



Diagnostic Exercise

From The Davis-Thompson Foundation*

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Answer Sheet

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Clinical History: An adult male Darwin's or Lesser Rhea (*Rhea pennata* – locally known as ñandu) showed claudication of the right leg. Clinical evaluation revealed multiple to coalescing, 1 to 5 cm diameter, nodular dermal masses on wings, limbs, thoracic and lumbar areas. Skin biopsies, blood tests, and x rays of the right tibio-tarsal joint were obtained. Biopsies resulted in a presumptive diagnosis of xanthomas. Blood chemistry showed elevated aspartate transaminase (AST), lactate dehydrogenase (LDH) and creatine phosphokinase (CPK). Complete blood count and x-rays were unremarkable. One month later, lameness persisted, and there was lower feed intake and significant increase in the extension of skin masses, most of them ulcerated. Due to the extension of the lesions and animal welfare concerns, the animal was euthanized. At necropsy, several round, well delimited, white to tan, 1 to 3 cm in diameter masses in different organs were found. In the oropharynx, at the base of the tongue, two contiguous, pedunculated, ulcerated masses of approximately 1.5 cm in diameter were found.

Morphologic diagnosis: Pharyngeal mucosa: Granulomatous stomatitis/pharyngitis, severe, focally extensive, chronic, with intracellular acid fast bacilli.

Microscopic Findings (Figure 2): Focally expanding the submucosa and ulcerating the epithelium of the oropharynx, there are two well circumscribed granulomas composed of aggregates and sheets of macrophages and multinucleated giant cells with peripheralized nuclei (Langhans type), separated by thin strands of connective tissue. These inflammatory cells have abundant, finely granular, basophilic cytoplasm and are mixed with small numbers of lymphocytes and plasma cells. Ziehl-Neelsen stain reveals myriad intracellular acid-fast bacilli. The same bacilli stain blue (i.e., positive) with Gram stain.

Microscopic images:

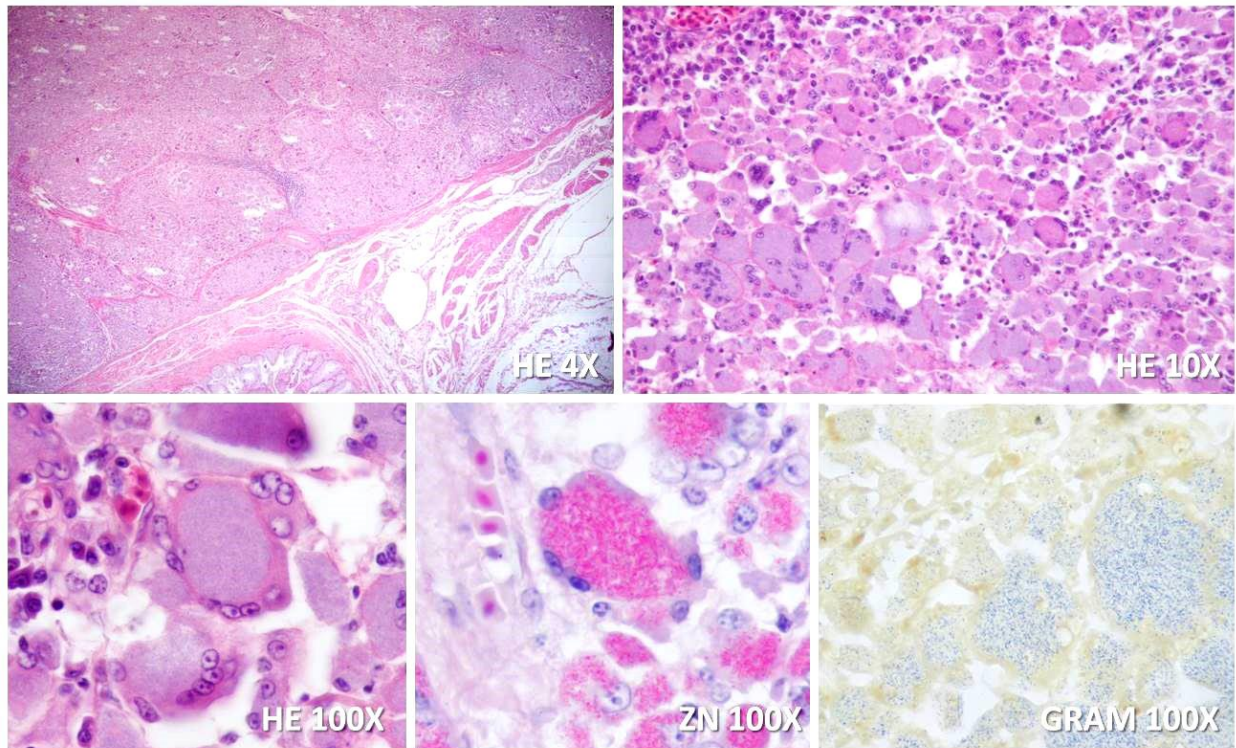


Figure 2. Photomicrographs of the oropharyngeal mass. HE: Hematoxylin and Eosin. ZN: Ziehl-Neelsen.

PCR: Positive for the 16S ribosomal subunit of the genre *Mycobacterium*, but negative for *M. avium*. Further species classification is pending.

Typical Gross Findings: Mycobacteriosis in birds frequently manifests as a progressive wasting disease, with emaciation and muscular atrophy. Mycobacterial granulomas usually present as nodules in different organs (prominently in the digestive system) with or without caseous necrosis. The lesions can be solitary or multifocal (systemic) and usually consist of pale, yellow to white to tan nodules of variable size. Larger nodules can have necrosis, but mineralization is uncommon. Granulomas are usually small (1 to 4 cm in diameter) but can be much larger, giving them the appearance of neoplastic masses.

Typical Microscopic Findings: In most birds, mycobacterial granulomas can present with a classic tuberculous conformation with central caseous necrosis surrounded by multinucleated giant cells and further layers of macrophages, lymphocytes, occasional plasma cells and heterophils. In chronic lesions, an outer layer of fibrous connective tissue is frequently observed. Non-caseating granulomas (more frequent in psittacines and passerines) are composed of sheets of epithelioid macrophages and multinucleated giant cells, without necrosis, conferring them a "lepromatous" pattern.

Differential diagnoses: Regarding the pharyngeal location, any oral neoplastic mass can be a potential differential diagnosis, although not many have been reported in this species or in this location. *Trichomonas gallinae* can produce nodular swellings in the oral cavity, with extensive

areas of necrosis and fibrin. Xanthomas can produce multinodular swellings in the skin and histologically can be similar, especially when compared to non-caseating granulomas.

Discussion: Mycobacteriosis is common in domestic and wild birds worldwide. The most common mycobacterium involved is *Mycobacterium avium* subsp *avium* but other species have been isolated from birds, including *M. genavense*, some mycobacteria from the *M. avium* complex and from the *M. tuberculosis* complex, among others [1]. The usual route of infection appears to be oral, since the majority of lesions are found in the digestive system; however, the respiratory route is also possible. This chronic disease is the result of a cell mediated immune response together with the ability of the microorganism to downregulate macrophage-killing mechanisms by preventing phagosome-lysosome fusion [2]. Lesions caused by mycobacteria in birds depend mainly on the affected avian species and its health status (immune status, concurrent infections) and not necessarily on the species of mycobacteria [3]. While uncommon in commercial poultry, mycobacteriosis appears to be more frequent in birds from zoological collections [1]. There are reports of mycobacteriosis in several species of wild birds, including ratites (ostriches, emus and rheas) [3, 4]. In rheas (ñandus), two reports mention granulomas mainly in the spleen and liver, all of them with a core of caseous necrosis. One of these reports studied the presence of mycobacteria in farmed rheas. The mycobacteria isolated in this study were *M. avium* subsp *avium* (great majority of cases), *M. intracellulare* (one case) and *M. vaccae* (one case). *M. avium* subsp. *avium* was also isolated from a subset of animals with no lesions [5-7]. In another report, pharyngeal granulomas were found in a 14-month-old ostrich, manifested as pedunculated masses. Microscopically, these were composed of several micro-granulomas with central caseous necrosis, surrounded by fibrous tissue. *Mycobacterium* sp. was identified by immunohistochemistry [8]. In our case we identified mycobacteria as the cause by PCR for the 16S ribosome subunit but *M. avium* PCR was negative. Further molecular characterization is pending. This case represents a combination of the lesion patterns previously reported in various cases.

References

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