Infection with *Mycobacterium simiae* complex in four captive Micronesian kingfishers

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**Case Description**—4 captive adult Micronesian kingfishers (*Halcyon cinnamominina cin-namominina*) at 3 zoologic institutions were examined routinely or because of dyspnea or lethargy.

**Clinical Findings**—All birds had marked hepatomegaly. Two birds had dyspnea caused by compression of air sacs by the enlarged liver, and 1 bird had generalized weakness and lethargy. Three birds had distended coelomic cavities, and 3 birds were thin or had lost weight. There were no consistent abnormalities in blood analytes. Results of most ancillary diagnostic tests such as acid-fast staining of cloacal or fecal swab specimens and culture of feces for acid-fast bacteria were negative. Results of examination of hepatic biopsy specimens in 2 of 4 birds were suggestive of mycobacteriosis.

**Treatment and Outcome**—3 birds died or were euthanized soon after diagnosis. One kingfisher was isolated and monitored for 4 months without treatment and died during anesthesia for disease monitoring. Postmortem histologic examination revealed histiocytic hepatitis and acid-fast bacteria in all 4 birds. Bacteriologic culture of liver specimens yielded *Mycobacterium simiae* complex in all 4 birds.

**Clinical Relevance**—Infection with *M. simiae* complex should be considered in ill Micronesian kingfishers, and further monitoring is warranted to determine whether this is an emerging pathogen in this species. *(J Am Vet Med Assoc 2007;230:1524–1529)*

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**A healthy 8-year-old female Micronesian kingfisher** was given a thorough physical examination after its mate of 2.3 years died of avian mycobacteriosis. Examination of a heparinized blood sample revealed no abnormalities via CBC and high plasma activity of AST (850 U/L; reference range, 338 to 583 U/L), compared with reference values for Micronesian kingfishers. Results of radiography and palpation of the coelomic cavity were normal. Three cloacal swab specimens yielded negative results for acid-fast bacteria, and 3 fecal specimens yielded negative results of bacteriologic culture for mycobacterial organisms. Five months later, an examination of the bird was performed prior to transfer to another zoologic institution. The kingfisher appeared healthy, had normal results of palpation and radiography, and had mild leukopenia (3,000 cells/µL; reference range, 3,274 to 13,112 cells/µL) and hypoalbuminemia (1.2 g/dL; reference range, 1.1 to 2.7 g/dL). A presumptive diagnosis of mycobacteriosis, radiography revealed substantial hepatomegaly, and body weight had increased to 0.067 kg (0.147 lb). Examination of blood revealed high total protein concentration (4.8 g/dL; reference range, 2.8 to 4.4 g/dL) and hyperglobulinemia (3.2 g/dL; reference range, 1.1 to 2.7 g/dL). The bird received LRS (30 mL/
kg [13.6 mL/lb], SC) and a cloacal swab specimen and bacteriologic culture of feces yielded negative results for acid-fast and mycobacterial organisms, respectively. Twenty-five percent of the bird’s body weight was lost during the next 3 weeks. The Hct (52%) was near the upper limit of the reference range, and heterophilia (49%; reference range, 18% to 35%) and monocytosis (13%; reference range, 8% to 12%) were evident, despite total WBC and absolute differential counts within reference ranges. Total protein (7.8 g/dL) and globulin (6.5 g/dL) concentrations were greater than previously measured, and albumin (1.3 g/dL) concentration was near the lower limit of the reference range. There was marked hyperphosphatemia (7.1 mg/dL; reference range, 0.4 to 4.6 mg/dL) and high plasma activity of AST (1,284 U/L). A cloacal swab specimen again yielded negative results for acid-fast organisms.

Despite losing weight, the bird was eating well and energetic during the following 3 weeks. The bird survived more than 4 months after the diagnosis of mycobacteriosis; however, while the bird was anesthetized with isoflurane for additional disease monitoring, cardiac arrest occurred and the bird died. Blood was not submitted for analysis. A cloacal swab specimen yielded negative results for acid-fast bacteria, and results of culture were negative, but cytologic examination of a liver swab revealed many acid-fast bacilli. At necropsy, the liver was enlarged (weight, 4 g [7.7% of body weight]) and tan-brown in color. Adipose stores were scant. Histologically, more than 75% of the hepatic tissue was replaced by rounded clusterings of histiocytes that contained occasional foci of lymphocytes. Histiocytes formed linear aggregations in the perivascular parenchyma. Many foamy macrophages with intracytoplasmic bacilli were in the spleen. The bone marrow had high cellularity because of numerous myeloid cells and occasional foamy macrophages. Use of Ziehl-Neelsen staining confirmed the presence of acid-fast bacilli in macrophages in all areas of histiocytic inflammation. Mycobacterium simiae complex was cultured from the liver by use of liquid culture media and confirmed via HPLC.

A 3-year-old female Micronesian kingfisher appeared healthy until acute onset of dyspnea, lethargy, and weakness. Despite thin body condition (body weight, 0.058 kg [0.128 lb], the coelomic cavity was distended and firm. Blood analysis revealed heterophilia (8,280 cells/µL; reference range, 385 to 4,587 cells/µL), band heterophilia (240 cells/µL; reference range, 0 cells/µL), and toxic changes. Plasma biochemical analysis was limited to 2 tests because of sample volume. Uric acid concentration was high (23 mg/dL; reference range, 4 to 18 mg/dL), and albumin concentration (1.2 g/dL) was low. The bird received enrofloxacin (5 mg/kg [2.3 mg/lb], IM) and LRS (17 mL/kg [7.7 mL/lb], SC). The following day, dyspnea was evident at rest, and the bird was placed in an oxygen chamber for preparation for fine-needle aspiration of the middle portion of the coelomic cavity. Cytologic examination of the aspirate revealed findings suggestive of granulomatous coelomitis, and the bird was anesthetized with isoflurane for exploratory surgery. The liver was markedly large, friable, and tan, and a liver lobe was resected. The bird died during the procedure.

At necropsy, the liver was pale tan with a grainy white pattern and was enlarged (weight, 3 g [6% of body weight]). Histologic examination revealed that approximately 5% of the liver parenchyma consisted of hepatocytes and approximately 95% was composed of macrophages. Similar macrophage infiltrates were also detected in the spleen, bone marrow, lungs, and mesovarium. Ziehl-Neelsen staining was used to detect acid-fast bacilli in macrophages in areas of histiocytic inflammation in the liver, lungs, and mesovarium. Bacteriologic culture of liver yielded M simiae complex at the same laboratory and by use of the same techniques as for the first bird of this report.

The bird had been housed with a male for 3.5 years prior to death. To screen for mycobacteria in this mate, the bird was evaluated immediately and 9 months and 13 months after the death of the female. Evaluations included blood analyses, radiography, cloacal swab specimens, and liver biopsies, and all results were unremarkable. Thirty months after death of its mate, the male bird was healthy and had results of blood analyses that were within reference ranges.

A 6.5-year-old, 0.059-g (0.130-lb) male Micronesian kingfisher had progressive episodes of open-mouth breathing during a 1-month period. The coelomic cavity became enlarged, and an intracoelomic mass was palpable. Isoflurane anesthesia was used to facilitate diagnostic procedures. Ultrasonography and contrast radiography performed with diatrizoate meglumine administered via gavage revealed displacement of the gastrointestinal tract, suggesting that the mass was the liver. A CBC revealed mild anemia and marked leukocytosis (31,700 cells/µL with heterophilia [21,239 cells/µL] and monocytosis [4,121 cells/µL]. Band heterophilia were detected but not counted. Plasma biochemical analyses revealed hyperproteinemia (5.9 g/dL), hyperglobulinemia (4.6 g/dL), and high activity of AST (1,754 U/L). A left lateral laparotomy was performed, and the liver was enlarged and tan in color; 2 biopsy specimens were collected. Enrofloxacin (15.7 mg/kg [7.14 mg/lb], PO, q 12 h) was prescribed. Histologic examination revealed diffuse histiocytic hepatitis with many acid-fast bacteria. Approximately 90% of the liver was composed of macrophages and giant cells; euthanasia was performed because of the poor prognosis.

At necropsy, the bird was in poor body condition with a markedly swollen, pale tan liver (weight, 16 g) that compressed the other coelomic organs (Figure 1). The spleen was also enlarged and pale tan. Histologic examination revealed that the liver, spleen, and pancreas were almost entirely effaced by macrophage and multinucleated giant cell infiltrates and only scant remnants of parenchymal cells remained (Figure 2). Perivascular and interstitial infiltrates of macrophages and giant cells were also evident in the lungs and kidneys. Fites acid-fast staining revealed many acid-fast bacilli within macrophages in all areas of histiocytic inflammation. The final histopathologic diagnosis was multifocal chronic active histiocytic inflammation with intracellular acid-fast bacteria. Mycobacterium simiae complex was cultured from the liver and spleen on liquid and solid culture media and confirmed via HPLC.

A 2.5-year-old 0.06-kg (0.13-lb) female Micronesian kingfisher was flushed and slightly lethargic. Re-
Results of a CBC indicated leukocytosis (13,600 cells/µL) with monocytosis (3,944 cells/µL); plasma biochemical analysis was not performed. Enrofloxacin (5 mg/kg [2.3 mg/lb], PO, q 12 h for 14 days) was administered, and 3 weeks later, results of physical examination, radiography, and monocyte count were unremarkable, although high plasma activity of AST (673 U/L) was detected. No acid-fast bacteria were seen in a cloacal smear specimen. Five weeks later, leukocytosis (13,600 cells/µL) with lymphocytosis (8,840 cells/µL; reference range, 688 to 7,044 cells/µL) was detected, although the bird did not have clinical signs of disease. Four months later, the bird was fluffed, had signs of depression, and was unable to fly to its perch. The bird was thin (weight, 0.056 kg despite having a distended coelomic cavity and palpable hepatomegaly. Examination of a fecal sample revealed many acid-fast bacteria. Because of the bird's distress, it was placed in an oxygen chamber; blood was not collected. The bird died overnight.

At necropsy, the bird was cachexic and had a markedly distended and firm coelomic cavity. The liver occupied approximately 80% of the coelomic cavity and was firm and pale tan with a few mottled red regions on the capsular surface. The enlarged liver compressed coelomic organs including the heart, intestinal tract, air sacs, and oviduct. The spleen was diffusely tan and firm, similar to the liver. Histologic examination revealed that approximately 90% of the hepatic parenchyma was replaced by infiltrates of macrophages and scattered multinucleated giant cells. Similar macrophage and giant cell infiltrates were evident in the spleen (replacing 40% of the splenic parenchyma), and mild infiltrates were evident in the lungs, adrenal glands, ventriculus, and small intestine. Pale basophilic slender bacilli were observed within macrophages in multiple tissues, and acid-fast staining revealed bacilli in the liver and ventriculus. Bacteriologic culture of a liver sample by use of liquid and solid culture media yielded M. simiae complex; identity of the bacterium was confirmed via HPLC.

Discussion

To the authors’ knowledge, this is the first report of infection with M. simiae complex in Micronesian kingfishers. Results of a recent study2 suggest that Mycobacterium avium and Mycobacterium intracellulare infections are rarely caused by direct or indirect bird-to-bird transmission and that exposure is most likely from an environmental source. It is unknown whether agents in the M. simiae complex are transmitted in a similar manner. Three of the 4 infected birds in the present report had previous exposure to another kingfisher infected with a mycobacterium. The first and third kingfisher described in this report both had mates with histologic evidence of avian mycobacteriosis, and the fourth kingfisher in this report was the offspring of a male kingfisher with histologic evidence of avian mycobacteriosis. Mycobacterial infection in the 4 studied kingfishers was initially suspected on the basis of acid-fast staining and histopathologic changes in the liver and other organs. Definitive diagnosis was made via mycobacterial culture of liver and HPLC analysis of the isolates, which had trimodal mycolic acid profiles of mycobacteria closely related to M. simiae. Mycobacterial nomenclature and phylogenetic relationships change frequently as new diagnostic and genetic tests become available. The National Jewish Medical and Research Center, which performed bacteriologic culture of one of

![Figure 1](image1.png)

Figure 1—Postmortem photograph of a Micronesian kingfisher infected with Mycobacterium simiae complex. The liver (L) is massively enlarged and pale. H = Heart. Bar = 2 cm.

![Figure 2](image2.png)

Figure 2—Photomicrographs of a portion of the liver from a Micronesian kingfisher infected with M. simiae complex. A—Notice that a thin remnant of hepatocytes (H) remains between massive sheets of foamy macrophages (M). H&E stain; bar = 100 µm. B—Notice that a thin remnant of hepatocytes (H) remains between sheets of macrophages containing numerous acid-fast bacteria (Mb). Fites acid-fast stain; bar = 100 µm.
The kingfisher samples, defined M simiae complex as including M simiae, Mycobacterium lentiflavum, Mycobacterium triplex, and Simiae-avium-like organisms, which is supported by other publications. At the time of the cases reported here, no generally accepted laboratory method for differentiating the species within the complex was available. However, recent work with HPLC, gas-liquid chromatography, DNA hybridization, 16S rRNA, and hsp65 gene sequencing indicates that M simiae, Mycobacterium genavense, M lentiflavum, M triplex, and a novel mycobacterium designated Mycobacterium sherrisi sp nov are genetically distinct but phylogenetically related. Of these 5 closely related mycobacteria, only M genavense has been previously reported in birds, to the authors' knowledge. Our review of the literature indicated that infections with M lentiflavum or M sherrisi sp nov have not been diagnosed in veterinary species, whereas M triplex has been found in fish and M simiae in monkeys, mice, and a domestic cat. Although M genavense is closely related to these mycobacteria, the laboratories in the present study were unable to culture M genavense; detect it by use of blood culture systems, or identify its characteristic profile via HPLC. Therefore, the 4 kingfishers were considered to have negative results for M genavense and positive results for M simiae complex, which is important because none of the other bacteria in the M simiae complex have previously been detected in Micronesian kingfishers or other avian species.

The mycobacteria in the M simiae complex are considered nontuberculous mycobacteria and are present in the environment as saprophytes. M simiae has been found in sphagnum, licks of healthy humans, and water. Nontuberculous mycobacteria are opportunistic pathogens with variable pathogenicity. A national study by the CDC found that 21% of M simiae isolates were associated with disease, immunocompromised individuals were more at risk, and there has been an increase in infections in certain geographic regions. Most published information on avian mycobacteriosis concerns M avium subsp avium (hereafter referred to as M avium), M intracellulare, and M genavense. A diagnosis of mycobacteriosis is a stepwise process, and a review of diagnostic testing has been published. Veterinarians must combine clinical signs and results of blood tests, radiography, laparoscopy, biopsy, cytology, culture, and advanced laboratory testing. The infected kingfishers reported here had typical clinical signs of avian mycobacteriosis of slowly progressive decline as seen in all 4 birds reported here, although none had typical radiographic bony lesions. There are no pathognomonic hematoxic or biochemical findings associated with avian mycobacteriosis. Birds infected with M avium may have leukocytosis, heterophilia, mononcytosis, or no WBC abnormalities despite clinical disease or mild to moderate anemia. Some infected birds may have high activities of AST, WBC abnormalities despite clinical disease or mild to moderate anemia, and plasma protein concentrations are variable. In a study of zoo birds, an arbitrary total WBC count of ≥ 18,000 cells/µL was used to suggest the possibility of mycobacteriosis. This critical value would not have been an accurate indicator of infection with M simiae complex in the birds reported here because only one of the WBC counts exceeded that value. Excluding that particular WBC count, the range of values was 3,000 to 13,600 cells/µL, which was similar to the reference range for Micronesian kingfishers. Therefore, mycobacteriosis caused by M simiae complex cannot be ruled out on the basis of the WBC count. Similar to M avium, high AST activity values can be detected in animals infected with M simiae complex, and because creatine phosphokinase values are often not abnormal, this may be attributed to liver damage.

Hepatosplenomegaly is a common finding on necropsy of birds infected with M avium and was seen in all 4 affected kingfishers. Similar to lesions caused by other types of mycobacterial infection, organomegaly caused by M avium infection can be the result of granuloma formation, histiocytic inflammation, or amyloid deposition; the granulomatous form is the most common. In contrast, organomegaly in the 4 birds reported here was caused by histiocytic inflammation; the pathogenesis of this lesion was unclear. In a report of M avium infection in a Micronesian kingfisher, hepatomegaly associated with histiocytic inflammation was observed. Thus, a histiocytic inflammatory response and associated organomegaly may be a unique response of Micronesian kingfishers to mycobacterial infection.

Because avian mycobacteria are often present within the gastrointestinal tract, feces should be collected for acid-fast staining and bacteriologic culture. Sensitivity of these tests may improve as the disease progresses and may be enhanced by collecting feces on sequential days to account for intermittent shedding. Of the cloacal and fecal smears submitted from the kingfishers, only 1 yielded positive results for acid-fast bacteria; that smear was obtained within 24 hours of death when the bird had advanced disease. Conversely, the first kingfisher of this report had histologic evidence of mycobacteria, was monitored aggressively for mycobacterial shedding, and had negative results for acid-fast bacteria in cloacal swab specimens (n = 4) and mycobacterial cultures of feces (2) during 5 months of screening after diagnosis. Although fecal testing has been successful in experimental avian mycobacteriosis, it was not helpful in the Micronesian kingfishers reported here; examination for hepatomegaly and bacteriologic culture of liver biopsy specimens were more informative.

Once a mycobacterial isolate is obtained via bacteriologic culture of appropriate tissues, a variety of laboratory techniques such as HPLC, genetic sequencing of 16S rRNA, or DNA-DNA hybridization are available to identify the species of mycobacteria. Disagreements are evident in the clinical microbiology literature regarding which testing methods are best. However, because mycolic acids of bacterial cell walls are species specific, HPLC is a rapid, accurate, and cost-effective method for identifying mycobacterial species and was used to diagnose infection with M simiae complex in liver tissues obtained at necropsy from the kingfishers reported here. At the time of this report, only 80 Micronesian kingfishers were in captivity; therefore, infection with M simiae complex was diagnosed in 5% (4 of 80) of the captive population. This is important from a conserva-
tiation standpoint because the Guam subspecies of the Micronesian kingfisher is extinct in the wild and captive propagation programs are used to help repatriate the species. We recommend that a bird with a suspected infection be quarantined for 1 year and noninvasive testing, such as use of cloacal swab specimens and feces for culture of acid-fast organisms, radiography, and blood analyses, be performed quarterly; liver biopsy for histopathologic examination and culture should be performed at 6 months and 1 year. Treatment of mycobacteriosis in birds is rarely pursued.8

Although we were unable to identify the particular mycobacterial agent within the M simiae complex responsible for the disease processes reported here, results indicated that the M simiae complex was pathogenic in Micronesian kingfishers. Additional studies are warranted to determine whether M simiae, M lentiflavum, M triplex, or M sherrisi sp nov can individually cause disease in birds as has been reported with M genavenae. Because nontuberculous mycobacteria are often environmental contaminants, it is proposed that wild birds are less likely to become infected than captive birds. Additional research is needed to determine whether other veterinary species, and birds in particular, are susceptible to the agents grouped together as the M simiae complex.

References

Selected abstract for JAVMA readers from the American Journal of Veterinary Research

Response of hypotensive dogs to dopamine hydrochloride and dobutamine hydrochloride during deep isoflurane anesthesia
Monica Rosati et al

Objective—To evaluate the dose-related cardiovascular and urine output (UrO) effects of dopamine hydrochloride and dobutamine hydrochloride, administered individually and in combination at various ratios, and identify individual doses that achieve target mean arterial blood pressure (MAP; 70 mm Hg) and cardiac index (CI; 150 mL/kg/min) in dogs during deep isoflurane anesthesia.

Animals—10 young clinically normal dogs.

Procedures—Following isoflurane equilibration at a baseline MAP of 50 mm Hg on 3 occasions, dogs randomly received IV administration of dopamine (3, 7, 10, 15, and 20 µg/kg/min), dobutamine (1, 2, 4, 6, and 8 µg/kg/min), and dopamine-dobutamine combination (3.5:1, 3.5:4, 7:2, 14:1, and 14:4 µg/kg/min) in a crossover study. Selected cardiovascular and UrO effects were determined following 20-minute infusions at each dose.

Results—Dopamine caused significant dose-dependent responses and achieved target MAP and CI at 7 µg/kg/min; dobutamine at 2 µg/kg/min significantly affected only CI values. At any dose, dopamine significantly affected UrO, whereas dobutamine did not. Target MAP and CI values were achieved with a dopamine-dobutamine combination at 7.2 µg/kg/min; a dopamine-related dose response for MAP and dopamine- and dobutamine-related dose responses for CI were identified. Changes in UrO were associated with dopamine only.

Conclusions and Clinical Relevance—In isoflurane-anesthetized dogs, a guideline dose for dopamine of 7 µg/kg/min is suggested; dobutamine alone did not improve MAP. Data regarding cardiovascular and UrO effects indicated that the combination of dopamine and dobutamine did not provide greater benefit than use of dopamine alone in dogs. (Am J Vet Res 2007;68:483–494)