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Mycobacterium chelonae Osteoarthritis in a Kemp's Ridley Sea Turtle (*Lepidochelys kempii*)

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ABSTRACT: A stranded Kemp's ridley sea turtle (*Lepidochelys kempii*) was rescued and treated at the National Aquarium in Baltimore (Maryland, USA) for inappetence and epidermal appendicular and plastral lesions. After 4 mo of care, the turtle developed a swollen left elbow joint. Within 1 mo of initial swelling, osteolytic lesions developed in the proximal radius and ulna. The elbow joint was surgically debrided, flushed, and cultured. The incision dehisced 10 days after surgery. *Mycobacterium chelonae* was cultured from the left elbow joint and from a skin nodule of the dorsum of the right front flipper. The turtle was euthanized due to apparent systemic infection with *M. chelonae. Mycobacterium chelonae* was isolated from cultures taken at necropsy of the lung, liver, spleen, kidney, and pericardium. Osteoarthritic infections with *M. chelonae* have not been reported in reptiles. Additionally, primary osteoarthritic diseases of synovial joints are uncommon in reptilian species. Due to the paucity of reports of mycobacterial diseases in sea turtles, the continued documentation of these cases will increase knowledge and understanding in caring for these endangered animals.

Key words: Case report, joint, Kemp's ridley sea turtle, *Lepidochelys kempii, Mycobacterium chelonae,* osteoarthritis, sea turtle.

A Kemp's ridley sea turtle (*Lepidochelys kempii*) was caught in a pound net where the Pocomoke River enters the Chesapeake Bay and brought to the National Aquarium in Baltimore (Maryland, USA) by the Maryland Department of Natural Resources. The turtle had a curved carapace width and length of 35 cm each, indicating that it was a sub-adult (Chaloupka and Musick, 1997).

The sea turtle was initially placed in a rehabilitation pool (3331.16 l, 3.66 m×1.52 m×0.46 m, of artificial sea water at 26 C, with a sand filter, and full water changes every 48 hr). Physical examination showed the sea turtle to be alert and responsive, with great muscular strength. Multiple 1–2 cm circular epidermal lesions were present on the carapace and all flippers. A full thickness (approximately 2 cm wide×3 cm long) portion of the keratinaceous maxillary beak was missing immediately below the nares. The initial complete blood count (CBC) and serum chemistries revealed a heterophilia ($11.65\times10^3/\mu l$) and hypoalbuminemia (0.3 gm/dl) when compared to in-house normal *L. kempii* reference values (National Aquarium in Baltimore, Maryland, sample size $n=10$). A transponder identification chip (American Veterinary Identification Devices, AVID, Norco, California, USA) was placed aseptically in the subcutaneous tissue of the left fore-flipper. Epidermal biopsies from the flipper and carapace were taken using a scalpel blade, incorporating the margin between damaged and normal tissue, and fixed in 10% neutral buffered formalin (NBF). The prepared tissue sections were stained with hematoxylin and eosin for evaluation. Initial therapy included cleansing of the lesions with organic iodine (Betadine® Solution, Purdue Frederick Company, Norwalk, Connecticut, USA); nutritional support included tube feeding (squid, fish, or crabmeat blended with fresh water and administered according to the turtle's metabolic needs), and offering occasional live crabs. Biopsy results from both samples contained numerous septate branching fungal hyphae, most consistent with an active fungal dermatitis acquired when in the wild. Other diagnostics performed at this time did not elucidate an initial cause of the dermatitis. One

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epidermal biopsy also showed some colonies of small bacterial rods; however, a separate sample submitted for Gram stain did not reveal the bacteria seen in the biopsies. These samples were not sufficient for additional fungal or bacterial isolation. Itraconazole (7.5 mg/kg orally every 24 hr; Janssen Pharmaceutical Inc., Titusville, New Jersey, USA) and enrofloxacin (5 mg/kg intramuscular (IM) every 24 hr; Baytril®, Bayer Corporation, Shawnee Mission, Kansas, USA) (Stein, 1996) were initiated.

The turtle was anorectic during the first month of rehabilitation, but began eating crabs by day 28 of rehabilitation. During that time the turtle maintained weight on tube fed gruel, defecated four times, and moved with normal strength. By wk 8 the carapace lesions resolved; however, the beak defect and several flipper lesions persisted. The epidermal flipper lesions were rebiopsied (mo 2), and submitted for histopathology; culture was not performed at this time. Histopathology showed mild to moderate acute hemorrhagic epidermitis; no etiologic agents were identified on these biopsies. Itraconazole and enrofloxacin therapy was discontinued.

A third biopsy and first culture was performed of the flipper lesions at wk 9, due to persistent flipper dermatitis and doubt that the infectious component had resolved. This biopsy indicated moderate subacute ulcerative dermatitis with septate and branching fungal hyphae and bacteria. *Flavobacterium meningoseptium,* sensitive to enrofloxacin, was isolated in culture. Fungal identification was unsuccessful; however, most fungal diseases are sensitive to Itraconazole. Itraconazole (7.5 mg/kg orally every 24 h) and enrofloxacin (5 mg/kg IM every 24 hr) therapy was resumed and then discontinued at 3 mo of rehabilitation when the turtle was stable and epidermal lesions were healing well.

After 4 mo of rehabilitation, the left elbow became swollen and a nodule developed on the dorsum of the right front flipper. Complete blood count and serum chemistry panel were within normal limits when compared to in-house values. Radiographs taken of the left elbow at this time showed soft tissue swelling but no bone involvement (Fig. 1). The identification chip was radiographically evident in the soft tissue distal to the swelling and the joint.

FIGURE 1. Initial radiographic evaluation of left elbow of a Kemp's ridley sea turtle (*Lepidochelys kempii*). Soft tissue swelling is present at the elbow joint.

Follow-up radiographs 1 mo later (rehabilitation mo 5) indicated osteolysis of the proximal radius and ulna (Fig. 2). A sterile preparation of the elbow was done with organic iodine and alcohol, and aspiration of the joint was attempted but sampling was unsuccessful.

The following day the sea turtle was anesthetized and surgery was performed to obtain a sample for culture and histopathology and to debride the joint. The cartilage of the distal humerus was abnormal in appearance and was removed along with the diseased portions of the proximal

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FIGURE 2. Recheck radiographic evaluation of left elbow 1 mo later. Notice the marked osteolysis of the proximal ulna and radius. The needle was used for attempted joint aspiration.

radius and ulna. The joint was lavaged extensively with sterile saline followed by closure of the joint capsule, muscles, and skin. Samples for culture were taken of whole blood, the left elbow cartilage and bone, and the right flipper nodule. Primary isolation was performed at the National Aquarium in Baltimore. Swabs of the left elbow joint and right flipper nodule were inoculated onto tryptic soy agar with 5% sheep's blood (BAP) and Lowenstein Jensen (L-J) agar (Remel, Inc., Lenexa, Kansas); media were incubated at room temperature (23–25 C). Whole blood was placed in blood culture media (Remel, Inc.) and incubated at 37 C. An incisional biopsy of the right flipper nodule was fixed in 10% NBF and submitted for histopathology.

The turtle was started on ceftazidime (20 mg/kg every 72 hr IM; Fortaz® Glaxo Wellcome, Inc., Research Triangle Park, North Carolina, USA) (Stein, 1996). Within 10 days post-operatively the surgical site began to dehisce, despite management in a dry, padded environment. Impression smears from the pending cultures at this time were positive with acid-fast gram-negative rods. Ceftazidime was discontinued, due to the lack of clinical response. Treatment began with amikacin sulfate (10 mg/kg IM every 24 hr) (Phoenix Scientific, Inc., St. Joseph, Missouri, USA) (Stein, 1996), enrofloxacin (5 mg/kg PO every 24 hr), intracoelomic fluid therapy, and wet to dry bandages changed daily. Amikacin was selected due to the reported sensitivity of rapidly growing mycobacteria to these drugs (Brown, 1985) and enrofloxacin for broad-spectrum activity.

Twenty-one days post-operatively *M. chelonae* was identified as the infectious agent of both the joint and the nodule, indicating a possible systemic mycobacterial infection was present. Additionally, the left fore-flipper's condition continued to decline rapidly. The turtle was euthanized due to poor prognosis for recovery (Frye, 1991) with intravenous (IV) pentobarbital (975 mg; Fatal Plus® Solution, Vortech) followed with potassium chloride (2 ml IV).

Gross findings included the swelling and dehisced surgical incision of the left flipper and overall poor condition of the animal. There were several small ulcerative lesions on all four limbs, the plastron, and the beak. Histologic examination revealed multifocal areas of necrosis of the left flipper involving skin, skeletal muscle, and bone. These necrotic areas were surrounded by lymphocytes, macrophages, multinucleated giant cells and contained acid-fast bacterial rods in a necrotic matrix. Hepatocytes showed moderate fatty change. *Mycobacterium chelonae* was isolated from the lung, liver, spleen, kidney, pericardium, and the identification chip.

Mycobacterium chelonae belongs to the Runyon Group IV atypical mycobacteria, which are characterized by rapid growth and high resistance to antibiotics (Jonge-

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vos et al., 1999). Isolates of *M. fortuitum* (also Runyon Group IV) have been found in water, soil, and dust, and *M. chelonae* is believed to have a similar ecology (Brown, 1985). *Mycobacterium chelonae* was first isolated by Friedman in 1903 from the lung of a turtle identified at that time as *Chelonia corticata* (common name unspecified) (Marcus, 1981; Miller et al., 1990).

Mycobacteria chelonae grows optimally in aquatic environments at 22–40 C, which is equivalent to the body temperature of poikilothermic species. However, reptilian tuberculosis is considered a sporadic disease with a very low annual incidence in well-managed collections (e.g., 0.1–0.5%) (Cowan, 1968; Brownstein, 1978). Reports of mycobacteriosis in chelonians indicate that plastral ulcerations and pulmonary tubercles are the most common findings (Brock et al., 1976; Rhodin and Anver, 1977; Keymer, 1978a, b; Leong et. al., 1989; Glazebrook and Campbell, 1990a, b).

In a large survey of chelonians (144 tortoises, 122 terrapins, and seven sea turtles) one case of mycobacteriosis was reported (a green turtle, *Chelonia mydas*) (Keymer, 1978a, b); however, method of mycobacteria identification and anatomical location of infection were not identified. In a survey of 141 sea turtles (*C. mydas, Eretmochelys imbricata,* and *Caretta caretta*) from farm, wild, and oceanarium-reared turtles (Glazebrook and Campbell, 1990a, b) two cases of mycobacteriosis were identified, using acid-fast stains of lung tissue in two farmed green sea turtles. Three of 120 captive reared hatchling Pacific green sea turtles had gross tuberculous lung lesions and were positive on acid-fast stain; one of these samples grew on culture and was identified as *Mycobacterium avium* (Brock et al., 1976).

The origin of *M. chelonae* in this immature Kemp's ridley sea turtle most likely occurred via hematogenous spread of bacteria from epidermal lesions. Hematogenous spread of bacteria in reptiles has been documented. A proposed pathobiology of hematogenous spread of infection to a joint was described in a leatherback sea turtle (*Dermochelys coriacea*) (Ogden et al., 1981), and cutaneous mycobacteriosis with suspected hematogenous spread to internal organs was also reported in a side-necked turtle (*Phrynops hilari*). Additionally, there is a similar case report of a sub-adult Kemp's ridley with epidermal lesions that progressed to a nocardial and unidentified fungal osteolysis of the metacarpal joint 2 mo after initial presentation (Harms et al., 2002). It is possible that the identification chip was the primary source of infection in both this case and the other Kemp's ridley case, but this is unlikely due to its sterile placement and location distant from the actual joint space. Additionally, it is unlikely that the joint aspirate was the initial source of *M. chelonae* because the area was prepared aseptically and the joint infection was present prior to the attempted aspirate.

Frequent occurrence of atypical *Mycobacteria* sp. in the environment combined with a low prevalence of mycobacteriosis in poikilotherms, suggests that these species may possess an innate natural resistance to these organisms. Thus, when atypical mycobacterial disease occurs in poikilotherms, it is unknown if the host is immunocompromised or a large number of organisms were introduced (Brownstein, 1978; Frye, 1991). The few reported cases of mycobacterial disease in sea turtles have mostly indicated single organ involvement such as the lung, liver, spleen, or epidermis. No reported mycobacteriosis in a sea turtle has yet been associated with bone or a joint or has identified the species as *M. chelonae* (Thoen et al., 1977; Drabick et al., 1990; Hines et al., 1995). The paucity of reports of mycobacteriosis in sea turtles may be due to the special stains and bacterial isolation techniques needed for identification of the disease.

This case illustrates how a perceived simple osteoarthritis was determined to be a widespread systemic mycobacterial in-

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fection when certain diagnostic studies were performed. To better prevent and possibly treat sea turtles with mycobacterial infections it is important that the scientific community continue to gather information on the specific strains of mycobacterium found in turtles and the pathologic effects each strain has on all the organs in the body. Due to the paucity of reports of mycobacterial diseases in sea turtles, the continued documentation of these cases will increase knowledge and understanding in caring for these endangered animals.

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