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## MYCOBACTERIUM AVIUM-RELATED EPIZOOTIC IN FREE-RANGING LESSER FLAMINGOS IN KENYA

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**ABSTRACT:** An epizootic in free-ranging lesser flamingos (*Phoeniconaias minor*) in Kenya resulted in more than 18,500 deaths from August through mid-November 1993. Disease was concentrated along the shores of Rift Valley Lakes Bogoria and Nakuru (Kenya) and did not involve any of the other avian or mammalian species frequenting the lakes. Coincidental to the outbreak was a bloom of algae on Lake Bogoria, toxins from which were first suspected to be causative. Discrete necrotic and granulomatous lesions were often noted in spleen and liver, and *Mycobacterium avium* serovar I was isolated from both organs. *Escherichia coli* and *Pseudomonas aeruginosa* also were often recovered in pure culture from liver. Gross and histopathological evaluation of the cases disclosed signs of acute sepsis and also chronic, potentially life-threatening lesions of mycobacteriosis, primarily involving the spleen and liver. Lesions typical for algae toxicosis were not seen in any birds. Deaths were attributed to septicemia, complicated in those affected, by mycobacteriosis.

**Key words:** Epizootic, lesser flamingo, mycobacteriosis, *Phoeniconaias minor*, septicemia.

### INTRODUCTION

Avian tuberculosis has been reported previously in lesser flamingos frequenting the Rift Valley lakes in Kenya (Cooper et al., 1975; Sileo et al., 1979). A survey conducted in 1974 indicated a high prevalence of the disease and many debilitated birds, while 2 yr later neither debilitated birds nor the disease were apparent (Sileo et al., 1979). The disease outbreak in 1974 was attributed to malnutrition-induced immunodeficiency, the result of a dramatic decline in the *Spirulina* spp. algae, upon which lesser flamingos primarily feed. Although mycobacteriosis has been reported only in lesser flamingos and one African fish eagle (*Cuncuma vocifer*) at Lake Nakuru (Kaliner and Cooper, 1973), white pelicans (*Pelecanus onocrotalus*), ruff (*Philomachus pugnax*), and greater flamingos (*Phoenicopterus ruber*) also have been affected (L. Sileo, unpubl. data).

### MATERIALS AND METHODS

An epizootic affecting lesser flamingos began on the shores of Lake Bogoria in Kenya (extending from 0°11' to 0°20'N, 36°05' to 36°05'E) in August 1993. Postmortem examinations were performed on 42 birds from the two lakes, tissues were fixed in 10% buffered formalin and submitted to the University of

Zimbabwe (Faculty of Veterinary Science, Harare, Zimbabwe) for histological evaluation. Tissues were trimmed, embedded in paraffin, cut at 6–10 µm, and stained with hematoxylin and eosin. In some cases, swabs of liver were taken aseptically in charcoal and Stewart's transport media (Oxoid, London, UK), and submitted for bacterial culture at the Nairobi Hospital Laboratory (Nairobi, Kenya).

Spleen and liver were frozen and submitted to the University of Zimbabwe for culture. After thawing, impression smears were stained with Ziehl-Nielsen and Gram stains (Barrow and Feltham, 1993). Spleen and liver were separately homogenized with a mortar and pestle and 5 g of each were placed in nutrient broth (Oxoid) treated with 4% NaOH, and centrifuged at 5,000 g for 15 min. The pellet was resuspended in phosphate buffered saline, neutralized with 1N HCl, and cultured on both Lowenstein-Jensen and Stonebrink's slants, prepared at the University of Zimbabwe following procedures described elsewhere (Barrow and Feltham, 1993). These were incubated in candle jars at about 10% CO<sub>2</sub> and aerobically. Aliquots of the suspensions were also placed on sheep blood agar plates at 37 C under aerobic and anaerobic conditions. The techniques employed for the isolation and identification of *Mycobacterium* spp. and other aerobic bacteria were essentially those described elsewhere (Barrow and Feltham, 1993; Vestal, 1977). Subcultures of the *Mycobacterium* spp. also were submitted to the Mycobacterial Reference Laboratory (Brisbane, Australia) for confirmation.

## RESULTS

Most deaths occurred in adults early in the epizootic but juveniles >6-mo-old were affected subsequently. As breeding occurs only in Tanzania, nestlings were not involved. Both sexes appeared to be affected equally. Nearly a month after the first reports of mortalities on Lake Bogoria, dead birds were discovered on the shores of Lake Nakuru (0°18' to 0°23'S, 36°03' to 36°07'E), 48 km south of Lake Bogoria. By the end of October, census indicated that approximately 18,500 deaths had occurred at both locations, while other avian and mammalian species frequenting the lakes did not exhibit unusual mortalities. More than 400 avian species have been recorded over a 2 day period at Lake Bogoria, alone.

Gross postmortem examinations revealed widespread petechiation in most birds. Other lesions present in some of the birds included gastrointestinal erythema, swollen, exudative tibiotarsal and humeroradial joints, hepatomegaly, splenomegaly, skin sloughing from the webs of the feet, and discrete foci of necrosis in the liver and spleen. Body condition varied, and was considered good in most of the birds, with the exception of those with advanced liver and splenic necrosis. Of the 42 birds examined histologically, 17 (>40%) had granulomata in a variety of organs, with the liver (6/42) and spleen (16/42) being affected most often. Granulomata also were occasionally found in kidney, lung, gastrointestinal tract, ovary, and bone marrow, although the latter two tissues were not always available for examination. The granulomata were characterized by extensive necrosis and prominent macrophage infiltration, in which acid-fast bacteria were numerous. In addition to chronic mycobacteriosis, acute inflammatory foci, with neutrophil infiltration, consistent with embolic seeding by conventional bacteria, were present in liver and kidney in 33 and 3 of the 42 birds, respectively. Mild, acute to subacute, non-

granulomatous enteritis was diagnosed in 13 birds, and fungal pneumonia, with filamentous, septate hyphae, typical of *Aspergillus* sp. co-existed with mycobacteriosis in one case.

Microbiology results from the 19 cases from which liver was submitted for bacterial culture, yielded essentially pure bacterial cultures in some cases. *Escherichia coli* was found in six, *Pseudomonas aeruginosa* occurred in another six, *Staphylococcus aureus*, was isolated from one bird and there was no isolation in six cases.

Ziehl-Nielsen staining of the impression smears of liver and spleen demonstrated numerous acid-fast coccobacillary rods in haphazard arrangements without beading. Gram-stained smears showed a small proportion (5 to 10%) of larger, often beaded organisms, morphologically similar to *Corynebacterium* sp. Milky-white colonies, suggestive of *Mycobacteria* spp., were visible after 10–12 days in culture under both microaerophilic and aerobic atmospheric conditions, and on the slants. *Staphylococcus* spp. and *Escherichia* spp., but not *Corynebacterium* spp., grew on blood agar. The mycobacterial isolates were neither photochromogenic nor scotochromogenic. Catalase-positive growth occurred at 22 C, 25 C, 37 C, 42 C, and 45 C, but was most luxuriant at 37 C. The organisms neither reduced nitrate nor produced urease, and the catalase resisted exposure to 68 C. Isolates from both spleen and liver appeared similar to, and behaved like *Mycobacterium avium* complex. The isolates sent to the Mycobacterial Reference Laboratory were confirmed as *Mycobacterium avium* serovar 1.

## DISCUSSION

The epizootic in flamingos occurred during a drought, a time when the population was considered unusually high, water levels were low, and ambient temperatures were high. The number of flamingos using the East African Rift Valley lakes varies, but census reports between 1974 and 1976 indicated between 350,000 and

550,000, while in 1969 the figure was about one million (Tuite, 1979). The population at Lake Bogoria, alone, was estimated at about 45,000 in January 1993 and 25,000 in September 1993. As an uncharacteristic bloom of algae (*Spirulina* sp.) coincided with the epizootic, it was initially suspected that algae toxicosis was responsible for the epizootic. The gross appearance of the liver supported this, but the splenic necrosis was inconsistent. Algal toxicosis is a well recognized cause of periacinar to massive hepatic necrosis, although it is typically associated with ingestion of the blue-green algae, *Microcystis aeruginosa*, during algal blooms (Kelly, 1993). *Spirulina* spp. have not been implicated in toxicosis, and are considered part of the normal diet for lesser flamingos. It is possible that the conditions resulting in the algae bloom also favored the proliferation of *P. aeruginosa* and *E. coli* to levels sufficient to overwhelm the birds on ingestion. *Pseudomonas aeruginosa* is saprophytic and aquatic, commonly found in free-standing water (Schlegel, 1993), and *E. coli* can exist in the environment for extended periods, causing disease upon ingestion of contaminated water or food (Baron, 1986). *Spirulina* spp. grow at just the water level at which lesser flamingos feed. Thus, the contamination of algae by large numbers of bacteria prior to ingestion, would explain both the route of infection for the flamingos, as well as the absence of infections in other species. The mycobacterial infections in many of the birds likely increased their susceptibility to conventional bacterial infections, and contributed to death due to debilitation, as mycobacteriosis and immune compromise are often linked (Jawetz et al., 1982).

The widespread petechiation noted grossly in many flamingos was consistent with sepsis, which was most likely directly related to death. The acute, microscopic inflammatory lesions in liver and kidney were typical for embolic spread of these bacteria, being characterized by intense multifocal neutrophil infiltration, unlike

the chronic histiocytic infiltrates associated with the mycobacterial lesions. The skin sloughs in the webs of some of the birds were retrospectively attributed to burns sustained at the Lake Bogoria hot springs where debilitated birds were often marooned.

When deaths were reported at Lake Nakuru, the main concern was whether or not the disease was surfacing there independently or was in some way linked to the Lake Bogoria epizootic. The similarities in necropsy findings suggested a common etiology, although a bloom of algae had not occurred at Lake Nakuru. However, flamingos are known to move between the Rift Valley lakes, although actual patterns have not been established. Affected birds were seen in flight at Lake Bogoria, making it conceivable that sick birds flew south to Lake Nakuru, where they eventually died, resulting in what then appeared to be the beginning of a second epizootic. Deaths also were recorded at Lake Solai (0°01'N, 36°10'E), in between Lakes Bogoria and Nakuru, where debilitated birds likely stopped, but not at Lake Elementaita (0°24' to 0°29'S, 36°12' to 36°15'E), approximately 20 km south of Lake Nakuru. By mid-November, with the arrival of the rains and the dispersal of the algae, the population of lesser flamingo again appeared healthy and no further deaths have been reported to date. The epizootic may represent part of the natural population dynamics and ecology for the lesser flamingo on the Rift Valley lakes.

Cultures from the 1979 report of mycobacteriosis in lesser flamingos on Lake Nakuru unfortunately were lost and the isolates were not typed (Sileo et al., 1979), but those isolated earlier from four birds were identified as *M. avium* type I (Cooper et al., 1975). The distribution of the granulomata differed in the earlier report, where lung and serosa were often affected and spleen and liver were not. The fact that many migratory birds frequent the Rift Valley lakes, makes it likely that this robust organism and the disease are trans-

ported long distances. Further studies to document the endemnicity of mycobacteriosis at Lake Nakuru and the migration patterns of birds between the lakes would provide useful information in understanding future recurrences of this disease.

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