



# Diagnostic Exercise

From The Davis-Thompson Foundation\*

Case #: 138 Month: February Year: 2020

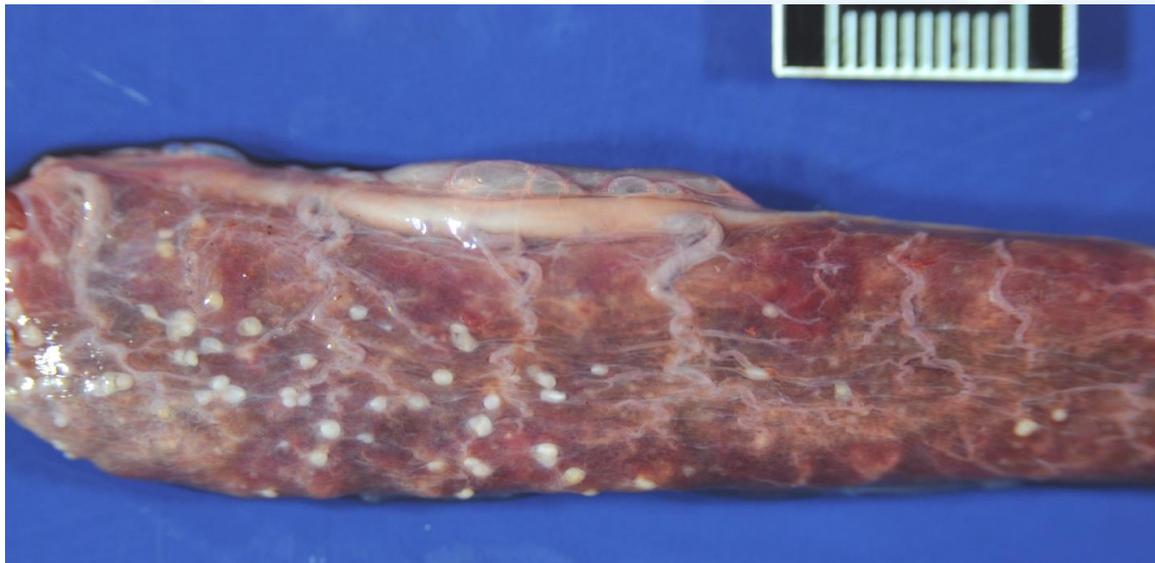
Answer Sheet

**Title:** *Chronic mycobacteriosis in a Gopher Snake*

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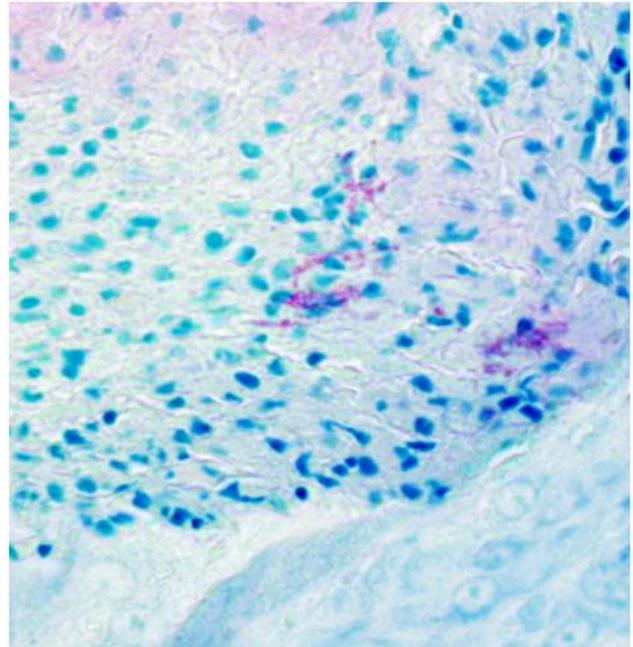
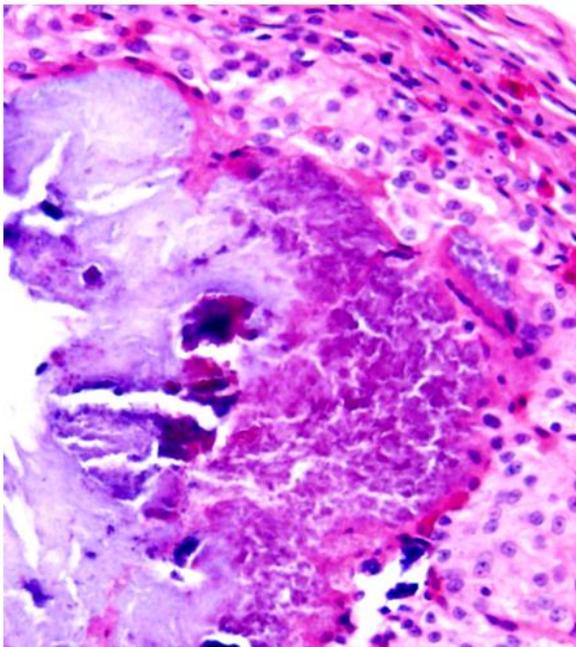
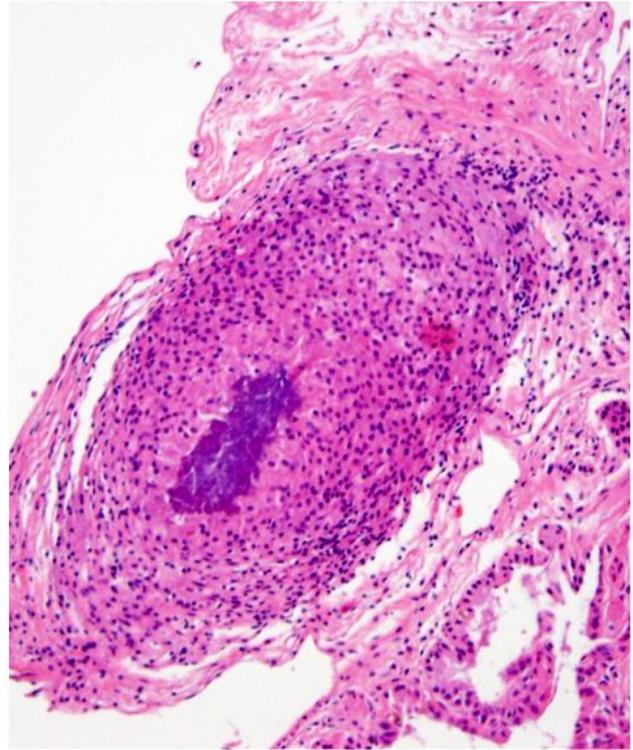
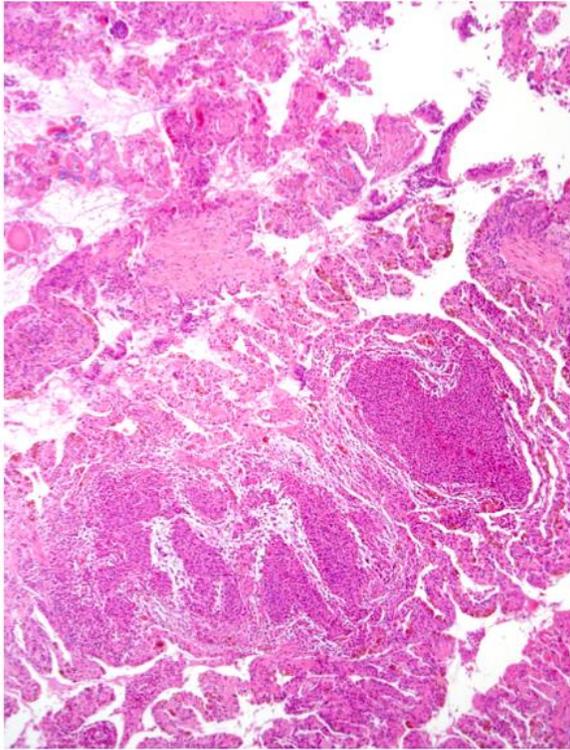
**Diagnosis:** Lung, spleen, pancreas: Severe, chronic, multifocal granulomatous inflammation with intracellular acid-fast bacteria (consistent with systemic mycobacteriosis)

**Typical Gross Findings:** Multisystemic, multifocal, distinct granulomas. In this case, a protracted history of *Mycobacterium* infection was documented.



What diagnostics are indicated (special stains, IHC, culture, etc.)?

Acid fast stains, such as Ziehl-Neelsen, are useful. Speciation requires either PCR or a combination of culture and PCR. In this case, there were small numbers of acid-fast positive coccobacilli bacteria within granulomas, and there were small numbers of *Mycobacterium* sp. cultured from the spleen, which were used as a basis for PCR amplification, which confirmed *Mycobacterium cheloniae*.



*What are key histologic features?* Key features of mycobacteriosis include mature granulomas, sometimes visible centrally located thin bacilli, and in many cases multinucleate giant cell formation.

**Microscopic Findings:** The pulmonary architecture contains multiple histiocytic-rich granulomas (Figure upper left, HE). The faveolar septa are markedly expanded by variably sized, individual to coalescing granulomas partially occluding the adjacent air spaces and faveoli. The

granulomas are predominantly composed by dense aggregates of foamy and epithelioid macrophages, which are surrounded by occasional lymphocytes and rare heterophils and multinucleated giant cells (Figure upper right). Few granulomas have central cores of necrotic debris admixed with mineralized material (Figure lower left). Multifocally, there are moderate amounts of proteinaceous fluid admixed with individual macrophages and karyorrhectic nuclear debris within airway spaces. Small numbers of macrophages, heterophils, and lymphocytes infiltrate the adjacent faveolar septa and islands of smooth muscle cells. Capillaries lining faveolar spaces are multifocally distended and filled with erythrocytes and red-brown pigment.

In an acid-fast stained section, thin, filamentous, beaded bacteria are present within the central region of granulomas (Figure bottom right).

**Discussion:** *Mycobacterium chelonae* is a gram-positive, aerobic, alcohol-acid-resistant opportunistic bacterium that belongs to the family of non-tuberculous mycobacterial species classified as fast-growing mycobacteria (Runyon group IV) that do not produce pigments (1, 2). Although mycobacteriosis in captive snakes is considered a rare finding (2, 3), there are several reports in the last two decades describing systemic disease and/or lesions consistent with mycobacteria infection in captive and wild caught snakes. The skin, oral cavity, and respiratory tract are commonly affected in snakes (1), which can be infected with *Mycobacterium chelonae* and other atypical mycobacterial species (4). Mycobacterial infection in snakes have been reported in boa constrictors (*Boa constrictor*), a royal python (*Python regius*), a black-tailed python (*Python molurus*), a Lichtenstein's green racer (*Philodryas olfersii*), and an Assam trinket (*Elaphe frenata*) (4).

*Mycobacterium chelonae* infection has been associated with ulcerative stomatitis and hepatic and pulmonary granulomas in a boa constrictor (2, 5). In turtles, it has been described in cases of septicemia and osteoarthritis (6, 7). A case-series showed that 14 of the 48 snakes examined had granulomatous lesions and molecular evidence of mycobacteriosis in multiple organs (4). The diagnosis of systemic mycobacteriosis in this case was made based on the histological appearance of the granulomas, Ziehl-Nielsen and aerobic culture result, and previous molecular findings (1). The granulomas in the lung, spleen and pancreas in this Gopher Snake are characterized by the accumulation of epithelioid macrophages. This histological feature is consistent with previous observations showing that histiocytic granulomas are common in snakes infected with atypical mycobacterium species (4, 8). Often mycobacterial granulomas in snakes progress to chronic forms that contain central cores of necrosis and mineral with loss of recognizable cellular components (4, 8).

Macrophages play a central role in the development of host defense mechanisms to control mycobacterial tuberculosis complex infections as well as the spread and persistence of intracellular mycobacterial species in tissues (9). Inside macrophages, pathogenic mycobacterial

species replicate by subverting phagocyte endocytic trafficking and resisting innate defense mechanisms (9). However, limited information is available about how fast-growing, atypical mycobacterial species interact with macrophages. Lipomannans from *M. chelonae*, a mycobacterial cell wall glycolipid, have shown to elicit a proinflammatory response in human macrophages (10). This observation is in agreement with previous studies performed with other fast-growing mycobacterial species, which also induce a potent innate immune response in macrophages. This proinflammatory phenotype may be the reason for the reduced pathogenicity of atypical mycobacteria species in their hosts (11, 12).

Limited information exists on whether *M. chelonae* could be transmitted from reptiles to humans (1). *Mycobacterium chelonae* in humans is often associated with skin and soft tissue infections, and these cases are commonly linked with hospital-acquired infections (13).

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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 non-members from any country of residence. Consider submitting an exercise! A final document containing this material with answers and a brief discussion will be posted on the CL Davis website ([http://www.cldavis.org/diagnostic\\_exercises.html](http://www.cldavis.org/diagnostic_exercises.html)).

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