MYCOBACTERIOSIS IN DOVES: A CASE REPORT AND REVIEW OF RECENT DISCOVERIES

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CASE REPORT

A 1.5 year old, male, spotted turtle-dove (*Streptopelia chinensis*) presented with a history of being fluffed up, lethargic and a tail bob. It was owned by a veterinary nurse and was provided with excellent care. The bird was slightly underweight (121 gms) with a mild to moderate degree of pectoral muscle atrophy. Haematological findings were within normal limits, although heterophil and monocyte counts were approaching the upper limits of normal (Table 1). A faecal Gram stain demonstrated significant numbers of budding yeasts. The dove was treated with azithromycin (once a day for 1 month) and nystatin (100,000 IU/kg, once a day for 7 days).

Three weeks after presentation the owner reported that the dove's condition had improved but still had a tail bob. The following week, however, the bird represented because of concern for weight loss and an increase in the respiratory effort. The dove had lost 11 grams and its respiratory rate increased dramatically with restraint. A complete blood count (Table 1) showed that the dove had a regenerative anaemia was hypoproteinaemic. The faecal Gram stain was unremarkable. A repeat chemistry panel revealed an increase in the creatine phosphokinase, a hyperglycaemia, and an elevated alkaline phosphatase (Table 1) (Fudge, 2000; Saggese et al., 2009). A bleeding ulcer of the digestive tract was suspected. Azithromycin was continued and sucralfate (20 mg/kg, q 8 hrs) was added to the treatment regimen. One week later the PCV was 35% (Table 1). Two weeks later all medications were stopped.

Two months after the initial presentation (two weeks after the end of treatment), the dove re-presented. The bird was now quiet, fluffed up and preferred to sit. When it did walk, it used its wings to support its weight. Radiographs demonstrated an enlarged liver silhouette. There was increased soft tissue density in the long bones of the wing and the legs. The shafts of the ulna and radius of both wings contained sharply defined radiolucent round foci. The complete blood count showed a marked increase in the total white blood cell count with a marked mature heterophilia (Table 1). Cytology of an aspirate of the tibiotarsus showed large numbers of histocytes, moderate numbers of multinucleated giant cells and occasional plasma cells. Erythropoeitic and myelopoeitic cells were not present. An acid fast stain of the bone marrow aspirate was positive for acid fast bacteria and a diagnosis of mycobacteriosis was made.

Treatment with rifampicin (45 mg/kg, PO, q 12 hours) and azitrhomycin (mg/kg q 24 hours) was initiated. Within one week of the onset of treatment with rifampicin, the dove's attitude was greatly improved, the swelling in the feet had resolved and it had gained 8 grams. After a second week of treatment, the dove was again quiet and fluffed up. The frequency of the rifampicin dose was reduced to once a day and the dove returned to normal behaviour. A total of eight months of treatment was completed at which time the complete blood count and heterophil count had dropped dramatically

(Table 1). The dove weighed 140 gms. For the first time, a mild eosinophilia was observed, the significance of which is unknown. At the time of this writing (3 months after the end of treatment) the dove is reported to be normal.

LITERATURE REVIEW

Mycobacteriosis occurs at a low frequency in a wide range of birds (Bush et al., 1978; Converse, 2007; Montali et al., 1976, Saggese et al., 2008; Van der Hyden, 1997). In the author's experience it a relatively common phenomenon in doves of the *Streptopelia* genus. There have been a significant number of reviews on the clinical manifestations, value of diagnostic assays, and treatment of mycobacteriosis in birds, most of this information has come from a limited number of surveys of a small number of clinical cases, often containing many different species (Bush et al., 1978; Tell et al., 2001; Van der Heyden, 1997). There has also been much discussion about the importance of the host's immune response versus the role of the infecting organism in the wide range of gross and microscopic lesions seen in birds with mycobacteriosis (Montali et al., 1976). Recently, an opportunity has arisen to study a large number of doves (*Streptopelia risoria*) from a single collection, all infected with a single genotype of *Mycobacterium avium avium* (Saggese et al., 2008; Saggese et al., 2009a; Saggese, 2009b) These studies have resulted in a clearer picture of this disease, at least in this species.

Susceptibility to infection and gross and microscopic lesions as a function of feather colour (Saggese, 2008)

This study found infection prevalence in coloured doves to be significantly higher than the prevalence in white doves, suggesting that even under conditions where a high level of exposure was likely to occur, white birds were more resistant to infection than birds with a gene for colour. Eighteen infected doves were identified and examined. Gross and microscopic lesions similar to those seen in previous reports were found. Lesions were consistently found in the liver and spleen of virtually every dove examined. Less commonly, the lesions were found in the lung and the kidney. Involvement of other organs, however, varied significantly between white and non white birds. Lesions of the proventriculus, ventriculus, bone marrow, ovary, heart, and trachea were only found in coloured doves. Also, the majority of the coloured doves had intestinal lesions; whereas intestinal lesions were rare in the white doves.

Microscopic lesions also varied between coloured and white birds, particularly in the liver. White birds had discrete granulomas that often contained many organisms. In contrast, the lesions in the coloured birds were characterized by diffuse amyloid deposition, a diffuse inflammatory reaction, and few organisms.

These results are some of the first to suggest that genes determining feather colour are linked to genes that influence susceptibility to infection and the nature of the host's immune response to infection with *M. avium avium*. These results also show that the host's immune response, and not necessarily the infecting organism, is responsible for the nature of the lesions seen in the bird. These findings also have significant diagnostic implications for the clinician. Mycobacteria were not found in the bone marrow or intestines of the white doves and therefore acid fast stains of bone marrow or faeces would not be expected to be positive, but bone marrow aspirates of coloured doves would much more be much more likely to be positive. It is uncertain if mycobacterial shedding was occurring in the doves with intestinal lesions as the organisms were found on the serosa, and within the muscularis and subserosa and not in the lamina propria of the villi. This contrasts with intestinal lesions seen in other species where a heavy infection of the lamina propria of the intestinal villi has

been observed (Montali et al., 1976; Schmidt et al., 2003; Van der Schaaf, 1976).

Ancillary diagnostic testing in the live bird

Non-invasive diagnostic testing. Signs of mycobacteriosis can vary significantly from bird to bird and are generally nonspecific. Mycobacteriosis is a chronic disease so most birds show weight loss and pectoral muscle atrophy. A delayed molt or decreased grooming may result in a poor quality of the feathers. Organomegaly, abdominal effusion, intra coelomic granulomas and lesions within the lungs can result in dyspnea. The presence of large volumes of fluid in the coelomic cavity has been a common finding in doves (Saggese et al., 2008). Uncommonly, cutaneous masses will also occur, these lesions; however, have not been reported in doves. Radiographically, liver and spleen enlargement may be demonstrated (Bush et al., 1978; Tell et al., 2001; Van der Heyden, 1997). Peritoneal fluid will result in a homogenous increase in soft tissue density in the caudal coelom. Involvement of the bone marrow is associated with increased soft tissue density of the marrow, interrupted by clearly defined round radiolucent zones and cortical thinning.

Because most birds are presented with nonspecific signs, ancillary diagnostic testing is often of benefit when attempting to differentiate mycobacteriosis from other diseases. In a second paper, studying doves from this collection Saggese et al., (2009) examine the diagnostic value of a number of ancillary diagnostic tests.

Eighteen infected doves were included in this study. Twelve were found to be in poor or fair condition and the rest were scored as being in good condition. Haematology revealed that infected doves tended to be anaemic (mean value of 32.3 ± 3.7 % [range: 11 – 49 %]), as compared to uninfected doves (41.9 ± 1.7 %; range: 30-49 %). Infected doves had also had higher WBC counts (23,320 ± 4,234 WBC/µl; range: 8,746-42,466 WBC/µl) than in uninfected doves (10,720 ± 1,033 WBC/µl; range 7,200-15,600 WBC/µl). The mean absolute count of heterophil count in infected doves was 11,380 ± 1,722 heterophils/µl (range: 3,410-20,400), this was significantly higher than the mean count in uninfected doves (4,875 ± 694.6 heterophils/µl; range: 1,913-7,494 heterophils/µl). No statistically significant differences were observed in the number of lymphocytes between infected (6,312 ± 1,661 lymphocytes/µl; range: 1,688-17,411 lymphocytes/µl) and uninfected doves (5,212 ± 690.8 lymphocytes/µl; range: 2,780-8,268 lymphocytes/µl). A significantly larger monocyte count was observed in infected doves (5,604 ± 1,561 monocytes/µl; range: 1,592-13,504 monocytes/µl) compared with the uninfected (662 ± 179.8 wbc/µl; range: 270-1,813 monocytes/µl).

Mean values of aspartate aminotransferase, creatine phosphokinase and uric acid values for the infected and uninfected birds were not statistically different. The mean total protein and mean globulin concentrations were significantly higher and mean albumin concentration was significantly lower in the infected doves compared with the uninfected. Results of plasma protein electrophoresis showed a significantly higher average plasma concentration of the alpha, beta and gamma globulins in the infected doves compared with the uninfected. Mean alpha, beta, and gamma globulins ranged from 2 to 6 times higher than those of the normal doves.[6]

Invasive diagnostic assays (biopsy and intestinal aspirates). Biopsy of the liver, particularly if it is enlarged, is a commonly used procedure and can be combined with an intestinal aspirate. Samples can then be used for histopathology, culture, and PCR based diagnostic assays. In the dove study, intestinal aspirates, bone marrow and wedge-shaped liver and spleen biopsies were collected immediately after the doves were euthanized. Each sample was split into three portions, for histologic or cytologic examination, PCR for the detection of mycobacterial DNA, and culture for actual

organisms. Intestinal aspirates were not cultured.

Culture proved to be the most sensitive assay, followed by histopathology, and lastly PCR. It should be noted, however, that traditional PCR was used and the use of real time PCR or multiplex PCR would have been likely to have improved the assay's sensitivity. In all cases, assays done on splenic biopsies were the most sensitive than any other source, with culture identifying 100% of the positive doves. Next in sensitivity were liver, then bone marrow, and then duodenal aspirates (Saggese et al., 2009a).

Treatment of birds with mycobacteriosis

Various treatments for mycobacteriosis have been described with varying degrees of success. All treatments used are based on protocols developed in humans and use a combination of three drugs to avoid the development of drug resistance. Treatments in humans can range from 6 to 12 months and similar periods of treatment have been recommended for birds. Initially, treatment regimens containing isoniazid were recommended, but *M. avium, avium* is resistant to isoniazid (Tell et al., 2001; Van der Heyden, 1997). Saggese et al. (2009b) treated 16 doves with mycobacteriosis using a combination of rifampicin (45 mg/kg), azithromycin (43 mg/kg) and ethambutol (30mg/kg) *per os* once a day for 180 days at which time the birds were euthanized and their tissues examined for infection with mycobacteriosis.

Five birds died during the course of treatment. The 11 surviving doves outwardly appeared to be in good health. However, 9 of 11 birds were still found to be infected at the end of the treatment period and most still had significant microscopic lesions. Reasons for treatment failure could be multiple. Isolates from treated and untreated doves, while susceptible to azithromycin were resistance to ethambutol and only intermediately sensitive to rifampicin. Also, the pharmacokinetics of these drugs has not been studied in doves and it is possible that higher doses or more frequent administration may be necessary if treatment is going to be successful. How other genotypes of *M. avium avium* might respond to treatment is not known.[7]

DISCUSSION

The case described in this report is that of a collared dove with mycobacteriosis. The clinical signs were generally nonspecific. Haematology initially was suggestive of an inflammatory disease with both total white blood cell counts and heterophil counts being elevated above the normal range. A marked increase in heterophils, as described in some doves with mycobacteriosis, did not occur until week 8. Monocyte counts stayed below the upper level of normal with the exception of week 5 and thus were never were suggestive of a chronic granulomatous disease (Saggesse et al., 2009a). Clinical chemistries provided little diagnostic information. A laboratory error resulted in the alkaline phosphatise being included in a second chemistry panel. This value was markedly elevated as compared to values reported for other birds and may represent the result of the diffuse bone disease (Fudge, 2000). Future investigations into the value of alkaline phosphatase as an indicator of bone involvement in birds with mycobacteriosis are indicated. The elevated glucose in the same chemistry panel remains unexplained. The bird never exhibited signs consistent with diabetes and repeat glucoses were not done. Retrospectively plasma electrophoresis may have been a useful ancillary diagnostic assay to have included in the work up of this bird (Werner and Reavill, 1999).

The marked anaemia that this bird experienced early in the clinical course of this disease was believed to be the result of a bleeding lesion in the gastro-intestinal tract. Whether this was associated with the observed yeast overgrowth of the gastrointestinal tract, mycobacteriosis of the intestines, or

another cause is not known. The usual presentation of unwillingness to walk appeared to be secondary to leg pain and swelling. The swelling appeared to be the result of a circulatory disturbance, possibly secondary mycobacterial lesions in the bone marrow.

The two most useful diagnostic tests preformed were radiology and a bone marrow aspirate. The radiographic lesions seen were identical to those seen by the author in doves and other species of birds with mycobacteriosis. The cytology was consistent with a granulomatous lesion of the bone marrow and many mycobacteria were identified with an acid fast stain. While useful in wild type and other doves with colour in their feathers, bone lesions may not occur in white birds, assuming studies with the ring neck dove are going to prove consistent across species (Saggese et al., 2008).

The decision to only treat with azithromycin and rifampicin and not add ethambutol to the treatment regime was made because of cost considerations and the previous studies suggesting that some genotypes of *M. avium avium* may be resistant to it (Sagesse et al., 2009b). Currently this dove has been off treatment for 2 months and is acting normally. Its white cell count has also decreased dramatically. Its total treatment time was 8 months, two more than the ring neck doves. Whether it is cured or just in remission is not known. Given the results of the controlled treatment trials in the ring neck dove, the owner has been given a guarded prognosis for a complete cure.

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Table 1: Haematological and Biochemical Findings in a Spotted Turtle Dove with Mycobacteriosis

Week	0	4	5	8	38	Normal Values (Range)*
PCV (%)	ND	18	35	45	43	11-49
Total Protein µg/L	ND	24	40	33	34	10-31
WBC†	28.4	19.0	16.0	72.0	4.0	7.2-15.6
Heterophils†	22.7	17.5	12.5	61.2	1.6	1.9-7.4
Lymphocytes†	3.97	0.4	1.6	10.1	0.9	2.8-8.2
Monocytes†	1.70	1.1	1.9	0.7	0.8	0.3-1.8
Eosinophils†	0	0.0	0.0	0.0	0.7	0
AST	89	85				30-344
ALP		992				
GGT	3	10				
TBA	14,3	26				
CPK	520	1692				20-1191
Uric Acid	0.39	0.29				1.7-11.0
Glucose		23.7				

* Saggese et al. 2009.[6] † Thousand cells per µL