Latin Comparative Pathology Group

The Latin Subdivision of the CL Davis Foundation

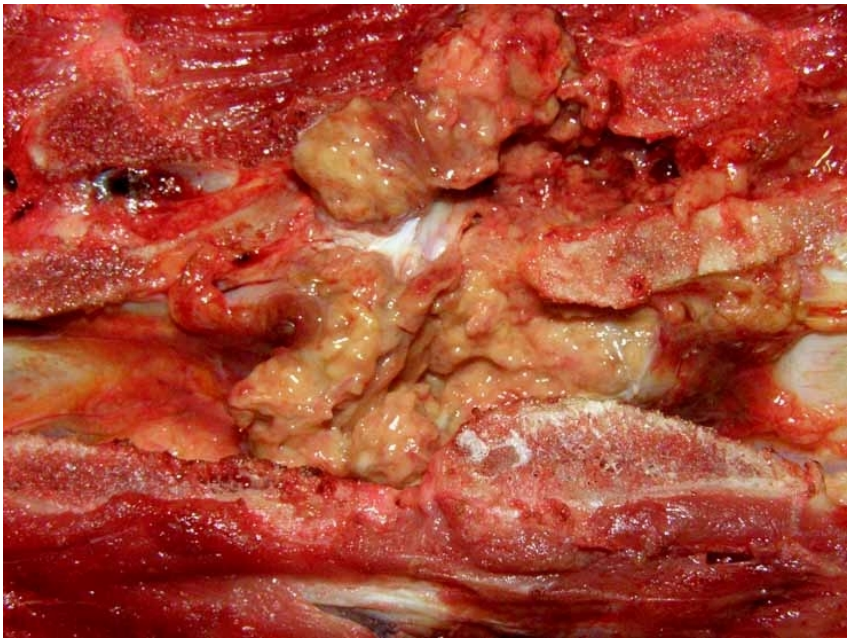
Diagnostic Exercise

Case #: 12 Month: August Year: 2011

Answer sheet

1. **Morphologic diagnosis:**

   Cervical vertebra: Severe, locally extensive, chronic pyogranulomatous osteomyelitis.



2. **Etiology:**

   Rhodococcus equi.

3. **Associated lesions:**

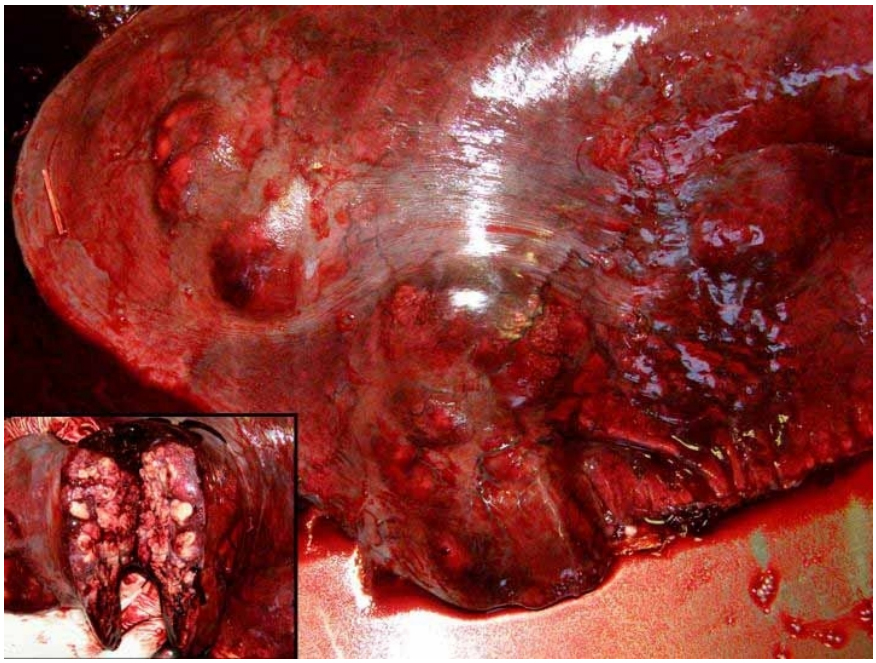
   - Lungs: Chronic pyogranulomatous bronchopneumonia.
   - Cecum and large colon: Chronic ulcerative typhlocolitis.
   - Related lymph nodes: Chronic pyogranulomatous lymphadenitis.

4. Microscopic lesion(s) in the spinal cord:

See microscopic findings.

Gross findings:

Postmortem examination of the filly revealed severe multifocal to coalescing chronic pyogranulomatous pneumonia, severe multifocal ulcerative typhlocolitis, and mild multifocal pyogranulomatous lymphadenitis, involving mediastinal, bronchial, and mesenteric lymph nodes. A 3 cm diameter pyogranuloma affected C5 vertebral body, protruding into the spinal canal and compressing the spinal cord.



Microscopic findings:

Microscopic examination revealed multifocal pyogranulomatous osteomyelitis, pneumonia, typhlocolitis, and lymphadenitis. Gram-positive small rods were within the cytoplasm of macrophages and giant cells in all the affected areas. The spinal cord at C5-C6 showed hemorrhages, necrosis, and wallerian degeneration of axons affecting both white and gray matter.

Microbiologic findings:

Rhodococcus equi was isolated in pure cultured from samples taken from lung, colic lymph node, and vertebra C5.

Discussion:

Rhodococcus equi is a Gram-positive, obligate aerobic, facultative intracellular pathogen that affects foals mainly under 4 months of age. *R. equi* is a soil organism; its multiplication depends on environmental temperatures, the presence of volatile fatty acids in herbivore feces, and on soil pH. The prevalence of *R. equi* infection is highly variable between farms, probably reflecting the level of contamination of the environment. Aerosol infection via dust is thought to be the main route of foal infection.

The ability of *R. equi* to induce disease in foals likely depends on both microbial and host factors. Among its virulence factors, *R. equi* contains an 80-90 kb plasmid, with three functional regions. The genes of two of these regions encode proteins involved in conjugation and in plasmid replication, segregation, and stability. The third region contains the genes of eight virulence-associated proteins designated Vap-A and VapC to VapI. The expression of these eight genes is complex and depends on at least five environmental signal, including temperature, pH, oxidative stress, magnesium, and iron. Other virulence factors of this microorganism include capsular polysaccharide, glycolipids containing long-chain mycolic acids, and cholesterol oxidase and phospholipase C ("*equi* factors"). Susceptibility to the young foals to *R. equi* infection is due in part to a declining title of maternal antibodies and an absence of fully competent cellular immune mechanism.

The bacterium engulfed by equine macrophages can survive by preventing phagosome-lysosome fusion, thus multiplying in and eventually killing the cells. In addition, there is a nonspecific degranulation of lysosomes in *R. equi*-infected macrophages that would contribute to the tissue destruction and neutrophil influx. Opsonization with specific antibodies enhances ingestion, phagosome-lisosome fusion, and killing of *R. equi* by cultured macrophages. Both CD4+ and CD8+ T cells are important in clearing the bacterium in mouse model of the disease. Lymphocytes T

CD4+ produce IFN-γ, which activates macrophages, and lymphocytes T CD8+ induce lysis of the infected macrophages.

The most common lesion in foals with *R. equi* infection is a subacute to chronic pyogranulomatous bronchopneumonia, in which multifocal to coalescing, white-yellow firm nodules occupy the cranioventral areas of the lung, although other parts of the lung may be affected. Usually the center of the pyogranulomas has liquefying or caseous necrosis. Microscopic lesions are characterized by macrophages filled with Gram-positive bacteria, neutrophils and necrotic foci. Approximately half of the foals with pulmonary lesions have lesions in the intestine, most commonly in cecum and colon, although the small intestine may be affected. Intestinal involvement without pulmonary lesions is much less common. Enteric infections may result from swallowing expectorated material from the lungs or ingestion of soil-contaminated feed. Lesions in cecum and colon consist in ulcers that are centered on lymphoid follicles, which are replaced by a pyogranulomatous infiltrate. Bronchial and colonic lymph nodes are enlarged and contain necrotic foci.

Occasionally there is a hematogenous spread of the infection that gives rise to arthritis, osteomyelitis, and hepatic or splenic abscesses. Osteomyelitis usually affects vertebra or metaphyses of long bones. *R. equi* has been also associated with metritis and abortion in mares.

Foals with pyogranulomatous bronchiopneumonia and/or multifocal ulcerative typhlocolitis are highly suggestive of suffering *R. equi* infection. Diagnosis is easily made by isolation of *R. equi* from the infection site, or by identifying intracytoplasmic Gram-positive bacilli in the macrophages on impression smears or tissue sections.

Recommended literature:

- Giguère, S. (2009) Update on *Rhodococcus equi* infections in foals. Proceedings of the 11th Geneva Congress on Equine Medicine and Surgery: 136-141.

- Maxie, M.G., ed. (2007) Jubb, Kennedy & Palmer´s pathology of domestic animals. Fifth ed., Saunders Ltd.

- Prescott, J.F. (1991) *Rhodococcus equi*: an animal and human pathogen. Clinical Microbiology Reviews 4 (1): 20-34.