Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2024-2025

Conference #16 — 15 January 2025

CASE I:

Signalment:
12-day-old, female, collared finchbill (*Spizixos semitorques*).

History:
A parent-raised chick was found dead on the ground outside of the nest without any premonitory signs. The carcass was covered by ants. The weather conditions on the day before were reported to be very hot.

Gross Pathology:
The chick was in fair body condition with small amounts of visceral adipose tissue. No major gross changes were identified except for some loss of skin, which was presumed to be due to postmortem scavenging.

Laboratory Results:
Bacteria were identified as *Clostridium piliforme* on the basis of conventional PCR and subsequent sequencing (target bacterial 16S rRNA) utilizing formalin-fixed paraffin-embedded brain tissue.

Microscopic Description:
Scattered throughout the cerebrum and affecting approximately 30% of the neuroparenchyma are multiple poorly demarcated to coalescing areas of hypercellularity. These areas have slightly prominent vasculature and are occasionally associated with hypereosinophilia or small lakes of extravasated erythrocytes. The cells infiltrating the neuroparenchyma are with a mix of granulocytes (heterophils), macrophages, and fewer lymphocytes and plasma cells, accompanied by increased numbers of glial cells and some necrotic debris. Occasionally, neurons are hypereosinophilic with smudgy or pyknotic nuclei. Other neurons relatively frequently contain stacks of faint, long, rod-shaped bacteria. Larger blood vessels within the inflammatory foci are frequently cuffed by dense aggregates of mononuclear cells that are up to five cell layers thick. The inflammatory cells additionally involve the overlying leptomeninges.

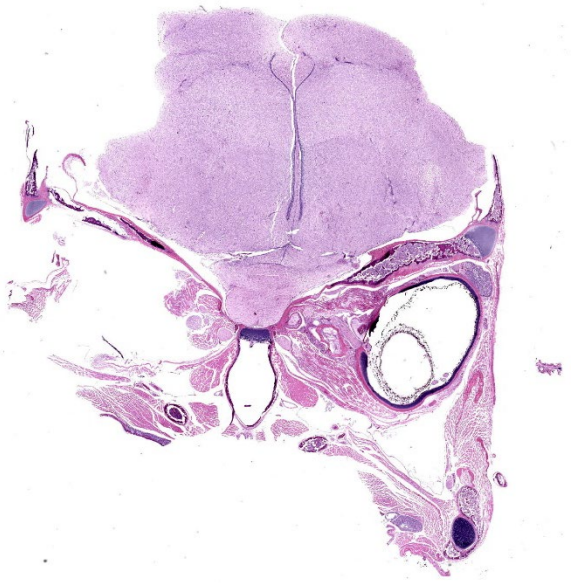

Figure 1-1. Head, collared finchbill. A slightly oblique cross section of the head is submitted for examination. (HE, 6X)

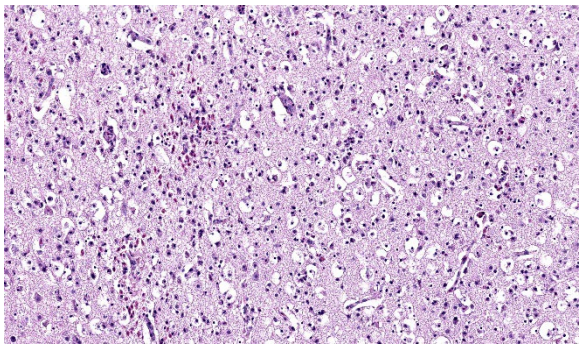

Figure 1-2. Cerebrum, collared finchbill. There are coalescing areas of hypercellularity, spongiosis, and small areas of hemorrhage within both cerebral hemispheres. (HE, 290X)

Steiner's silver and Gram stains were performed to better characterize the intracellular bacteria. Myriads of strongly argyrophilic, curvilinear to filamentous bacilli were highlighted on the silver stain, mapping to the areas of inflammation and necrosis. On the Gram stain, these bacteria were gram-negative.

Contributor's Morphologic Diagnosis:
Brain, cerebrum: moderate, multifocal to coalescing, subacute heterophilic and necrotizing meningoencephalitis with myriad intracellular argyrophilic and gram-negative filamentous bacilli, consistent with clostridial encephalitis

Contributor's Comment:
The characteristic histologic appearance of the intracellular filamentous bacilli was highly suggestive of Tyzzer's disease, caused by *Clostridium piliforme*. The etiologic diagnosis was further supported by the strongly argyrophilic and gram-negative nature of the bacteria and subsequently confirmed by molecular techniques. There were no bacteria morphologically compatible with *C. piliforme* or necrotizing inflammation in the other examined organs however.

Tyzzer's disease has been reported in a wide variety of mammalian species and is a major differential diagnosis for cases of hepatitis, myocarditis, and colitis, especially when this triad of lesions is noted concurrently.[7] While *C. piliforme* is not often thought of as a pathogen affecting birds, encephalitis in young birds is a known manifestation of Tyzzer's disease.[1,2,4,5] The condition is reported to affect both wild and captive birds from several orders, including passerines, psittacines, and piciformes. Affected birds can present with neurologic signs such as head tilt and torticollis. Histopathologic changes are often localized to the brain and characterized by multifocal to coalescing areas of mixed inflammation and necrosis.[1-2] Less commonly, birds can present with lesions in the liver, heart, and gastrointestinal tract, similar to mammals.[4]

At our institution, we have identified seven cases of avian Tyzzer's disease to date, including five metallic starlings (*Aplonis metallica*) between 11 to 25 days-old and two other collared finchbills (12 days old and 3 years, 5 months old). Six of these cases had encephalitis with no evidence of argyrophilic bacteria in the other examined tissues. The one case without brain involvement was the only adult bird in this list. This adult finchbill had multifocal random hepatitis with intracellular argyrophilic bacteria. Tyzzer's disease should be on the list of differential diagnoses in young birds with encephalitis.

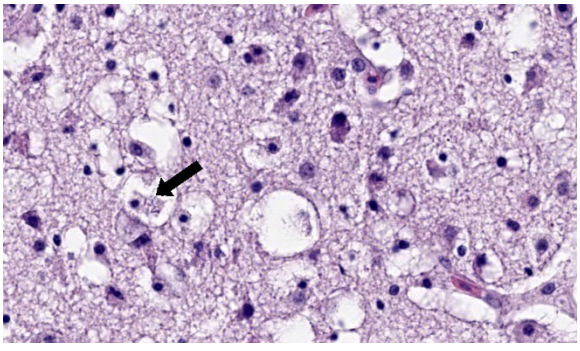

Figure 1-3. Cerebrum, collared finchbill. In areas of necrosis, glial cells occasionally contain 1x3 bacterial rods within their cytoplasm (arrow). (HE, 756X)

Contributing Institution:
San Diego Zoo Wildlife Alliance

Disease Investigations

https://science.sandiegozoo.org/disease-investigations

JPC Diagnosis:
Brain: Meningoencephalitis, heterophilic and necrotizing, subacute, multifocal, moderate with intracytoplasmic filamentous bacilli.

JPC Comment:
This week's moderator was Dr. Francisco (Paco) Uzal, Distinguished Professor of Veterinary Diagnostic Pathology at UC Davis and a in gastrointestinal and clostridial diseases.

This first case was recently shared at the Davis-Thompson Foundation's 2024 Northeast Veterinary Pathology Conference and we are pleased to share it with a wider audience. Conference participants homed in on the prominent cuffing of vessels and heterophilic inflammation within the neuroparenchyma and meninges. The increased cellularity is notable, even for an avian brain (which are typically more cellular than their mammalian counterparts.)Differential diagnoses from the group favored viral etiologies (e.g. highly pathogenic avian influenza) and avian chlamydiosis. Dr. Uzal noted that visualizing intracytoplasmic filamentous bacteria was difficult on H&E, though either a Gram or argyrophilic stains were helpful for making a definitive diagnosis.

The contributor nicely summarizes the current literature on avian cases of Tyzzer's disease and adds several observations from their own collection. We have covered *C. piliforme* many times in the WSC, most recently in a horse in Conference 4, Case 1, 2023-2024. Neurologic involvement remains rare,although there is a recent case report in a cat of systemic involvement with cutaneous and neurologic infection.[3] In this report, immunosuppression by concurrent infection with feline panleukopenia virus and trafficking of bacteria by macrophages aided dissemination of *C. piliforme*. This is not a feature of the present case. Finally, there is a single case report (from the archives of the AFIP) of zoonotic transmission of Tyzzer's disease to a human patient infected with HIV-1 with cutaneous involvement,[6] though this lesion was benign.

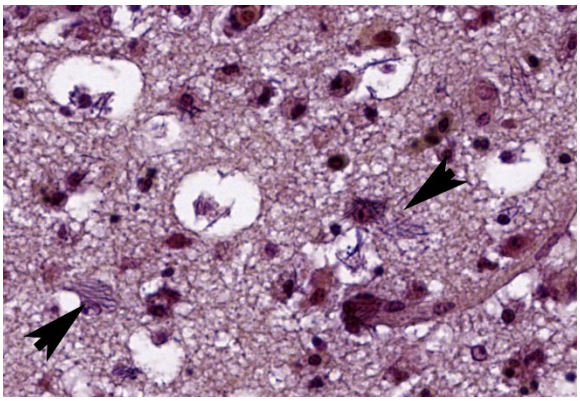

Figure 1-4. Cerebrum, collared finchbill. A tissue Gram-stain demonstrates aggregates of 1x3um rods within the cytoplasm of glial cells. (BB, 400X)

References:

1. Mete A, Eigenheer A, Goodnight A, Woods L. Clostridium piliforme encephalitis in a weaver bird (Ploceus castaneiceps). *J Vet Diagn Invest*. 2011;23(6):1240–1242.
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4. Raymond JT, Topham K, Shirota K, Ikeda T, Garner MM. Tyzzer's disease in

a neonatal rainbow lorikeet (*Trichoglossus haematodus*). *Vet Pathol*. 2001;38(3):326–327.
5. Saunders GK, Sponenberg DP, Marx KL. Tyzzer's disease in a neonatal cockatiel. *Avian Dis*. 1993;37(3):891–894.
6. Smith KJ, Skelton HG, Hilyard EJ, et al. Bacillus piliformis infection (Tyzzer's disease) in a patient infected with HIV-1: confirmation with 16S ribosomal RNA sequence analysis. *J Am Acad Dermatol*. 1996 Feb;34(2 Pt 2):343-8.
7. Uzal FA, Plattner BL, Hostetter JM. Alimentary system. In: Maxie MG, ed. *Jubb, Kennedy & Palmer's Pathology of Domestic Animals*. 6th ed. Vol. 2. Elsevier; 2016:1-257.

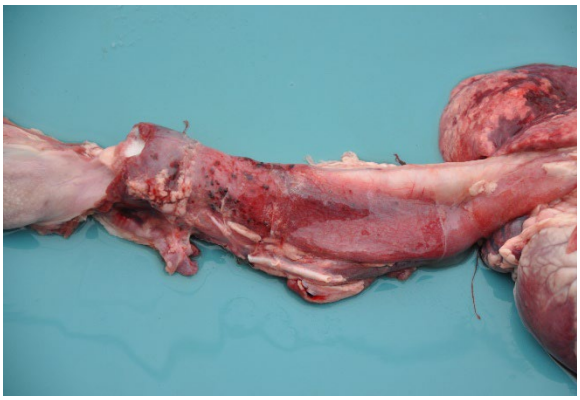

Figure 2-1. Esophagus, lamb. The cervical esophagus exhibits dilatation and sagging with congestion and petechiae on the serosa.. (*Photo courtesy of*: Setor de Patologia Veterinária, Universidade Federal do Rio Grande do Sul, Brazil (http://www.ufrgs.br/patologia/)

CASE II:

Signalment:
Less than 1 year-old female Texel ewe lamb (*Ovis aries).*

History:
Animal found dead with greenish serous nasal discharge and frothing. No previous clinical signs were reported by the owner.

Gross Pathology:

A female Texel ewe lamb was submitted to necropsy examination presenting good body condition and pale ocular mucosa. In nasal planum, a large amount of greenish serous nasal secretion was noticed. Submandibular and retropharyngeal lymph nodes presented moderate enlargement and showed diffuse dark red coloration. In the nasal cavity, moderate amount of inert plant fibers and ruminal content were seen and turbinates were diffusely hyperemic. The thoracic cavity contained a small amount of translucent liquid (hydrothorax). The lungs were not collapsed showing elastic consistency and abundant amount of foamy liquid in bronchi and trachea associated with inert plant fiber, as well as multifocal areas of consolidation found predominantly in the cranioventral region of the lungs. In the cervical segment of the esophagus moderate dilatation and sagging were noticed; the adventitia presented a moderate diffuse coloration with multifocal pinpoint hemorrhages smaller than 1 cm (petechiae). Moderate hydropericardium and multifocal areas of hemorrhage in epicardium were noted. In addition, discrete amount of hairlike parasites compatible with *Haemonchus contortus* in the abomasum

Laboratory Results:
Blood and tissue samples were tested for BTV RNA detection by RT-qPCR. Bacterial analysis was performed in lung tissue and *Mannheimia sp.* was isolated.

Microscopic Description:
A section of esophagus is examined. In muscular layer, there is severe multifocal to coalescing hyaline and flocculate degeneration and necrosis. Hyaline degeneration and necrosis are characterized by hypereosinophilic and swollen myofibers, with rounded edges

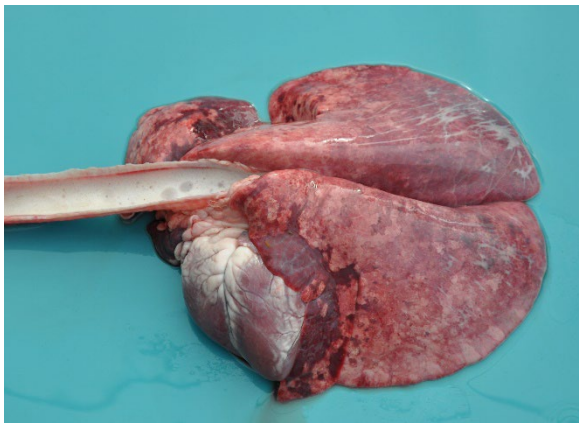

Figure 2-2. Lung, lamb. The lungs failed to collapse and there is cranioventral consolidation. (*Photo courtesy of*: Setor de Patologia Veterinária, Universidade Federal do Rio Grande do Sul, Brazil (http://www.ufrgs.br/patologia/)

in cross-section, sometimes showing hypercontracted and segmented cytoplasm with loss of striations, as well as pyknotic nuclei; eventually, myocytes display fragmented and flocculate sarcoplasm (flocculate degeneration and necrosis). Moreover, multifocally, macrophages are noted infiltrating cell sarcoplasm, as well as regenerating myofibers, which are characterized by elongated muscle cells with a row of central closely spaced nuclei containing myoblasts. In addition, moderate to severe inflammatory infiltrate of macrophages, lymphocytes, and few neutrophils, edema as well as moderate multifocal congestion were associated with necrotic areas and mild fibroblast proliferation. In some sections, marked multifocal to coalescing areas of hemorrhage are observed in tunica adventitia. Thin-walled structures consistent with *Sarcocystis sp.* were observed in some slides.

Contributor's Morphologic Diagnosis:
Esophagus: severe diffuse subacute necrotizing esophagitis.

Contributor's Comment:
The gross and microscopic findings observed in the present case were compatible with bluetongue disease, which was confirmed by detection of BTV RNA through RT-qPCR. Bluetongue virus (BTV) is a non-enveloped arbovirus, a member of the Reoviridae family, and is the prototype of the genus Orbivirus.[1] Bluetongue (BT) is a hemorrhagic disease caused by BTV, which affects domestic and wild ruminants. Among domestic species, sheep are the most susceptible and the severity of clinical signs can vary according to breed, age, and immune status of the affected flock.[2] In cattle, bluetongue is hardly noticed[6] though cattle are defined as amplifiers and reservoir hosts.[8] Virus replication in endothelial cells of small vessels results in distinct disease findings associated with vascular injury, such as tissue infarction, hemorrhage, vascular leakage, and edema.[2]

BTV is a vector-borne virus, mainly transmitted by biting midges from the genus *Culicoides*. South America has ideal climatic conditions for the survival and proliferation of *Culicoides* spp., and as reported by indirect evidence (serological investigations), BTV has spread since 1978 all over the American continent, with the exception of Uruguay.[3] BT cases are likely underreported due to the presence of very mild clinical signs, which can be mistaken for other similar endemic diseases.[3]

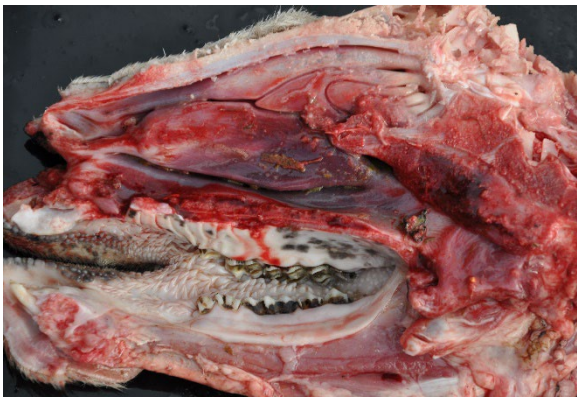

Figure 2-3. Nasal cavity, lamb. There are plant fibers and diffuse hyperemia of the turbinates within the nasal cavity. (*Photo courtesy of*: Setor de Patologia Veterinária, Universidade Federal do Rio Grande do Sul, Brazil (http://www.ufrgs.br/patologia/)

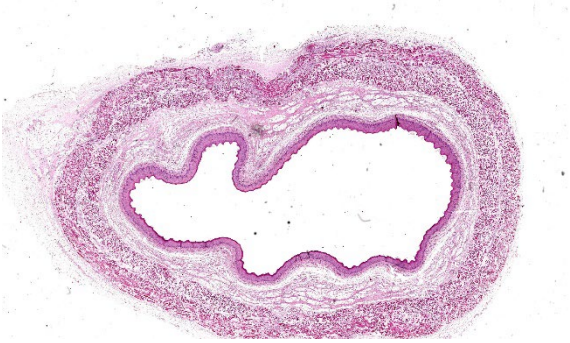

Figure 2-4. Esophagus, lamb. One section of the esophagus is submitted for examination. There is a diffuse retiform pattern of pallor within the muscularis at subgross magnification. (HE, 7X)

Typical signs in affected sheep include pyrexia, facial edema, ocular and nasal discharge, crusting of the muzzle, dyspnea, oral erosions and ulcers, coronitis, lameness and weakness.[2] The main gross findings described by Antoniassi et al[4] were esophageal dilation associated with non-collapsed enlarged lungs and foamy fluid within the trachea and bronchi, occasionally mixed with ruminal content. Hydropericardium, pale areas in the myocardium, and hemorrhagic foci scattered in the endocardium, epicardium and at the base of the pulmonary artery were also reported, as well as hyperaemia and erosions in the oral mucosa, and subcutaneous edema of the face. Aspiration pneumonia in BTV infection is related to aspiration of rumen content after reflux episodes resulting from severe injury of the esophageal muscles[4,5] which leads esophageal paralysis.[6] The disease is named bluetongue due to the fact that the tongue may become edematous, congested or cyanotic[6] though this gross lesion was not seen in the present case.

Histologically, edema, hemorrhage and microvascular thrombosis can be seen in areas with macroscopic lesions in acute presentations. These microvascular lesions are related with muscular necrosis.[6] Bianchi et al[7] described that the most common lesions found were located in lungs, esophageal striated muscle, cardiac and skeletal muscles, primarily in the neck and forelimbs.

The differential diagnosis for BTV in sheep should include foot-and-mouth disease, contagious ecthyma, sheep pox,[8] photosensitization, and peste des petits ruminants.[6] Vitamin E and selenium deficiency may be considered, once muscular necrosis associated with mineralization may resemble the changes observed in nutritional muscular dystrophy (NMD).[8] Another orbivirus to take in consideration is the Epizootic Hemorrhage Diseases Virus (EHDV), that may occasionally be responsible to mild clinical signs that resemble BTV in sheep.[6]

BTV 12,[5] BTV-1, BTV-4 and BTV-17[9] have been described in previous outbreaks in the State of Rio Grande do Sul. Until the moment, the genotyping of the virus responsible for this case has not been performed.

Contributing Institution:
Setor de PatologiaVeterinária, Universidade Federal do Rio Grande do Sul, Brazil (http://www.ufrgs.br/patologia/).

JPC Diagnosis:
Esophagus, muscularis: Degeneration and necrosis, monophasic, diffuse, moderate, with edema.

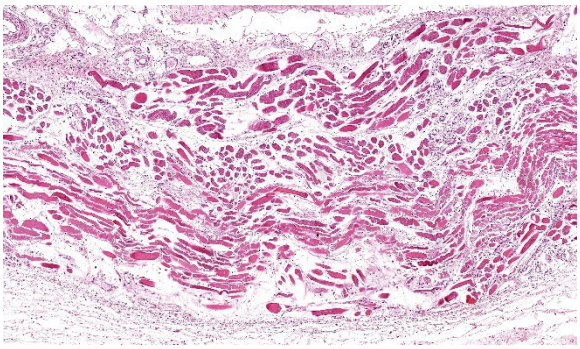

Figure 2-5. Esophagus, lamb. There is diffuse shrinkage and separation of myofibers with expansion of the interstitium. (HE, 109X)

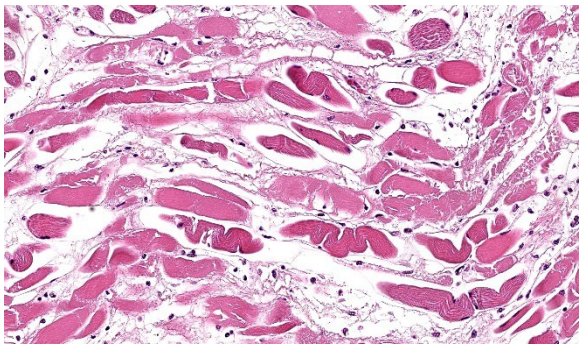

Figure 2-6. Esophagus, lamb. Myocytes exhibit one or more of the following: variation in fiber size, hypereosinophilia, loss of cross striations (degeneration), cytoplasmic granularity, fragmentation, nuclear pyknosis (necrosis.) There is edema and infiltration of the interstitium by macrophages. (HE, 475X)

JPC Comment:
This second case prompted spirited discussion among conference participants. The contributor lays out a detailed summary of bluetongue with characteristic histologic features to look for, though these are challenging to confirm in this section. Although there is notable edema and myocyte degeneration and necrosis, the cause is not apparent. We did not identify vasculitis and/or thrombosis in this particular section which would be expected in this case, as BTV is an endotheliotropic agent.

Conference participants offered ionophore toxicity as a potential etiology in this case, which is a ruleout for monophasic muscular injury in small ruminants. Vitamin E/selenium imbalance would likely result in polyphasic injury which would result in additional lesions of mineralization and fibrosis, which are lacking in this case. Early degenerative changes are present in myocytes, to include the early reversible change of hyalinization and hypereosinophilia resulting from glycogen depletion (and lack of replenishment). We covered monensin and associated muscular changes in a recent WSC in a brahman calf (Conference 5, Case 3, 2024-2025).

Our diagnosis is different from the contributor in this case. We framed this case as necrosis and degeneration (vice myositis) due to the lack of significant inflammation this section. In this case, the main driver of the lesion is the presumed vasculitis and ischemic damage – inflammation is mild at best in this lesion. Additionally, the lack of involvement of the esophageal mucosa makes the focus of the lesion more selective, and we prefer to restrict the morphologic diagnosis to the muscular tunics of the esophagus alone.

References:

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CASE III:

Signalment:
11-month-old, male Doberman, *Canis lupus familiaris*, dog.

History:
The dog was presented to the referring veterinarian with a 1-month history of persisting vomiting, diarrhea, and weight loss. On abdominal ultrasound, an approximately 16 x 6 cm echogenic, heterogeneous, poorly vascularized mass was in the mesogastrium extending from the caudal aspect of the liver to the area of the urinary bladder. Peritoneal effusion and reactive intestinal serosa were also appreciated. The dog underwent laparotomy for excision of the mass, but died during surgical procedure.

Gross Pathology:
On necropsy, an 18 x 7 x 5 cm firm, poorly demarcated, tan mass was firmly adhered to the greater curvature of the gastric wall, capsule of the pancreas and serosa of the duodenum and jejunum and gastric lymph nodes. On cut surface, the mass was tan with multiple 1-4 mm yellow to green caseous areas

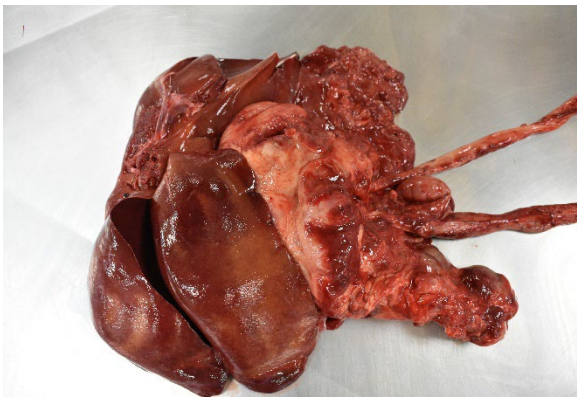

Figure 3-1. Stomach, dog. An 18 x 7 x 5 cm firm, poorly demarcated, tan mass was firmly adhered to the greater curvature of the gastric wall, capsule of the pancreas and serosa of the duodenum and jejunum and gastric lymph nodes. (*Photo courtesy of:* Setor de Patologia. Departamento de Clinica e Cirurgia Veterinárias, Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil)

and up to 8 cm cystic necrotic foci with viscous, yellow to brown exsudate. Along the serosa of the stomach, duodenum, and jejunum, there were multiple 2-4 cm in diameter nodules similar to the larger mass. The mucosa of the stomach and intestines was grossly normal, without ulcerations. The peritoneum was diffusely dark red with engorged blood vessels. The left testicle had an approximately 2 cm firm tan nodule within the parenchyma.

Laboratory Results:
The bloodwork revealed leukocytosis (23.160/mm^3) with neutrophilia (17.602/mm^3) e monocytosis (1853/mm^3). Chemistry results were normal.

Frozen fragments of the mesenteric mass were submitted to DNA extraction and panfungal PCR using internal transcribed spacer (ITS) primers. Amplified DNA product was submitted for sequencing and aligned

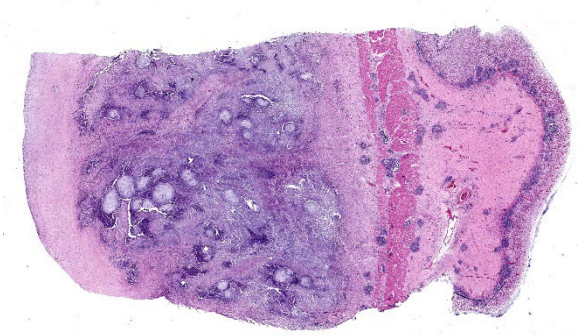

Figure 3-2. Stomach, dog. One section of stomach is submitted for examination. The serosa (left) is expanded up to 1.5cm and contains multifocal to coalescing pyogranulomas. There are numerous aggregates of lymphocytes and plasma cells within the overlying mucosa, expanded fibrotic submucosa, and muscularis. (HE, 7X)

with *Scedosporium apiospermum / Pseudoallescheria boydii* with 98.9% identity.

Microscopic Description:
Stomach: expanding the serosa and extending into the muscularis layer and submucosa is a dense inflammatory infiltrate forming multifocal to coalescing pyogranulomas. The center of the pyogranulomas contains myriads of fungal hyphae surrounded by degenerated and intact neutrophils, cellular debris, epithelioid macrophages, macrophages with foamy cytoplasm, fewer lymphocytes, eosinophils, plasma cells, and occasional Langhans multinucleated giant cells. Hyphae are 2-3 µm in diameter wide with thin non-parallel walls containing septations, irregular branching and bulbous dilations up to 9 µm in diameter. Hyphae were positive on periodic acid Schiff and Gomori methenamine silver stain. Melanine was not detected on Fontana Masson stain. The muscularis externa and serosa are expanded by reactive fibroblasts and abundant deposition of fibrous connective tissue. Lymphoid follicles in the lamina propria are hyperplastic.

Contributor's Morphologic Diagnosis:
Stomach: severe, multifocal to coalescing pyogranulomatous gastritis with numerous intralesional hyphae.

Contributor's Comment:
Fungi within the genus *Scedosporium* are saprophytes distributed worldwide in soil and fresh water, especially in environments rich in organic matter and manure.[1,11,17] *Scedosporium apiospermum* was previously known as *Scedosporium boydii*, but they are currently classified as distinct species. *Pseudoallescheria* is the teleomorph or sexual stage of *Scedosporium* spp. and usually is not present in tissue samples.[7,9]

Scedosporiosis is an emerging opportunistic fungal infection described in humans and animals.[5,17] In humans, infection with *Scedosporium apiospermum, Scedosporium boydii* and *Scedosporium aurantiacum* (collectively, the *S. apiospermum* species complex) can result in two distinct diseases: mycetoma and systemic scedosporiosis (pseudallescheriasis). Mycetoma is a chronic infection of the skin and subcutaneous tissue characterized by the production of grains.

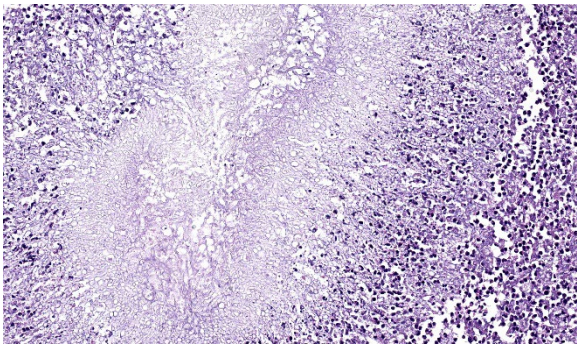

Figure 3-3. Stomach, dog. Pyogranulomas are centered on aggregates of 3-4um septate fungal hyphae with terminal conidia. (HE, 381X)

Most human patients with systemic infection are immunocompromised and common affected organs include lungs, nasal sinuses, bone, joints, and the brain[10]. Infection is usually through inhalation or traumatic inoculation through the skin. *Scedosporium*-related pneumonia is reported after near drowning in contaminated water.

In animals, few cases of cutaneous and systemic scedosporiosis are described in dogs, cats, horses, cattle, and an elephant seal.[3,4,8,14,18,21,22] In dogs, cutaneous infections are more often described in the digits, face and around the joints.[5,6,15] Infections with *Scedosporium* spp. have been reported after cutaneous traumatic injuries and surgical procedures.[6] In the present case, the distribution of the lesions suggests that the infection occurred through ingestion, although the mucosa of the affected gastrointestinal tract was intact. Gastrointestinal foreign bodies were not appreciated on gross examination and the dog had no history of previous surgical procedures. Immunosuppression and concomitant diseases are common predisposing factor for systemic infection in human patients, including infection by human immunodeficiency virus, diabetes, cystic fibrosis, neutropenia, those receiving prolonged high-dose corticosteroid therapy, or those who have undergone allogeneic bone marrow transplantation.[2,13,16,17,20] In this dog, all major organs were evaluated by histopathology and there was no evidence of concomitant diseases that could have predisposed to infection or history of previous use of immunosuppressants.

Although special stains (periodic acid-Schiff and Gomori methenamine silver) highlight characteristic morphologic features of the hyphae, these can be impossible to differentiate from *Conidiobolus* spp., *Basidiobolus* spp., *Aspergillus*, and *Fusarium* spp.[12,19] Fungal identification of *Scedosporium* spp. requires

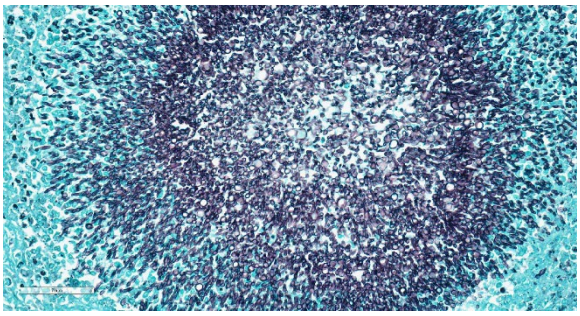

Figure 3-4. Stomach, dog. Fungal hyphae are stained well with Gomori-methenamine silver. (GMS, 200X) (*Photo courtesy of:* Setor de Patologia. Departamento de Clinica e Cirurgia Veterinárias, Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil)

culture along with DNA sequencing or matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry.[10] Scedosporiosis should be considered as a differential diagnosis for fungal pyogranulomatous gastroenteritis and peritonitis in dogs, especially when numerous fungal hyphae with bulbous dilations are observed.

Contributing Institution:
Escola de Veterinária, Universidade Federal de Minas Gerais (UFMG).

www.vet.ufmg.br

JPC Diagnosis:
Stomach: Serositis, pyogranulomatous, chronic-active, multifocal, severe, with numerous hyphae.

JPC Comment:
The contributor provides an interesting slide supported by an exhaustive background on *Scedosporium apiospermum*. The major microscopic features were readily apparent on H&E and special (fungal) stains alike. We interpreted the multifocal presence of lymphoid

aggregates as secondary to the profound serosal fibrosis and the resulting decrease in gastric motility.

We differed from the contributor's morphologic diagosis on two minor points. Similar to the discussion from Case 2, only one layer of the gastric wall is involved in the primary inflammatory process - for this reason, we describe this case as a serositis versus a gastritis. Additionally, we also noted the morphologic features of hyphae were best observed with histologic stains and not as well visualized on HE (which forms the basis of the JPC morphologic diagnosis, as special stains are not available to conference participants in advance of the conference. In this case, a silver stain best highlights the parallel walls, regular septation, irregular branching, and bulbous dilations that were generously present in section. We agree with the contributor that PCR is ideal for confirming identity of fungal agents, though morphology can help to create a prioritized list of differentials. Dr. Uzal suggested to conference participants that *Zygomyces* was unlikely in this case as hyphae have non-parallel wall hyphae and are pauciseptate[23] whereas the hyphae in this section were regularly septate and had conspicuously (and almost perfectly) parallel walls.

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CASE IV:

Signalment:
5-year-old, female, Scottish blackface sheep (*Ovis aries*).

History:
Presented to the Veterinary School farm animal clinic for investigation of ill thrift. Treatment was given in response to a high faecal parasite egg count and the numbers decreased. The submitting farm has a history of ovine pulmonary adenocarcinoma.

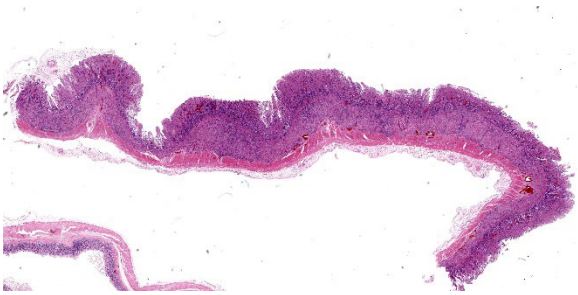

Figure 4-1. Cecum, sheep. A section of cecum is submitted for examination. (HE, 7X)

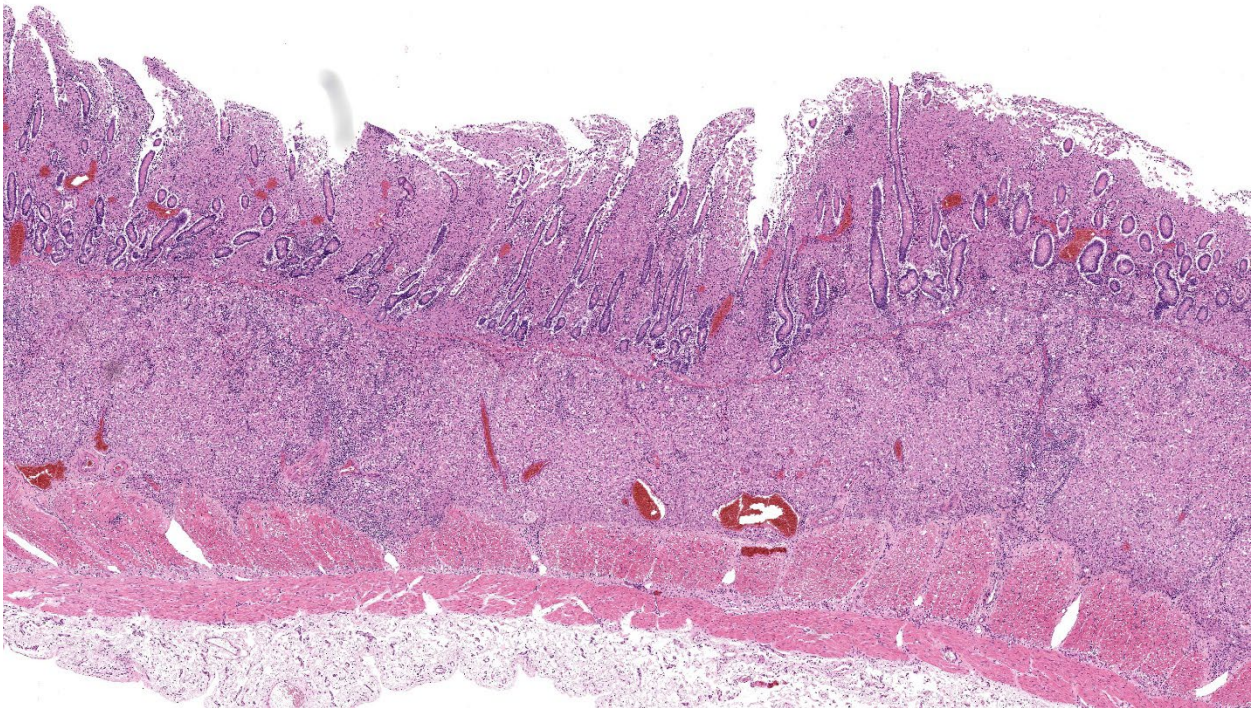

Figure 4-2. Cecum, sheep. The mucosa and submucosa are markedly expanded by a dense eosinophilic inflammatory exudate. (HE, 50X)

Gross Pathology:
Within the abdomen approximately 1 litre of yellow-tinged clear, watery fluid (hydroperitoneum) is noted.

Diffusely, the walls of the ileum and the distal third of the jejunum are moderately to markedly thickened, the mucosal surface exhibits a corrugated pattern and is bright yellow in colour. The mucosa of the caecum, diffusely, and colon, segmentally, are mildly to moderately thickened.

Multifocally, the mesenteric lymph nodes are moderately enlarged, and upon cut section show moderate multifocal expansion of the cortex by firm white-grey tissue.

Laboratory Results:
Parasitology:

- McMaster slide method: 2000 strongyle spp. eggs per gram (epg), 500 *Moniezia* spp. epg, 1100 Coccidia spp. epg, 100 *Nematodirus* spp. epg
- Baermann technique: positive for *Dictyocaulus filaria* larvae.

Serology:

- *Corynebacterium pseudotuberculosis*: Negative
- Maedi-Visna virus: Negative
- *Mycobacterium avium subsp. Paratuberculosis* (MAP): Positive

Microscopic Description:
Caecum: Separating intestinal crypts, the lamina propria and submucosa are diffusely markedly expanded by large numbers of epithelioid macrophages. Within the cytoplasm of these cells there are large numbers of 4x1µm bacilli that fail to stain with HE stain but are acid-fast positive on Ziehl-Neelsen stain. Admixed with the macrophages are small numbers of

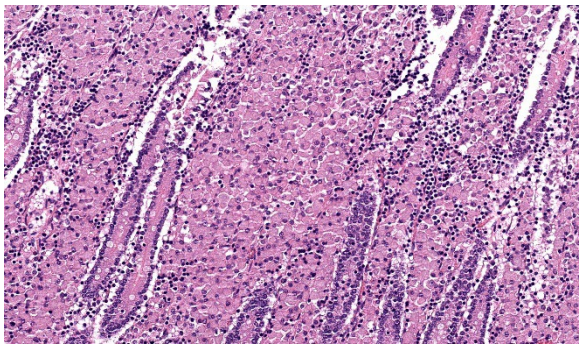

Figure 4-3. Cecum, sheep. Remaining mucosal glands are markedly separated and replaced by sheets of macrophages. (HE, 381X)

lymphocytes, plasma cells and occasional multinucleate giant cells. Similar inflammatory cell infiltrates extend, multifocally, into the inner circular and outer longitudinal muscular layers of the caecal wall. The serosa is expanded, diffusely, and the connective tissue is separated by clear, material (protein poor oedema) and contains small numbers of lymphocytes, plasma cells and macrophages, that latter containing acid-fast bacilli, which are present rarely in presumed lymphatics. Small numbers of intestinal crypts are lined by flattened enterocytes. The blood vessels are filled with moderate numbers of erythrocytes (congestion).

Contributor's Morphologic Diagnosis:
Caecum: Typhlitis, chronic, histiocytic, diffuse, marked with intra-histiocytic acid-fast bacteria.

Contributor's Comment:
The microscopic features of this enteritis are consistent with diffuse multibacillary *Mycobacterium avium subsp. paratuberculosis* (MAP) infection, also known as Johne's disease.[3,7,12]

MAP is a Gram-positive, acid-fast, aerobic, non-motile, non-spore forming bacterium of the genus *Mycobacterium*, family *Mycobacteriacea* and a member of the *Mycobacterium avium complex* which comprises *M. avium avium*, *M. avium sylvaticum*, *M. colombiense* and *M. intracellulare.*[10]

Premortem diagnosis is challenging, and the gold standard is based on faecal culture, which takes several weeks to months,[3] or other tests such as Ziehl-Neelsen stained faecal smears, ELISA, PCR, identification of MAP specific peptides, and expression of gamma interferon all of which will provide more rapid results. Liver biopsy has also been shown to have high sensitivity and specificity for identifying sheep with advanced disseminated infection.[9] On post-mortem examination, the gold standard is based on culture and isolation of the bacteria although PCR of affected intestinal tissue samples for Insertion Sequence 900 (IS900) provides faster results and is MAP specific.[3,6,11]

Infection is thought to occur in neonates and juveniles via ingestion of contaminated food, pasture, water, colostrum, and milk. MAP is thought to cross the intestinal mucosal barrier via preferential uptake by M-cells present overlying Peyer's patches and mucosal lymphoid domes. Bacteria then invade subepithe-

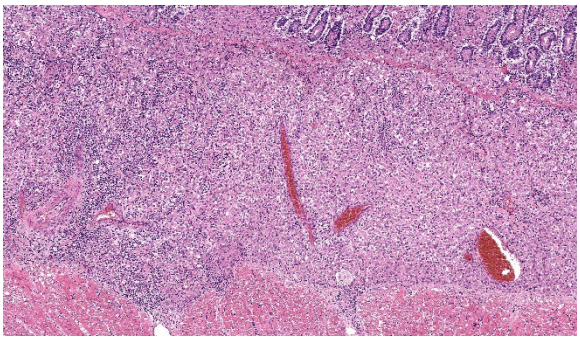

Figure 4-4. Cecum, sheep. The histiocytic infiltrate markedly expands the submucosa and extends into the superficial muscularis. (HE, 142X)

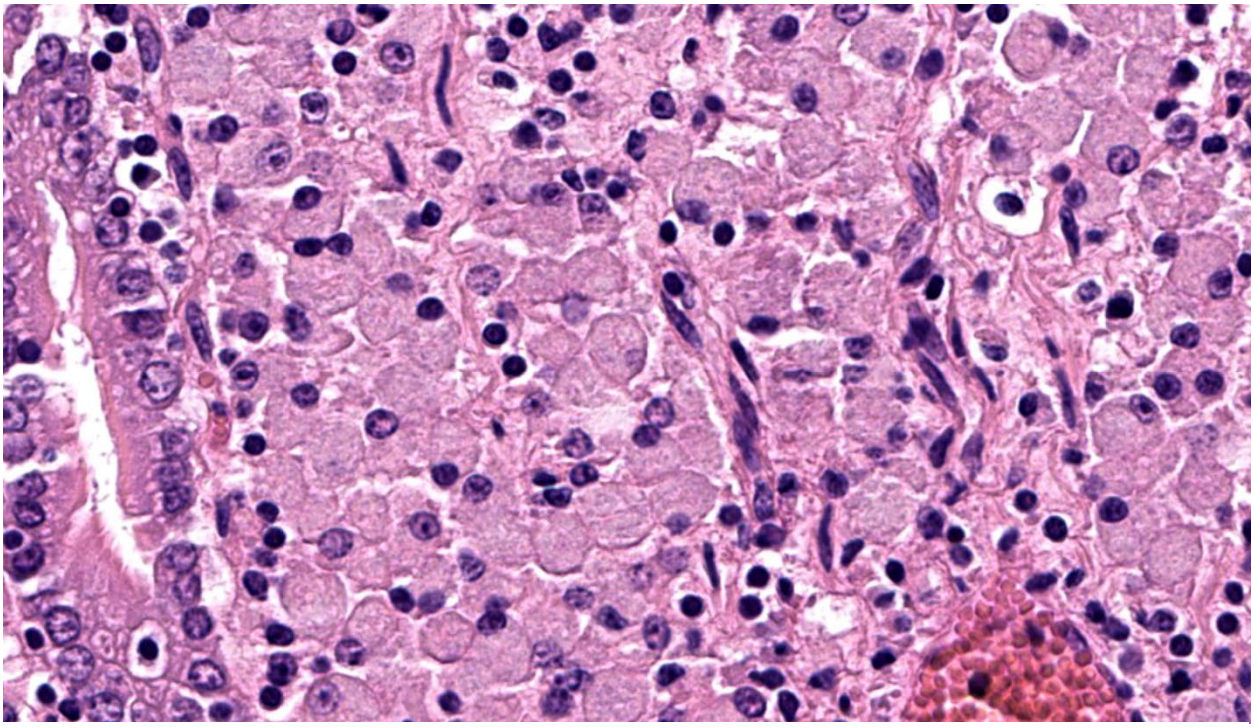

Figure 4-5. Cecum, sheep. Histiocytes have abundant grey-pink granular cytoplasm. (HE, 1250X)

lial macrophages and modulate the immune system[3,10,12] whilst concurrently inhibiting phagolysosome fusion, acidification and macrophage activation to avoid degradation.[4] Typically, the infection takes months to years before causing macroscopic lesions and clinical signs. Experimental challenge in sheep usually requires an incubation period of 4 to 8 months before the onset of clinical signs. Animals infected before 2 years of age exhibit greater pathological and clinical manifestations compared to those infected when adult.

Depending on the ensuing immune response to infection, different morphological presentations of disease develop. The paucibacillary form of the disease is driven by a Th1 cell-mediated interferon gamma response whereas the multibacillary Th2 form is a humoral response driven by IL-10. The multibacillary morphology is thought to be caused by a switch from a Th1 response towards a Th2 response or may be caused by a simultaneous Th1 and Th2 response with failure of the Th1 component of inflammation.[7] Depending on the inflammatory response, the affected macrophages display different phenotypes, either M1 iNOS (inducible nitric oxide synthase) and TNF-alpha positive activated macrophages driven by Th1 mediated inflammation or M2 CD163, IL-10 and TGF-beta positive activated macrophages mediated by a Th2 response.[2] Additionally, 3 different subsets of M2 macrophages have been described: M2a is present in allergies and helps with killing and encapsulating parasites, M2b is driven by a Th2 inflammatory response induced by IL-1 and M2c is induced by IL-10 and TGF-beta and is believed to be implicated in Johne's disease progression towards the multibacillary form.[2]

MAP is subdivided in type I/III and type II strains which affect, predominantly, sheep and cattle, respectively. Additionally, types II and III have broader host ranges.[6,11] MAP has been reported in numerous species including

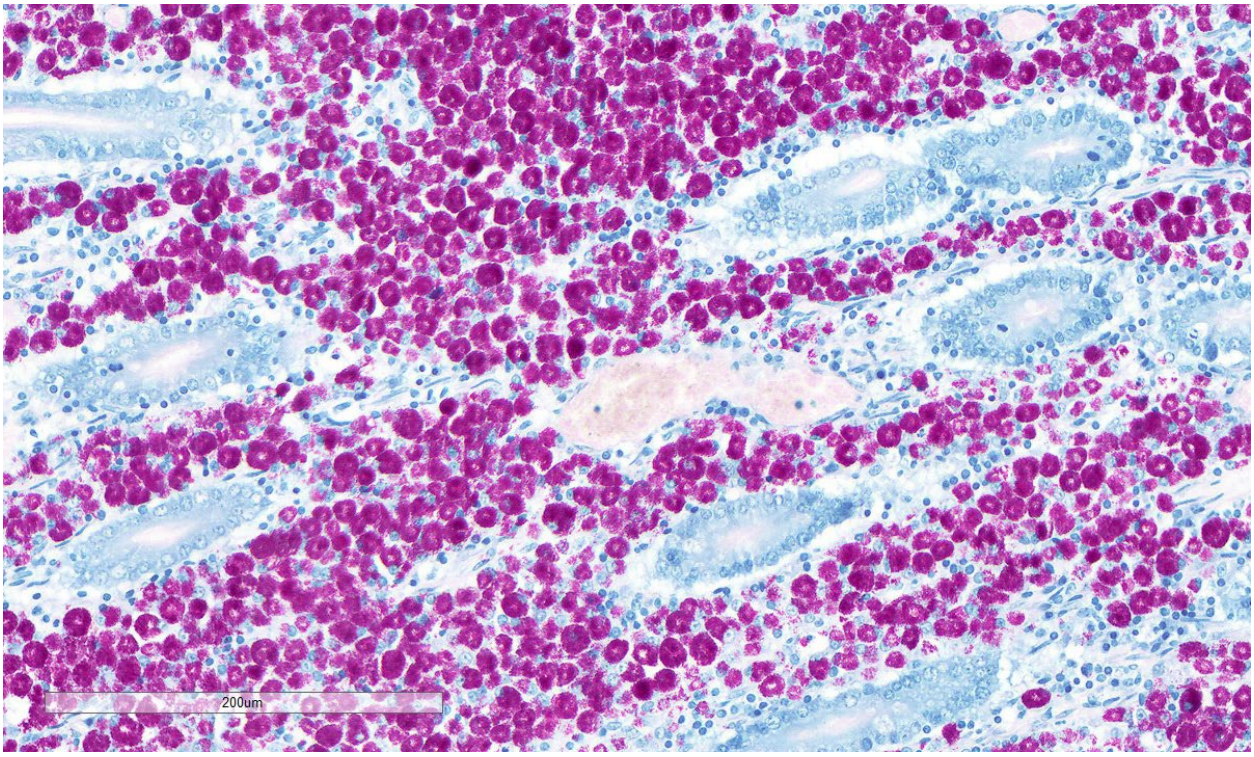

Figure 4-6. Cecum, sheep. Histiocytes contain large numbers of cytoplasmic acid-fast bacilli. (HE, 400X). (*Photo courtesy of:* Veterinary Diagnostic Services, College of Medicine, Veterinary and Life Sciences, University of Glasgow)

a wide range of captive and wild ruminants, camelids, rabbits, equids, swine, lagomorphs and primates. It has also been detected in rodents, carnivores and several species of wild birds but without evidence of disease.[11] Rabbits may play a role in the epidemiology of the disease although it is not clear if they are naturally susceptible to the disease.[1] Furthermore, MAP has been isolated from humans suffering from Crohn's disease by PCR and FISH, but in human tissues it is in a mycobacterial cell wall deficient state, so is not acid-fast.[6,11]

Contributing Institution:

Veterinary Diagnostic Services
School of Biodiversity, One Health & Veterinary Medicine
College of Medicine, Veterinary and Life Sciences
University of Glasgow
https://www.gla.ac.uk/schools/vet/cad/

JPC Diagnosis:

Intestine: Enteritis, granulomatous, chronic, diffuse, severe, with edema.

JPC Comment:
The conference concludes with a classic entity that has been a frequent submission to the WSC. There was spirited debate on tissue identification in this case as participants were divided on whether or not there were villi present (i.e. consistent with small intestine) – Dr. Uzal was unable to resolve this question definitively, so we hedged with the lessspecific "intestine" in our morphologic diagnosis.

The microscopic features of this case align

with the Th2 multibacillary pathogenesis that the contributor nicely summarizes. Sheets of macrophages expand the mucosa, lamina propria, and submucosa and ultimately result in a malabsorptive diarrhea that corresponds to the ill thrift noted clinically for this ewe. Dr. Uzal discussed that the submucosal involvement in this case was not unusual for MAP, though it may be overlooked in the description.

One feature not reported in this case is mineralization of the aorta and endocardium which is reported to occur in up to 25% of paratuberculosis cases.[8] Macrophage activation, particularly of M1 macrophages, likely drives calcification of elastic fibers via deposition of vitamin D metabolites in response to cytokines such as TNF-α, IL-1, and IL-8.[6] Plasticity of vascular smooth muscle to differentiate towards an osteoblastic phenotype under the influence of this same cytokine milieu likely also plays a role in vascular mineral deposition.[6] Conversely, M2 macrophages impair osteogenesis and/or enhance osteolysis and mineral degradation. The interplay of secreted matrix metalloproteases, available matrix constituents, and concurrent injury to the vessel via reactive oxygen species (ROS) has also been evaluated.[6] Although not as well studied as in human atherosclerosis in which these processes have been more extensively studied, an understanding of this basic process is helpful for understanding mineralization in other diseases. Important ruleouts in this case include ingestion of calcinogenic plants (e.g. *Cestrum diurnum* and *Trisetum flavescens*) and hypervitaminosis D.

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