Diagnostic Exercise

From The Davis-Thompson Foundation*

Case # **213**; Month: **May**; Year: **2023**
Answer Sheet

Title: Mycobacterial septicemia by non-granulomatous mycobacteriosis in a freshwater turtle.

Contributors: Dr. Cleide Spröhnle-Barrera DVM, DVClinSc (candidate); Professor Robert Doneley, BVSc, FANZCVS (Avian Medicine); Dr. Viviana Gonzalez-Astudillo, BVSc (hons), MNR, PhD, Dipl. ACVP (Anatomic Pathology); School of Veterinary Science, The University of Queensland, Gatton QLD 4343 Australia.
*Address correspondence to Dr. Viviana Gonzalez-Astudillo:
v.gonzalez@uq.edu.au

Clinical History: A 4-month-old, male, Murray River short neck turtle (*Emydura macquarii*; Figure 1) was presented with swelling around the thoracic inlet and pre-femoral fossa, suspected to be secondary to a renal infection secondary to water quality. In-house laboratory testing revealed anemia and hypoproteinemia (packed cell volume 8% [reference interval, 18%-30%] and total protein 9 g/dl [reference interval, 2.5-4.2g/dL]). The turtle died suddenly while in hospital after a week of antimicrobial therapy.

Necropsy Findings: The subject was moderately dehydrated. Within the coelomic cavity was approximately 3-4 ml of clear translucent watery fluid (transudate). Diffusely, the liver was severely discolored pale brown (Figure 2). The intestines were distended up to 2 mm in diameter, filled with approximately 2 ml cream-colored to pale brown content.

Gross and Microscopic Images:

Figure 1. Freshwater turtle, non-granulomatous mycobacteriosis.

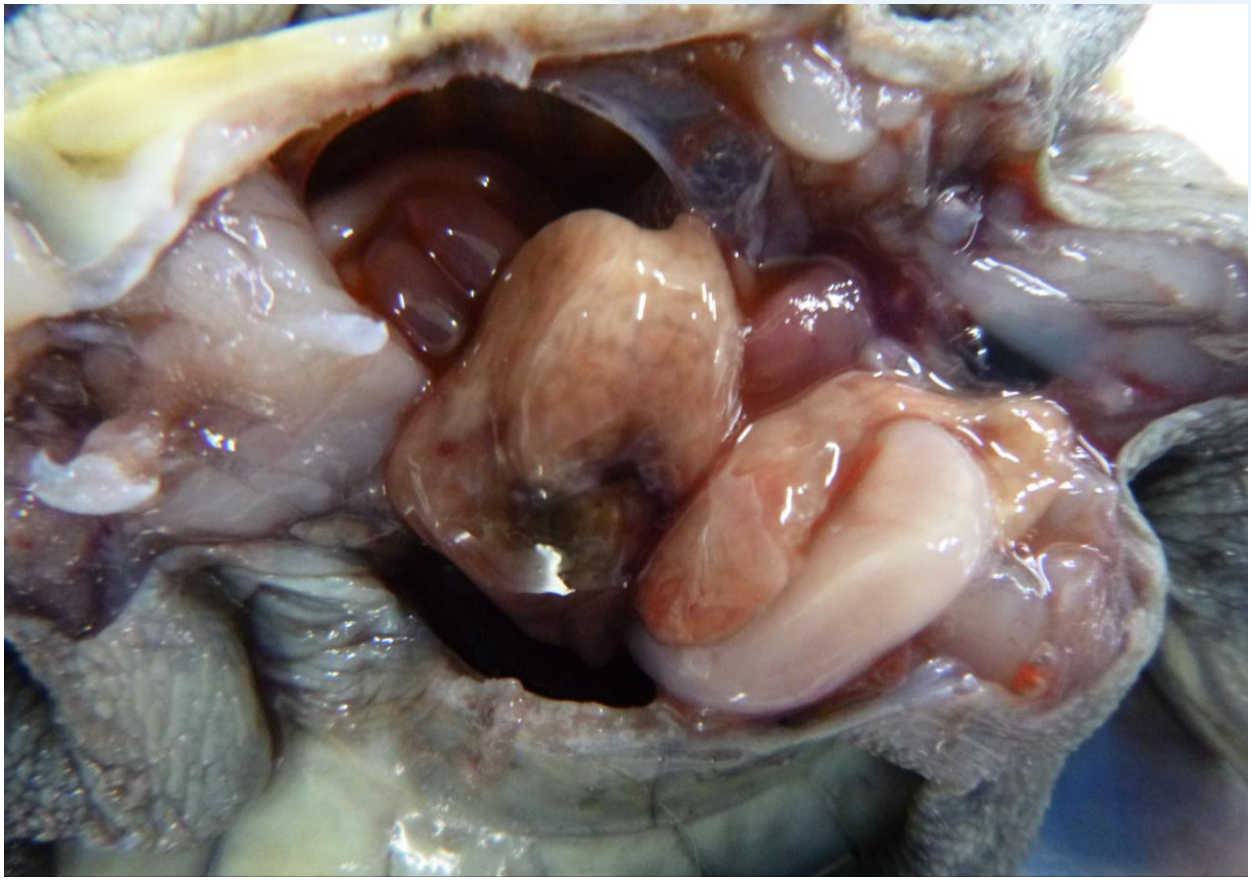

Figure 2. Freshwater turtle, non-granulomatous mycobacteriosis.

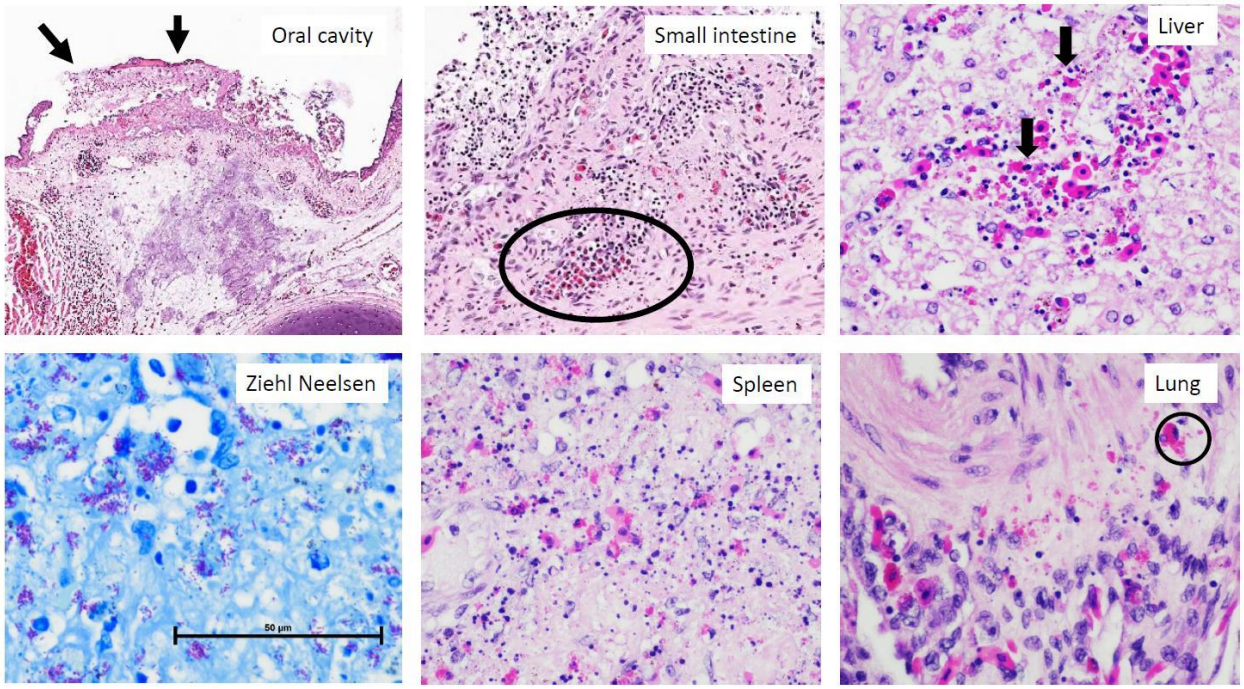

Figure 3. Freshwater turtle, non-granulomatous mycobacteriosis. Microscopic findings

Microscopic Findings:

Liver: There are numerous degranulated heterophils filling sinusoids and infiltrating/replacing necrotic hepatic cords trapped in a beaded eosinophilic meshwork (fibrin). There is frequent hepatocellular fragmentation (necrosis) and disassociation. There are occasional random vascular walls displaying moderate fibrinoid necrosis. Small numbers of hepatocytes display few discrete clear cytoplasmic vacuoles (macrovesicular degeneration).

Lungs: Multifocally, about 20% of faveolar outlines are mildly to moderately replaced by necrotic debris. In approx. 30-40% of the interstitial capillaries, there is intravascular degranulated heterophilic debris (intravascular heterophilic degranulation) which extends to the faveolar space admixing with necrotic debris and fewer extravasated erythrocytes (microhemorrhages).

Spleen: The entirety of tissue is diffusely necrotic and randomly, about 50% of the splenic parenchyma and small to moderate caliber vasculature contain aggregates of degranulated heterophils admixed with necrotic debris (intravascular heterophilic degranulation).

Oral cavity: There are focally extensive erosions and ulcerations of the mucosa with numerous degenerate heterophils admixed with necrotic debris trapped in fibrin. The submucosa is comprised by loosely-arranged collagen fibers (edema) along a few viable and degenerate heterophils reaching the adjacent stratum spinosum. Ulcerated foci are covered by fibrin and necrotic debris.

Intestine: There is multifocal to coalescing necrosis of the deep crypt epithelium, with replacement by heterophilic and cellular debris, and occasional erosions and ulcerations of the mucosa. Immediately adjacent lamina propria, there is also multifocally effaced. Within all the segments, there is sloughing of enterocytes into lumen; about 50% of sloughed off enterocytes are degenerated and necrotic.

Ziehl-Neelsen stain: **Liver:** Myriads of intracellular and occasionally extracellular 0.5 x 2-micron acid-fast bacilli are observed in necrotic foci, including parenchyma and biliary epithelium.

Morphological diagnoses:
Liver: 1. Cholangiohepatitis, heterophilic/necrotizing, acute, multifocal, random, moderate to severe with fibrinoid necrosis of vascular walls; 2. Macrovesicular lipid degeneration, diffuse, moderate.
Lungs: Pneumonia, necrotizing, diffuse, moderate with intra-vascular and faveolar heterophilic degranulation and microhemorrhages.
Spleen: Splenitis, necrotizing, acute, diffuse with intra-vascular heterophilic degranulation.
Oral cavity: Stomatitis, ulcerative/erosive, subacute, focally extensive, moderate with acute hemorrhages, segmental mild vasculitis, fibrinoid necrosis, and secondary bacterial colonization
Small Intestine: Enteritis, necrotizing/ulcerative, acute, multifocal to coalescing, severe with crypt ectasia/degeneration and superficial coccobacilli colonization.

Potential Etiology: *Mycobacterium chelonae*

Discussion: The cause of death of this turtle was a septicemia, caused by a non-granulomatous mycobacteriosis. In turtles, it has been reported to be caused by *M. chelonae*. Some of the key features supporting septicemia, and its suggested etiology include systemic distribution, acute nature of the disease, and histochemical characterization. Additionally, the prominent heterophilic degranulation observed supports not only a disseminated/systemic process but a feature of toxic granulocytic change, known to occur in non-mammalian vertebrates [1].
M. chelonae is a non-motile, non-spore forming, gram positive, acid-fast bacillus with a beaded appearance. This agent is considered a non-tuberculous mycobacterium (NTM), that is ubiquitous in the environment and often found in soil, water, and aquatic animals [2]. It has become a significant infection in humans, commonly associated with skin and soft tissue infections, especially causing cellulitis and abscesses in immunosuppressed patients [3].
M. chelonae was first isolated from a turtle in 1903 by Friedmann, named after the sea turtle *Chelona corticata* presenting as a hemorrhagic coelomic

effusion, with petechiae on serosal surfaces, and can lead to disseminated infection causing death [4]. There are only very few cases reported in freshwater and sea water turtles. An easter spiny softshell turtle (*Apalone spinifera spinifera*) was reported to have died with acute mycobacteriosis, displaying numerous emboli of bacteria associated with fibrinocellular thrombi disseminated in blood vessels of multiple organs [5]. Another report of disseminated infection was made in a stranded Loggerhead Sea turtle (*Caretta caretta*) [6].

More recently, a rare case in a Kemp's ridley sea turtle (*Lepidochelys kempii*) that was euthanized for inappetence and epidermal appendicular and plastral lesions with isolation of *M. chelonae* from multiple organs, including the bones [4]. This is particularly interesting, since a recent case of an Eastern Long-neck turtle (*Chelodina longicollis*) diagnosed with an apparently localized mycobacterial infection in the right foot, was successfully treated with clarithromycin and rifampicin given orally for 9 months, that appeared to successfully resolve the infection [7].

Thus, although mycobacteriosis in reptiles is typically a chronic, granulomatous disease [8], this case report demonstrates that atypical non-granulomatous mycobacteriosis should be considered in reptiles presenting clinical signs and lesions compatible with septicemia.

References:

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*The Diagnostic Exercises are an initiative of the **Latin Comparative Pathology Group (LCPG)**, the Latin American subdivision of The Davis-Thompson Foundation. These exercises are contributed by members and

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