1997

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Abstract: A whooping crane (*Grus americana*) confirmed as suffering from *Mycobacterium avium* infection was treated for 1 year with daily doses of rifampin (45 mg/kg) and ethambutol (30 mg/kg) and 2 doses of *M. vaccae* antigen. Remission of disease occurred during therapy; however, recrudescence to active infection was suspected by 10 months after the antitubercular drugs were discontinued when the crane exhibited weight loss and had thickening of bowel wall as seen on radiographs. A second therapeutic regimen using azithromycin was then initiated (40 mg/kg fed daily) and was accompanied by a second remission within 6 weeks. After 16 weeks of azithromycin therapy, ethambutol (30 mg/kg daily) was added to the azithromycin to reduce the probability of emergent drug resistance. Three weeks later the crane developed severe hepatic dysfunction as suggested by blood chemistry values. This contributed to the crane's collapse and eventual death. All tissues cultured were negative for *M. avium* infection. A severe hepatopathy and chronic fibrosing cardiomyopathy may have resulted from the drug combinations. This case suggests azithromycin as a promising therapeutic agent in treatment of avian tuberculosis and warrants further investigation.

PROC. NORTH AM. CRANE WORKSHOP 7:253-255

Key words: avian tuberculosis, azithromycin, ethambutol, *Grus americana*, *Mycobacterium avium*, *Mycobacterium vaccae*, rifampin, treatment, whooping crane.

Treatment of avian tuberculosis is seldom attempted because of the long time interval for assessing outcome of treatment and the potential for suppression of disease rather than cure. This may result in recrudescence, further transmission of infection, and increased zoonotic exposure (Bush et al. 1978, Farer 1978, Beehler 1990). However, endangered species or otherwise valuable birds may justify special consideration. Recent reports suggest treatment can be successful in some cases of avian mycobacteriosis (Rosskopf and Woerpel 1991, VanDerHeyden 1994) although these diagnoses were not confirmed as *M. avium* infection by bacterial isolation. Drugs used in treatment of psittacine species were various combinations of isoniazid, rifampin, ethambutol, streptomycin, clofazimine, and cyclocerine. This report follows the course of treatment and outcome over 29 months of a whooping crane infected in the wild with *M. avium*.

We thank J. P. Thilsted, New Mexico Veterinary Diagnostic Services (NMVDS), New Mexico Department of Agriculture, for biopsy histopathology and R. B. Ferguson, Tuberculosis and Mycology Section, Scientific Laboratory Division, New Mexico Department of Health, for isolation and identification of *M. avium* complex. D. G. Teubert, Senior Research Specialist, Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, verified the isolate as *M. avium* by DNA probe. R. L. Cromie, The Wildfowl and Wetlands Trust, United Kingdom, provided *M. vaccae* antigen, and S. B. Kadin and D. J. Williams, Pfizer Laboratories, Inc., Groton, Connecticut, donated azithromycin. Helpful comments were given by J. C. Lewis, U.S. Fish and Wildlife Service (USFWS), and by other reviewers of the manuscript.

CASE REPORT

A debilitated 5-year-old male whooping crane from the experimental Rocky Mountain flock (Drewien and Bizeau 1978) was captured at the Monte Vista National Wildlife Refuge, Colorado, and transported to the Rio Grande Zoo, Albuquerque, New Mexico, on 5 November 1992 by University of Idaho biologists. Details of the initial case presentation, diagnostic workup, and patient management have been reported (Snyder and Richard 1994). At initial examination the bird was thin (weight 5.78 kg) and had a large, easily palpable, mid-coelomic mass and a chronic cloacal prolapse. A firm 2-cm tissue mass was palpable within the prolapsed tissue.

Initial diagnostic tests included blood samples for hematology and serum biochemistry, feces for ova and parasites, whole body radiographs, and biopsy and culture of the tissue mass in the wall of the prolapsed cloaca. Radiographs and biopsy were done under isoflurane anesthesia. The prolapse was reduced and held in place with a purse-string suture.

Radiographs confirmed a large, mid-coelomic soft tissue density and splenomegaly. The initial white blood cell count (WBC) was estimated to be elevated (8–10 WBC/oil immersion field). Dissection of the cloacal mass revealed pockets of brownish, inspissated exudate within a firm fibrous-like stroma. Histologically, there were numerous coalescing granulomas with necrotic centers surrounded by zones of macrophages. An acid fast strain showed rod-shaped, acid-fast bacteria in the centers of many of the granulomas. The preliminary diagnosis was avian tuberculosis, and a decision was made to attempt treatment based on reports of success in

some psittacine species (Rosskopf and Woerpel 1991, VanDerHeyden 1994). Mycobacteriosis or other granulomatous diseases were differential diagnoses. *M. avium* complex was confirmed in 3 weeks by isolation from the biopsy tissue (organism on file, NMVDS). The isolate was later verified as *M. avium* by use of a specific DNA probe for this organism.

First Treatment Regimen

Antitubercular treatment was initiated on 12 November 1992 when the bird began to self feed, and was continued for 1 year. Rifampin (Rifadin, Marion Merrell Dow, Inc.) and ethambutol (Myambutol, Lederle) were started at 30 mg/kg daily. The measured portion of powder and tablet were loaded into gelatin capsules and placed into the body cavity of a mouse or herring, which the bird ate. The dosage of rifampin was increased to 45 mg/kg daily (on 10 December 1992) for the remainder of the treatment year. Isoniazid (Isoniazid, USP, Rugby Laboratories) was unsuccessfully added as a third antitubercular drug at a dose of 30 mg/kg daily on 2 occasions early in treatment. On both attempts the bird became anorectic within 3 days but resumed eating when isoniazid was discontinued.

The purse-string suture at the cloaca was removed in mid-January when pericloacal swelling had regressed sufficiently. *M. vaccae* antigen (Cromie 1991) was started at that time. Two 0.05-ml doses of the vaccine were given intradermally 8 weeks apart in the thick skin on top of the head.

Monitoring of the patient throughout treatment was by physical examinations, radiography, hematology, serum biochemistry, and feces collection for demonstration of acid-fast bacilli by staining and by mycobacterial culturing. These data were collected at various intervals from monthly to quarterly throughout treatment. The mid-coelomic mass and spleen were markedly reduced in size by 9 March 1993, 4 months after treatment with antitubercular drugs was begun. At this time the cloacal mass was palpable at less than 1 cm. Nine months after treatment was started (6 August 1993), all tests were within normal parameters, including radiographs. The cloacal mass was no longer palpable. The WBC (by eosinophil Unopette method) had decreased from 32,787/μl on 16 December 1992 to 6,206/μl on 6 August 1993.

The bird continued to appear normal on 10 June 1994, 6 months after administration of antitubercular drugs was discontinued, although it had experienced a weight loss of 0.22 kg from 25 February 1994. This weight loss was possibly an early manifestation of recrudescence. Sequential blood values during treatment and observation have been reported (Snyder and Richard 1994).

Second Treatment Regimen

By 15 September 1994 there was additional weight loss of 0.28 kg and an increase in the WBC from 10,304/μl in June 1994 to 20,280/μl. Radiographs taken 23 September 1994 showed prominent loops of intestine in the pelvic region which were interpreted as uniform thickened bowel wall. No other abnormal findings were evident; the bird's appetite was normal and fecal cultures were negative for mycobacteria.

Although *M. avium* was not again isolated from the bird by fecal culture, we considered recrudescence of infection as the cause of the recurrent wasting illness and bowel wall thickening. Treatment with azithromycin (Pfizer Laboratories, Inc.) was initiated 26 October 1994 at 20 mg/kg given in food daily. After 1 week the dose was increased to 40 mg/kg and was continued 16 weeks until 15 February 1995 with no ill effect. We decided to initially give azithromycin alone to test its potential usefulness in treatment. The bird made a dramatic improvement; weight gain was 0.57 kg over the first 6 weeks on azithromycin (26 October to 8 December 1994) and the WBC dropped to $<10{,}000/\mu$l. The bird again appeared in full remission.

Third Treatment Regimen

The drug manufacturer recommended using azithromycin in combination with other antibiotics to reduce the probability of emergence of drug resistant *M. avium*. Thus, after 16 weeks of azithromycin therapy, a second antibiotic, ethambutol (30 mg/kg daily), was added (starting 15 February 1995). After 3 weeks of treatment with this combination (8 March 1995), the bird was found collapsed and unable to stand; both antibiotics were discontinued. Body weight had dropped 1.5 kg (to 4.69 kg), WBC was 10,442/μl, and serum aspartate aminotransferase (AST) was >760 mg/dl. The serum AST had been 310 mg/dl in June 1994 and 321 mg/dl in September 1994. Tube feeding and intensive supportive care were initiated and continued for 3 weeks as the bird's condition worsened until euthanasia was performed on 4 April 1995. The carcass was submitted to National Wildlife Health Center, Madison, Wisconsin, for complete necropsy.

Feces were collected for acid-fast bacilli (AFB) stain and mycobacterial culture on 17 occasions between 12 November 1992 and 10 March 1995 (29 months). AFB stains were negative on all samples. Cultures were negative for mycobacteria in 9 of the samples; *M. fortuitum* was isolated from 1 sample (28 January 1994), *M. terrae* was isolated from 2 samples (23 August 1994 and 23 November 1994), and 5 samples were overgrown by contaminant bacteria.

Necropsy Findings

A complete necropsy was performed including gross and histopathology, virology, parasitology, bacteriology, and TB culture. Gross necropsy revealed significant lesions involving the heart and liver. There was serosanguinous fluid within the pericardial sac, a mildly cloudy pericardium, and a pale area on the left ventricular wall. The liver was large with rounded borders and was orange/red and mottled. Bile was red-tinged and watery. There was no evidence of the coelomic or cloacal masses found at initial presentation and no tubercular-type lesions were seen in the gastrointestinal tract, liver, or spleen. Histologic lesions were a severe chronic active fibrosing cardiomyopathy and a severe hepatopathy that was suggestive of a toxic reaction. Acid-fast stains failed to reveal evidence of acid-fast bacteria, and no mycobacteria were recovered from cultures of spleen, liver, and ceca.

DISCUSSION

Avian tuberculosis, confirmed by isolation of *M. avium* from granulomatous tissue, was resolved in this whooping crane before its death from an apparent adverse drug reaction (azithromycin + ethambutol). The first drug regimen (rifampin + ethambutol + *M. vaccae*) induced remission of disease, but 10 months after the antitubercular drugs were discontinued, the recurrence of wasting illness suggested recrudescence of infection. Treatment with azithromycin brought about a rapid and pronounced second remission. It is unknown if the final combination (azithromycin + ethambutol) contributed to the control of infection or if the apparent cure had already been effected. Although the results of necropsy suggest that mycobacterial infection was resolved, a sufficient time interval to assess the long-term outcome was not possible.

Azithromycin, a macrolide antibiotic, has shown in vitro and in vivo activity against *M. avium* complex infection in persons with AIDS (Young et al. 1991, Cynamon and Klemens 1994). The results of this clinical trial suggest further investigation of azithromycin as a promising therapeutic agent for *M. avium* infection in birds.

The etiology of the hepatopathy and cardiomyopathy found at necropsy cannot be proven, but the toxic nature of the changes and their temporal relationship to drug therapy (azithromycin + ethambutol) suggest an adverse reaction to the drugs. A recent report describes fulminant hepatic failure in humans undergoing antitubercular therapy with rifampin and/or isoniazid (Mitchell et al. 1995). A similar event likely occurred in this bird when given azithromycin and ethambutol.

Fecal AFB strains and cultures were not reliable for either diagnosis or surveillance of infection in this whooping crane. These tests were negative before and during treatment as well as during recrudescence. Accurate diagnosis required biopsy of affected tissue.

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