Two 7-year-old, captive-bred male Australian snake-necked turtles (*Chelodina longicollis*) were presented for multiple excoriations on the carapace and plastron. The animals were housed in a 1,235-L (325 gallon) public display aquarium with 1 male and 2 female conspecifics, 2 Lake Kutubu rainbowfish (*Melanotaenia lacustris*), and 4 red rainbowfish (*Glossolepis incisus*), none of which exhibited any clinical signs at that time. All turtles originated from the same clutch. Water quality was tested weekly with pH maintained between 7.59 and 7.83, alkalinity between 66 and 70.5 ppm, nitrates < 13.5 ppm, and temperature between 23.3 and 24.4 °C. A 12-hour photoperiod was provided with visible light from a metal halide bulb and ultraviolet light from 2 mercury vapor bulbs on opposite ends of the enclosure providing basking spots appropriate for Ferguson Zone 3 species. Ultraviolet index was monitored monthly and maintained between 1 and 4. The turtles were feed between 15 and 25 g of food 3 times per week. Food items included mealworms, crickets, superworms gut-loaded with a commercial formula (Superload Bug Burger; Repashy Ventures Inc), smelt, rainbow trout and shrimp fortified with a commercial vitamin supplement (Vita Fish; Marine Enterprises International), and earthworms.

**Clinical and Gross Findings**

On presentation, both turtles had multiple round, pitting excoriations measuring between 1 and 4 mm in diameter on the carapace and plastron. The centers of the lesions were soft with necrotic cores that extended from the surface of the scutes to the underlying dermal bone. Some defects had bleeding margins. One turtle had a single focus of cutaneous depigmentation and swelling associated with the nail of the first digit of the left forelimb. No other clinical signs were observed in either turtle.

Hematologic findings were consistent with azurophilia (2.43 X 10^3 azurophils/μL; reference range, 0.298 to 0.458 X 10^3 azurophils/μL) in 1 animal but were otherwise unremarkable. Results of plasma biochemical testing for each animal were unremarkable.

All lesions were debrided, flushed copiously with dilute iodine solution, and treated topically with a compounded enrofloxacin-nystatin-triamcinolone paste. Both animals were treated empirically with ceftazidime (20 mg/kg, IM, q 72 h) for 30 days. The salinity of the system was increased to 2 to 3 ppm for the duration of treatment. Topical treatment was repeated every 4 days. No improvement or worsening of clinical signs was observed over the course of treatment.

Approximately 1 month following initial presentation, the animals died within 6 days of one another. The first animal was found dorsally recumbent with clear, foamy discharge coming from the nares and...
mouth and died before a more-thorough evaluation could be performed. The second animal was found dead in its enclosure.

At necropsy, gross findings for both turtles included multifocal, round, 2- to 5-mm-diameter, opaque white plaques involving the mucosa of the distal portion of the trachea and mainstem bronchi (Figure 1). The tracheal lumen was not occluded. The lungs were pink with multifocal white and yellow mottling of the caudal margins, and 15 to 20 mL of fluid was recovered from the cranial coelom of each turtle. In both turtles, the liver was mottled brown to green to white and was mildly friable. Hemopericardium was observed histologically in both turtles but was obvious on gross evaluation in only 1 turtle.

Formulate differential diagnoses, then continue reading.

**Histopathologic Findings**

Tissue samples were collected, fixed in neutral-buffered 10% formalin, routinely processed, and embedded in paraffin; 5-μm-thick sections were cut and stained with H&E stain for histologic evaluation. Both turtles had mild to severe granulomatous tracheitis (Figure 2), bronchopneumonia, and hepatitis, with moderate numbers of intralesional acid-fast bacilli in the trachea and lungs. The inflammatory plaques in the trachea were composed of epithelioid macrophages, multinucleated giant cells, and granulocytes forming variably discrete granulomas. Sections of the heart, lungs, and plastron were stained with Gomori methenamine-silver stain, and no argyrophilic fungal organisms were found. Mild to moderate, multifocal, necrotizing, and heterophilic myocarditis and epicardial mesothelial hyperplasia were also observed in both individuals. Both turtles had hemopericardium, which was more severe and accompanied by vasculitis in one. Severe, multifocal, ulcerative, and heterophilic to granulomatous dermatitis and osteomyelitis were observed in sections of the plastron. No other clinically relevant histopathologic abnormalities were found. Formalin-fixed, paraffin-embedded tracheal tissue was submitted to the University of Florida College of Veterinary Medicine’s ZooMed Diagnostic Laboratory for testing with a PCR assay and direct sequencing, which confirmed the presence of *Mycobacterium marinum* complex.

**Morphologic Diagnosis and Case Summary**

Morphologic diagnosis: multifocal myocarditis, granulomatous tracheitis, bronchopneumonia, and hepatitis with severe ulcerative dermatitis and osteomyelitis of the carapace and plastron accompanied by hemopericardium in 2 Australian snake-necked turtles.

Case summary: disseminated mycobacteriosis caused by *Mycobacterium marinum* in 2 snake-neck turtles.

**Comments**

*Mycobacterium marinum* is 1 of > 170 *Mycobacterium* species. Generally, *Mycobacterium* spp are slow growing, aerobic, acid-fast staining, and rod shaped. Unlike many other nontuberculous mycobacteria, *M. marinum* can be a primary as well as an opportunistic pathogen. It is commonly associated with both saltwater and freshwater and thrives better in cooler temperatures (< 30 °C). *M. marinum* is the *Mycobacterium* species most often isolated from reptiles and is zoonotic. In humans, *M. marinum* is usually...
localized to the skin and may cause granulomatous skin lesions. It is assumed to be introduced via injuries to the skin and can be transmitted by direct contact with contaminated water, fomites, or infected animals, including reptiles.  

*Mycobacterium marinum* is capable of causing both chronic and acute disease. Chronically infected animals may be infected for years before clinical signs manifest. Conversely, acute disease is rare and is associated with rapid morbidity and mortality.

In fish, *M. marinum* infection presents as a chronic wasting disease with a course of months to years and is characterized by emaciation and ulcerative lesions of the integument. In amphibians, clinical signs are similar, including ulcerative skin lesions and nodules indicative of granulomatous inflammation. Disseminated disease is common in affected reptiles. These animals often are presented with nonspecific clinical signs, including anorexia and lethargy, but swellings associated with mycobacterial granulomas may also be observed. Other commonly reported findings include oropharyngeal lesions in squamates, pneumonia in snakes, and musculoskeletal lesions of the limbs and joints in lizards.

Chelonians are the most commonly reported reptiles to have mycobacteriosis. Chronic mycobacteriosis most commonly results in cutaneous lesions early in the disease course and progresses to systemic granulomatous disease. However, acute, nongranulomatous disease has been reported in a captive-born Eastern spiny softshell turtle (*Apalone spinifera*) that had no lesions of the carapace or plastron. This turtle was presented with a 4-day history of lethargy and was found to be obtunded with petechiae on all limbs and pale mucous membranes. The causes of death were bacterial septicemia and disseminated intravascular coagulation, which are otherwise not commonly associated with mycobacteriosis. The snake-necked turtles in the present report only had carapace and plastron lesions without any other clinical signs and lived for a month following initial presentation, indicating that they were probably affected by the more chronic form of the disease.

The 3 other snake-necked turtles in the exhibit did not develop any clinical signs despite being hatched in the same clutch and subjected to identical husbandry conditions as the 2 affected animals. Three months after the 2 turtles died, physical examination and histologic observation of intralesional acid-fast rods in samples of granulomatous lesions collected by biopsy or at necropsy may support a diagnosis of mycobacteriosis. Acid-fast staining of fecal samples has poor sensitivity (32% to 34%) and should therefore not be used alone to determine the presence of mycobacteria. Only low-level mycobacterial shedding occurs in infected animals in the early stage of the disease process, which may also contribute to inaccuracy in detection. Culture is difficult and time-consuming owing to the fastidiousness and slow-growing characteristics of the organism. Molecular diagnostic tests, such as the PCR assay used in this case, yield faster results and have been shown to be more sensitive than histopathologic examination and fecal staining and culture. For these turtles, mixed fungal-bacterial infections that can then progress to disseminated infectious disease. These infections are often associated with gram-negative organisms, including *Pseudomonas* spp, *Aeromonas hydrophila*, *Citrobacter freundii*, *Serratia* spp, *Morganella* spp, and *Providencia* spp. Secondary infection of traumatic wounds is especially common in freshwater turtles owing to the high microbial load of aquatic environments. In this case, microbial load was mitigated by good water quality, appropriate husbandry practices, and adequate monitoring. Given the diffuse distribution of lesions over the carapace and plastron, infection with *M. marinum* was more consistent with hematogenous spread of the bacteria, as opposed to secondary infection. Ultimately, the cause of death in both individuals was likely hemopericardium resulting in cardiac tamponade as a complication of mycobacteriosis. This may have been caused by bleeding secondary to sepsis, which was suggested by the severe inflammation seen throughout multiple organs.

*Mycobacterium marinum* can be shed from integumentary or branchial lesions or from the gastrointestinal tract of infected hosts. It is disseminated in an environment by contaminated water and fomites. In aquaria, mycobacteria have been identified on food items, cage decorations and furniture, and live plants. Susceptible animals become infected when the pathogen contacts defects in the skin or epithelial mucosa of the gastrointestinal, genitourinary, or pulmonary tract. Mycobacteria are resistant to many disinfectants including quaternary ammonium compounds, chlorine, benzalkonium chloride, cetylpyridinium chloride, and phenolicand glutaraldehyde-based disinfectants. This makes mycobacteria challenging, if not impossible, to eliminate once established in a captive environment. In reptiles and turtles specifically, it is thought that the most common source of exposure is from contaminated water and fomites.

Mycobacteriosis can be difficult to diagnose, especially because isolation of mycobacteria alone is not necessarily an indication of disease given that these organisms are common environmental contaminants. Histologic observation of intraslesional acid-fast rods in samples of granulomatous lesions collected by biopsy or at necropsy may support a diagnosis of mycobacteriosis. Acid-fast staining of fecal samples has poor sensitivity (32% to 34%) and should therefore not be used alone to determine the presence of mycobacteria. Only low-level mycobacterial shedding occurs in infected animals in the early stage of the disease process, which may also contribute to inaccuracy in detection. Culture is difficult and time-consuming owing to the fastidiousness and slow-growing characteristics of the organism. Molecular diagnostic tests, such as the PCR assay used in this case, yield faster results and have been shown to be more sensitive than histopathologic examination and fecal staining and culture. For these turtles,
tracheal tissue collected at necropsy was submitted for a PCR assay. It may have been beneficial to perform antemortem diagnostic testing such as fecal culture or a fecal PCR assay. Fecal culture is highly specific but can require up to 8 weeks to observe growth, which may be impractical for diagnosis in a clinical setting. In contrast, results of PCR assays of fecal and cloacal swabs can take only days to receive and have been found to have high specificity and sensitivity for confirming the presence of mycobacteria. Ancillary diagnostic tests, such as hematologic and plasma biochemical testing, may suggest infection but often do not reflect the severity of systemic disease, as in this case. Evaluation of hematologic and plasma biochemical test results is further complicated by the lack of species-specific reference intervals from a representative population of healthy animals as well as variations attributable to sex, reproductive status, age, and environmental conditions.

If mycobacteriosis is confirmed in a population of fish, amphibians, or reptiles, euthanasia of clinically affected individuals should be considered as no treatment regimens have been reported to be successful. For reptiles, reported treatments have included erythromycin and amikacin along with debridement of lesions and lavage with chlorhexidine or iodine antiseptic solutions. Mycobacterium marinum has been shown to be susceptible to various systemic bactericidal antimicrobials such as clarithromycin, imipenem, isoniazid, and fluoroquinolones. However, even with attempted treatment, the prognosis remains guarded, as in this case. Treatment may improve superficial skin infections, but deep tissue and systemic infections are much harder to treat especially because antimicrobials cannot penetrate the necrotic tissue in the core of granulomas. Overall, treatment is not advisable owing to the commonly chronic and progressive nature of the disease, expense of antimicrobials, and risks of potentiating antimicrobial resistance and transmitting the organism to other animals and humans.

References