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Diagnostic Exercise

From the Latin Comparative Pathology Group and the Davis-Thompson Foundation

Mycotic rumenitis in feedlot cattle

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History:

Feedlot with 3,700 animals. About 20 animals died in one month (December 2023). In January 2024, 7 new animals died within 2 days, prompting a visit to the establishment. On physical examination, clinical signs included apathy, lethargy, marked ptyalism and sialorrhea, watery diarrhea, and dyspnea with superficial tachypnea. The affected animals often adopted a kyphotic posture and refused to move when stimulated (Fig.1). Clinical signs began 15 to 45 days after arrival at the feedlot. Treatments with tilmicosin, tylosin, florfenicol, and tulathromycin were administered without a favorable response. The cattle had been fed a low-fiber, high-cereal diet containing monensin.

Autopsy findings:

Autopsy were performed on three animals, all of which displayed similar lesions. Severe cranioventral congestion and edema of the lungs were observed. In the rumen, multiple well demarcated foci, 5-15 cm in diameter, were noted on the serous surface. Many of the foci exhibited a pale center (Figures 2 and 3), sometimes with traces of fibrin, and were surrounded by a hyperemic halo. Other lesions were bluish black with a dark red center (Figures 2 and 3). These lesions were transmural, corresponding to well-defined, brownish friable plaques on the rumen mucosal surface, also bordered by a hyperemic area (figures 3,4,5,6,7). Additionally, much of the rumen mucosa appeared gray and slightly thickened, with abundant liquid content present.



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Figure 1. Animals in the feedlot. The two animals at the center of the image exhibit ptyalism and sialorrhea. Also, the one on the right manifests a kyphotic posture.



Figure 2. Multiple well demarcated foci, ranging from 5 to 10 cm in diameter, with a pale center, surrounded by a hyperemic halo and sometimes with traces of fibrin. In some foci, fusion between the omentum and ruminal serosa is evident (fibrinous peritonitis).



Figure 3. Large infarcted area with a dark red center and rough texture. The necrotic core is surrounded by a broad black-blue halo.





Figures 4 and 5. Ruminal mucosa with friable plaques of dark brown necrotic tissue surrounded by a hyperemic border (transmural ulcers).



Figure 6. Ruminal mucosa with extensive focus of necrosis. The area appears greyish brown, slightly thickened and surrounded by a hyperemic halo.

Follow-up questions:

- Microscopic description
- Morphologic diagnosis
- Etiologic diagnosis
- · Most likely etiology

ANSWERS

Microscopic description:

Rumen: mucosa with extensive areas of epithelial necrosis. Preserved epithelial regions show marked hydropic degeneration with multifocal subepithelial cleavage and detachment of the superficial mucosa (Figure 8).



Figure 7. Transmural lesion corresponding to a necrotic friable plaque, light brown, surrounded by a hyperemic border.

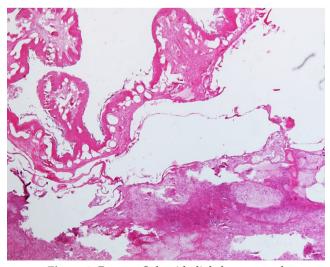


Figure 8. Rumen. Subepithelial cleavage and detachment of the superficial mucosa. Lamina propria is thickened with marked edema, lymphangiectasia and acute inflammation. Obj 4x. H&E.

The lamina propria is thickened, with moderate to marked edema, lymphangiectasia and areas of hemorrhage. There is intense diffuse mixed inflammatory cellular infiltration (lymphocytes/plasma cells with a predominance of neutrophils), more evident around blood vessels, extending into the submucosa and affecting the muscular and adventitial layers (Figure 9). Mainly in the submucosa, numerous blood vessels with hyalinized walls, with cellular infiltration and extensive thrombi (fibrin, erythrocytes, and neutrophils) are recognized (Figures 10). Occasionally, structures compatible with fungal

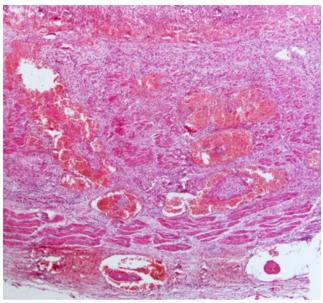


Figure 9. Rumen. Submucosa, muscular and adventitial layers with intense mixed inflammatory cellular infiltration of lymphocytes, plasma cells and a predominance of neutrophils. Mainly in the submucosa and adventitia, numerous blood vessels were congestive and some with thrombi (arrowheads). Obj 4x. H&E.

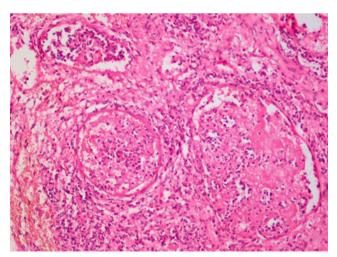


Figure 10. Rumen. Mainly in the submucosa, numerous blood vessels with hyalinized walls, cellular infiltration and extensive thrombi (fibrin, erythrocytes, and neutrophils) are recognized. Obj 20x. H&E.

hyphae are identified in negative images in the thrombosed vessels (Figure 11). In the adventitial layer, there is fibrino-purulent exudate with numerous neutrophils, mostly necrotic.

Special Stains:

PAS Stain: pauci-septate hyphae with walls that are tapered or non-parallel are recognized in thrombotic blood vessels in the submucosa (Figure 12) and in the connective tissue and muscle fibers of the muscular layer (Figure 13). Occasionally, irregular and bulbous non-dichotomous dilations are identified (Figure 13).

Grocott Stain: Structures compatible with fungal hyphae are recognized in the thrombosed vessels (Figure 14) and the muscular layer (Figure 15).

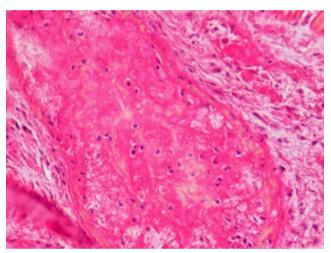


Figure 11. Rumen. Occasionally, structures compatible with fungal hyphae are identified in negative image in the thrombosed vessels (arrowheads). Obj 20x. H&E.

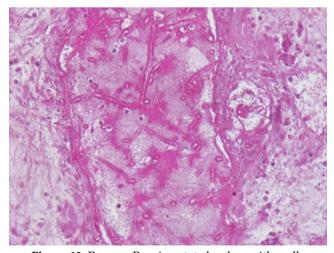


Figure 12. Rumen. Pauci-septate hyphae with walls that are tapered or non-parallel are recognized in thrombotic blood vessels in the submucosa, muscular and adventitial layers. Obj 20x. PAS stain.

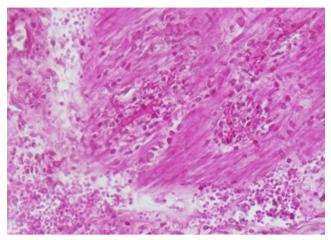


Figure 13. Rumen. In the connective tissue and muscle fibers of the muscular layer occasionally hyphaes were also seen. Irregular and bulbous non-dichotomous dilations are identified (red arrowheads). Obj 40x. PAS stain.

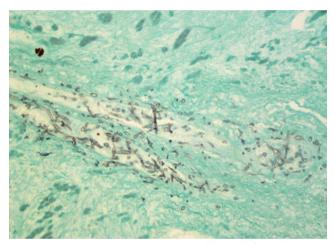


Figure 14. Structures compatible with fungal hypha are recognized in the thrombosed vessels. Obj 40x. Grocott's methenamine silver stain.

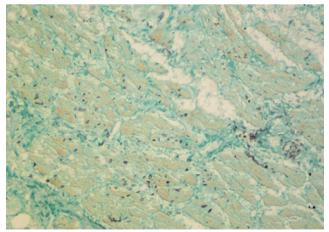


Figure 15. Structures compatible with fungal hypha are recognized in the connective tissue and muscle fibers of the muscular layer. Obj 20x. Grocott's methenamine silver stain.

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Morphologic diagnosis:

Rumen: severe, acute, focally extensive, transmural, necrotizing rumenitis with vasculitis and thrombosis and intralesional fungal hyphae consistent with *Mucorales* (former *Zygomycetes*).

Etiologic diagnosis:

Mycotic rumenitis (ruminal mucormycosis or ruminal zygomycosis)

Most likely etiology:

Mucorales of the genera *Mucor*, *Absidia*, and *Rhizopus* (members of the family *Mucoraceae*).

Comments:

Mucormycosis is a saprophytic opportunistic infection caused by fungi of the order *Mucorales* (former class *Zygomycetes*), of which several genera can cause disease, such as *Rhizopus* sp., *Mucor* sp., and *Lichtheimia* sp. (formerly *Absidia* sp. and *Mycocladus* sp.). Differentiating between these genera is not possible through histological study (3). There are normal gastrointestinal habitants, and opportunistic infections occur secondary to mucosal damage caused by ruminal acidosis (grain overload), as well as in cases of septic processes, prolonged antibiotic therapy, reflux of acidic abomasal contents, mucosal disease (bovine pestivirus), immunosuppression, and other conditions (5).

In diets high in carbohydrates (e.g., cereals), a reduction in ruminal pH occurs due to an increase in volatile fatty acids and changes in ruminal microflora, as an increase

in *Streptococcus bovis* which produces lactic acid. This is associated with reduced ruminal motility, impaired fluid transport to the rumen, and hypovolemia. These conditions contribute to the development of ruminal mucosal ulceration, which facilitates the invasion of the submucosa and blood vessels by opportunistic fungi (5). Fungal organisms exhibit a notable affinity for arterioles, leading to the onset of acute vasculitis. This condition results in thrombosis and infarction of the ruminal wall. In severe cases, the lesions can progress to complete transmural necrosis of the ruminal tissue (1, 2, 4).

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