed in the more extensive areas of inflammation. Many to massive numbers of acid-fast organisms were observed in these lesions. The second pattern consisted of mild focal or multifocal histiocytic nodules, in some cases accompanied by multinucleated giant cells. Central necrosis was not common in these birds. No or few acid-fast organisms were observed in this form of inflammation.

Focal or multi-focal granulomatous pneumonia was observed in seven non-white doves and two white doves, respectively. Two white doves and two non-white doves had mild multifocal, lymphocytic nephritis but necrosis was not a component of the kidney lesions. A severe, diffuse, air sacculitis characterized by large amounts of necrotic debris, exudate and severe histiocytic infiltration with massive numbers of acid-fast bacteria was observed in three non-white birds.

Moderate to severe multifocal serosal and mucosal granulomatous enteritis was observed in 13 birds. Mild multifocal to severe diffuse bone marrow inflammation was observed in 11 non-white doves, characterized by replacement of normal bone marrow by histiocytes and multinucleated giant cells.

Very mild lesions in the liver, spleen or lung, without detectable mycobacteria in acid-fast stained tissues,
smears, cultures and PCR, were observed in three white birds and one non-white bird.

Results of culture and PCR. Overall, 18 out of 29 (62%) birds were considered infected, based on positive results from cultures and PCR and/or by the detection of acid-fast bacilli in one or more tissues or smears (Table 1). An amplicon of expected molecular mass (236 base pairs) was amplified by PCR from the liver or spleen from four white and six non-white doves. These sequences were identical and had 100% identity with the sequevars of *M. avium* subsp. *avium* that contains serotypes 2, 3, 4 and 9 (Morita et al., 2004).

Health and infection status. Based on the presence of gross and microscopic lesions, 21 of 29 (72.4%) birds were considered diseased. Seventeen birds were considered severely diseased while the four birds that had microscopic lesions were considered mildly diseased. With the exception of one bird, all of the 18 infected birds showed microscopic lesions and were considered diseased. This negative dove was considered infected based on positive culture and PCR of liver and spleen samples, but classified as healthy infected in the absence of significant lesions.

Differences between white and non-white coloured doves. The prevalence of infection in the white doves (36.4%) was significantly lower than in the non-white morphs (77.7%). White doves had on average fewer organs affected than the non-white doves. The pattern of inflammation was significantly different between the two groups. The association between a diffuse pattern in the liver and spleen was significantly different between white and non-white doves (Table 1). This diffuse pattern of inflammation was rare in the white doves. No statistically significant differences in the prevalence of disease between white doves and non-white doves were observed.

Discussion

The present study investigates a natural presentation of mycobacteriosis in ring-neck doves. Important observations are the variability observed in susceptibility to infection, organ distribution and nature of lesions between white and non-white doves. In the presence of identical environmental conditions, these differences may be explained by genetic differences associated with the phenotypic characteristic colour morph. Previous findings in another group of ring-neck doves (Saggese & Phalen, 2005) as well in other species of doves (Hejlicek & Treml, 1993) support a genetic cause for these differences. While the age and time of infection for each individual dove was unknown, all were adults kept under identical food and housing conditions. The presentation of this disease, constant exposure and the chronic nature of the lesions of mycobacteriosis together with the sample size make it unlikely that age or time of infection influenced our results.

There are several reports of mycobacteriosis in pigeons and doves (Feldman, 1938; Pond & Rush, 1981; Hejlicek & Treml, 1993, 1995; Morita et al., 1994; Bougiouklis et al., 2005; Saggese & Phalen, 2005), but the prevalence of infection and/or disease in captive populations of ring-neck doves have not been previously reported to the author’s knowledge. Overall, the prevalence of mycobacteriosis in captive collections of birds is rarely higher than 15% (Montali et al., 1976; Van der Heyden, 1997; Tell et al., 2001). Nevertheless, while the prevalence of infection (62%) and disease (72.4%) was considered very high in this flock, the finding that not all the birds were infected suggests that exposure alone under the conditions observed in this flock (overcrowding, poor hygiene) was apparently not sufficient to cause infection or disease in some birds.

The distribution of gross and microscopic lesions in these birds was similar to that previously reported in other birds with mycobacteriosis. The liver, spleen, lung, bone marrow are common sites of mycobacterial infection in birds (Feldman, 1938; Francis, 1958; Thoen, 1997; Tell et al., 2001; Fulton & Thoen, 2003; Converse, 2007). Focal or multi-focal granulomatous inflammation is the most common form observed in this disease (Montali et al., 1976; Fulton & Thoen, 2003; Schmidt et al., 2003). The diffuse pattern of granulomatous inflammation observed in this study is consistent with the non-tuberculoid form described by Tell et al. (2001),
in which diffuse infiltration of the organ with inflammatory cells occurs. The diffuse enlargement observed in 12 doves with diffuse histiocytic and multinucleated giant cells and severe amyloidosis is consistent with this description. A diffuse form of granulomatous inflammation similar to that observed in the non-white doves was previously observed in wild-type ring-neck doves but not in white birds (Saggese & Phalen, 2005). This pattern of inflammation resembles the lepromatous form of granulomatous inflammation observed in human leprosy (Connor et al., 1997). The presence of this diffuse form contrast with reports in other species of doves, where the tuberculoid or nodular form of granulomatous inflammation has been reported (Feldman, 1938; Pond & Rush, 1981; Morita et al., 1994; Bougiouklis et al., 2005). Alternatively, it has been stated that tubercles rarely develop in Columbiformes with mycobacteriosis, although specific details were not provided (Ramis et al., 1996). Nevertheless, the presence of both types of inflammation in the doves of our series may explain these conflicting opinions.

Twelve birds had amyloidosis of the liver. Massive amounts of amyloid were observed in doves with diffuse inflammation, but very little was observed in the birds with multifocal inflammation. Amyloidosis is a pathological condition characterized by the deposition of insoluble fibrillar proteins in various tissues and organs of the body following prolonged inflammation or infection (Cotran et al., 1999). Amyloid deposits have been reported previously in birds with chronic inflammatory diseases such as mycobacteriosis and aspergillosis. Several forms of amyloid have been described in mammals, but only amyloid AA (amyloid associate) has been found in birds (Landman et al., 1998; Cotran et al., 1999; Schmidt et al., 2003). Amyloid AA is a product of the proteolytic cleavage of serum AA (SAA), an acute phase-protein produced by hepatocytes (Landman et al., 1998). The concentration of SAA in the blood increases within a several hours of the onset of injury, infection, or inflammation. Production of SAA is directly stimulated by the cytokines interleukin-1, interleukin-6 and tumour necrosis factor-α produced in response to tissue injury and inflammation (Petersen et al., 2004). The persistent inflammation caused by chronic mycobacteriosis is a probable cause of the deposition of amyloid in these organs (Saggese et al., 2007). Amyloid was not a feature in the mildly diseased doves with focal or multifocal granulomatous inflammation, suggesting that an inflammatory process in these birds was insufficient or of too short duration to trigger amyloidosis, supporting the suggestion of an infection arrested at early stages.

There were significantly fewer organs with gross and microscopic lesions in the white doves compared with other birds. Lung and intestines were rarely affected in the white doves compared with the non-white doves. These findings suggest that lesion distribution, as well as the severity of the lesion and the type of the lesion, are influenced by factors linked to feather colour.

Four doves had mild lesions consistent with a mycobacterial infection but were negative on all tests for the organisms. It is possible that these birds had low levels of mycobacteria in their tissues and that they were not detected. However, it is more likely that these birds had recently overcome a mycobacterial infection through a mild but adequate cell-mediated immune response. It is probable that the mild lesions observed in these four birds represented a controlled or at least an arrested infection, similar to that seen in other mycobacterial infections (Jubb et al., 1993; Cotran et al., 1999; Dannemberg, 2006). It suggests that at least some birds may recover from natural infection. The fact that three out of these birds were white is consistent with other observations made between different colour morphs.

A single dove with confirmed mycobacterial infection of the liver and spleen did not have lesions. The significance of this is unknown, but could represent the early stage of infection that was prior to the onset of detectable lesions or that a lesion was present but not detected. This case shows that, in some circumstances, culture or PCR may be a necessary adjunct to histopathology to detect all infected birds.

The present study represents the first attempt to examine the association between feather colour and susceptibility to infection, disease and pathology. The white doves had a lower prevalence of infection, fewer infected organs affected and a different pattern of inflammation as compared with the coloured doves. These data are consistent with epidemiological and genetic studies showing immune response polymorphism to mycobacterial infections in humans and other mammals (Bellamy, 2005; Dorman et al., 2004; Barthel et al., 2000; Pan et al., 2005; Di Pietrantonio & Schurr, 2005) and in different strains of chickens (Hu et al., 1997; Bacon et al., 2000). Specific genes that have been associated with differing immune responses to mycobacterial infections include those that code for the major histocompatibility receptors, cytokines, T-cell receptors, immunoglobulins and NRAMP1 (Zekarias et al., 2002).

The lack of information available about the role of genetics and susceptibility to tuberculosis in birds contrasts to what is known in humans and other mammals (Bellamy & Hill, 1998). Both environmental and genetic factors and their interaction influence susceptibility to tuberculosis in humans (Casanova & Abel, 2002; Hell, 2007) and in domestic mammals (Barthel et al., 2000; Phillips et al., 2002; Di Pietrantonio & Schurr, 2005; Dannemberg, 2006). For example, in humans infected with M. tuberculosis, less than 10% of infections progress to clinical disease (Cole et al., 2004). Resistance to clinical disease has been linked with ethnic background and race (Bellamy et al., 2000; Lim, 2000; Casanova & Abel, 2002; Van Helden et al., 2006). Genes coding for natural-resistance-associated-macrophage-protein 1 (NRAMP1), vitamin D receptor, interferon-γ receptor, interleukin-1, interleukin-10 and interleukin-12, HLA class II molecules, Toll-like receptor 2 and tumour necrosis factor-α, are all considered to influence immunity to mycobacterial infection. Their deficiency, functional defect or genetic polymorphism has been associated with altered susceptibility to mycobacteriosis (Taffik, 2001; Casanova & Abel, 2002; Phillips et al., 2002; Acevedo-Whitehouse et al., 2005; Bellamy, 2005; Okada & Shirakawa, 2005; Hill, 2006; Naik, 2006). The chromosome location of some of these genes has been elucidated (Cervino et al., 2002; Bellamy, 2005; Baghdadi et al., 2006).

Ring-neck doves have been selected for different phenotypic traits, mainly colour, for more than 2000 years, and more than 45 colour morphs are currently recognized by pigeon fanciers (Oliver, 2005). The genetics that govern the colouration of ring-neck doves have
been partially deduced by Miller (2007). There are three basic colour morphs in ring-neck doves: dark or wild type, blond and white. Dark is dominant both over blond and white, and blond is dominant over white. The alleles coding for colour are sex-linked, and are located on the sex (Z) chromosome. Females are single sexual chromosomed (ZW or Z-) and males are double sexual chromosomed (ZZ). Females' with a single white gene and homozygous males will be white. It is very probable that the gene or genes involved in immunity to M. avium subsp. avium infection in these doves could be associated with these loci or at least located in the same (Z) chromosome.

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