Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2023-2024

Conference #4 13 September 2023

CASE I:

Signalment:
1-month-old, Quarter Horse colt, horse (*Equus caballus*)

History:
This foal presented to the Equine Emergency Service acutely ill with a fever, tachypnea, diarrhea, and seizures. Laboratory data revealed elevated liver enzymes and bile acids, left shift neutropenia, elevated fibrinogen, hypoglycemia, hyperlactatemia, and electrolyte abnormalities (see details below under laboratory results). Abdominal ultrasound identified hepatomegaly. The foal was humanely euthanized due to poor prognosis.

Gross Pathology:
On postmortem examination, the foal was in good body condition. The sclera and mucous membranes were mildly yellow (icterus). The liver was diffusely enlarged, mottled pale red to dark purple with disseminated pinpoint to ~1 mm diameter pale tan foci scattered throughout all lobes. The wall of the right dorsal colon was moderately thickened by edema, and the mucosa was diffusely dark purple. The right dorsal colon contained a moderate amount of opaque, homogeneous, bright yellow, watery digesta. The small colon contained small, soft, partially formed fecal balls. The skeletal muscle was diffusely pale tan to red. The lungs were slightly wet, heavy and mottled pale pink to dark red. On section, they oozed a small amount of serous fluid. A complete postmortem was performed, and no other significant gross lesions were identified.

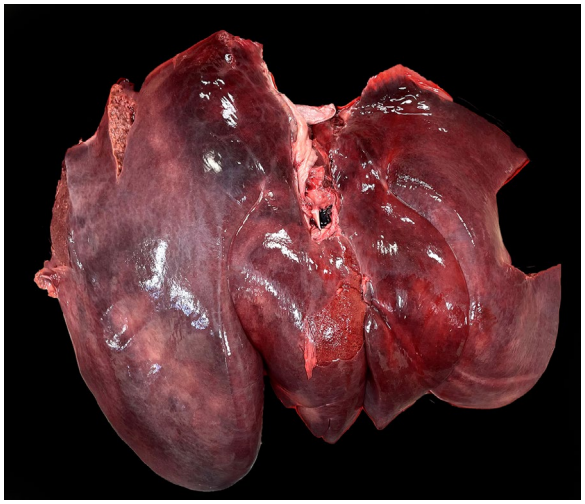

Figure 1-1. Liver, foal. The liver is diffusely enlarged and mottled pale red to dark purple. (Photo courtesy of: National Institutes of Health Comparative Biomedical Scientist Training Program (CBSTP) in collaboration with Colorado State University Veterinary Diagnostic Laboratory, https://nih-cbstp.nci.nih.gov/; https://vetmedbiosci.colostate.edu/vdl).

Laboratory Results:

CBC	
Neutrophils	100 cells/µl (3,000-7,000)
Bands	400 cells/µl (0-100)
Fibrinogen	800 mg/dL (100-400)
Chemistry	
AST	1593 IU/L (185-375)
T-bilirubin	3.3 mg/dL (0.4-1.8)

SDH	223 IU/L (0-10)
GGT	54 IU/L (10-25)
Glucose	62 mmol/L (70-135)
Sodium	123 mEQ/L (132-142)
Chloride	84.4 mEQ/L (97-104)
Bicarb	20 mEQ/L (26-33)
Anion gap	22 mmol/L (8-15)
Icterus	6 mg/dL (1-3)
Lactate	7.2 mmol/L (1.11-1.78)
Bile acids	12 µmol/L (0-8.0)

PCR testing performed on fresh liver tissue at the University of Kentucky for *Clostridium piliforme* was positive.

PCR testing on fresh feces performed at University of California Davis was negative for the following: *Clostridium difficile* toxin A and B, *Lawsonia intracellularis*, *Salmonella* spp., *Cryptosporidium* spp., *Rhodococcus equi*, *Clostridium perfringens* antigen, *C. perfringens* alpha toxin, *C. perfringens* beta toxin, *C. perfringens* beta2 toxin, *C. perfringens* cytotoxin netF, and *C. perfringens* enterotoxin. PCR testing for *C. piliforme* was not performed on the feces.

Microscopic Description:
Liver: Multifocally and randomly affecting approximately 80% of the hepatic parenchyma are numerous 150-300 micron diameter foci of lytic necrosis characterized by fibrin, cellular debris, and high numbers of degenerate neutrophils. Necrotic foci are often coalescent, forming larger discrete sites (up to ~1 mm) of necrosis. There are hypereosinophilic and shrunken necrotic hepatocytes with pyknotic or karyorrhectic nuclei at the interface between necrotic and viable tissue. Peripheral to regions of necrosis, less affected hepatocytes occasionally contain numerous intracytoplasmic, stacked, pale, basophilic, filamentous bacilli. The portal interstitium is expanded by edema and variable numbers of lymphocytes and plasma cells. Few neutrophils and lymphocytes infiltrate through the hepatic capsule, which is also mildly edematous. The mesothelium is mildly reactive. Steiner's stain reveals numerous, stacked, argyrophilic elongate bacilli within hepatocytes at the periphery of necrotic foci.

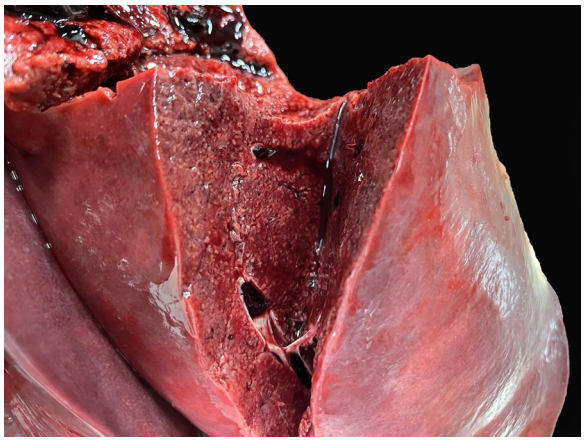

Figure 1-2. Liver, foal. The liver is diffusely enlarged, mottled pale red to dark purple with disseminated pinpoint to ~1 mm diameter pale tan foci scattered throughout the parenchyma of all lobes. (Photo courtesy of: National Institutes of Health Comparative Biomedical Scientist Training Program (CBSTP) in collaboration with Colorado State University Veterinary Diagnostic Laboratory).

Contributor's Morphologic Diagnosis:
Liver: Severe acute multifocal random necrosuppurative hepatitis with intrahepatocellular argyrophilic filamentous bacilli, etiology consistent with *Clostridium piliforme*.

Contributor's Comment:
Tyzzer's disease is a bacterial infection caused by *Clostridium piliforme*, an anaerobic, generally gram-negative, argyrophilic, pleomorphic, obligate intracellular bacillus. It can cause clinical disease in a variety of animals, including rodents, foals, calves, dogs, nonhuman primates, and cats.[5] One case of Tyzzer's disease has also been reported in an

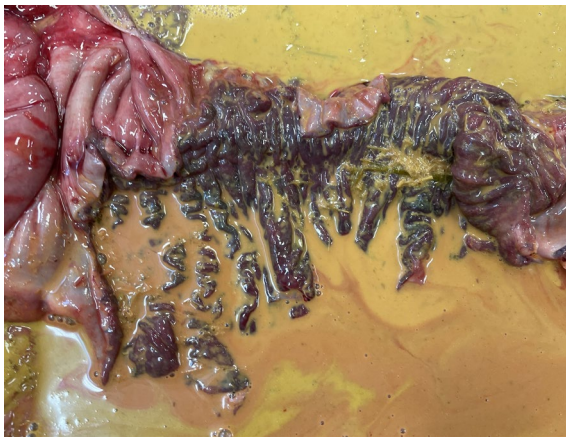

Figure 1-3. Colon, foal. The wall of the right dorsal colon is moderately thickened by edema, and the mucosa is diffusely dark purple. The right dorsal colon contains a moderate amount of opaque, homogeneous, bright yellow, watery digesta. (Photo courtesy of: National Institutes of Health Comparative Biomedical Scientist Training Program (CBSTP) in collaboration with Colorado State University Veterinary Diagnostic Laboratory).

HIV-1 infected human.[7] Clinical disease generally occurs in young or immunocompromised animals and can cause lethargy, fever, anorexia, icterus, seizures, coma and death.[8] Foals generally succumb to fatal disease between 2 and 4 weeks of age.[8]

The pathogenesis of Tyzzer's disease is not fully elucidated. However, it is postulated that fecal-oral transmission of spores leads to gastrointestinal infection of mucosal enterocytes in the ileum, cecum and colon. Following necrotizing enteritis/typhlocolitis, portal circulation of bacteria leads to hepatic and systemic involvement.[6] Adult horses are generally resistant to disease but can harbor and shed *C. piliforme* in their feces. Foals commonly consume the dam's feces within the first few weeks of life, and it is likely that in many cases contaminated feces from the dam is a primary source of infection.[8] However, spores of *C. piliforme* can also persist in the environment for at least 5 years, so long-term environmental contamination can also be problematic.[1]

Tyzzer's disease classically causes a triad of hepatitis, colitis and myocarditis. The prevalence of each of these lesions varies between species. In foals, the most common finding is multifocal necrotizing hepatitis that is occasionally accompanied by necrotizing lymphohistiocytic colitis and less frequently a multifocal necrotizing myocarditis. In a recent case series of 25 cases, all foals had hepatitis, 10/25 had colitis, and only 8/25 had myocarditis.[3] The triad of concurrent lesions is more commonly observed in rodents and lagomorphs.[6] In this case, there was necrotizing hepatitis, a mild to moderate necrotizing lymphohistiocytic colitis and mild patchy gray matter edema with proliferation of Alzheimer type II astrocytes. There were no remarkable microscopic findings in the myocardium. The changes in the brain are compatible with hepatic encephalopathy. Hepatic encephalopathy in cases of Tyzzer's disease results from hyperammonemia secondary to acute liver failure. Horses, in contrast to carnivores and ruminants, do not exhibit spongy vacuolation of myelin (status spongiosis) with hyperammonemia.[4]

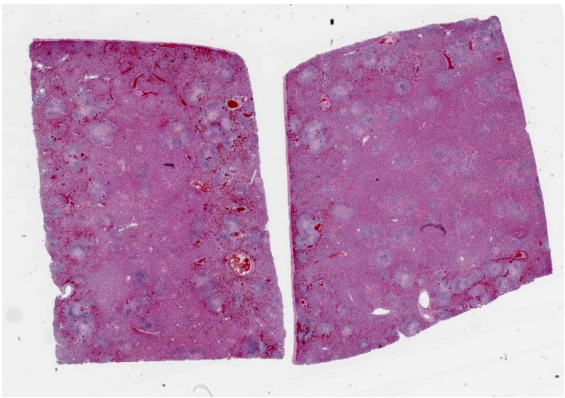

Figure 1-4. Liver, foal. Two sections of liver are 50% effaced by areas of lytic necrosis. (HE, 5X)

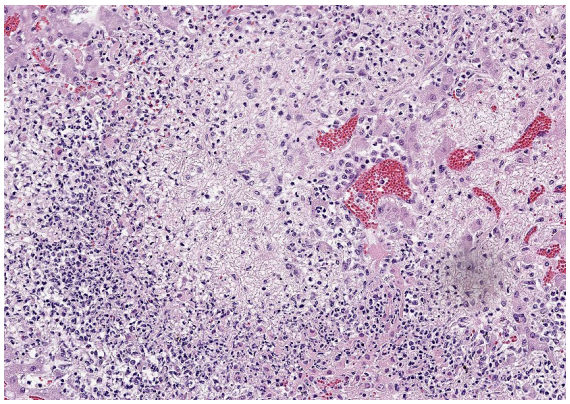

Figure 1-5. Liver, foal. Higher magnification of areas of lytic necrosis. There is loss of hepatocytes and infiltration of large numbers of necrotic and viable neutrophils and fewer macrophages admixed with abundant cellular debris. (HE, 5X)

The diagnosis of Tyzzer's disease can be made with gross and histologic findings with the presence of intracellular filamentous bacteria within hepatocytes. The bacteria can be faintly visualized on H&E and highlighted by silver stains (such as Steiner's or Warthin-Starry), Giemsa, and periodic acid-Schiff stains.[6] PCR amplification of the 16S rRNA gene of *C. piliforme* can be performed to further support Tyzzer's disease. Considering the close phylogenetic proximity to other nonpathogenic clostridia, such as *C. colinum*, the PCR results should be interpreted with the gross and histologic findings. *C. piliforme* is very difficult to culture but can be isolated by inoculation of embryonated chicken eggs or specific primary cell lines.[1]

Other differentials that may be considered with multifocal necrosuppurative hepatitis without distinct intracellular filamentous bacteria in a foal include EHV-1 or bacteria that lead to septicemia (e.g. *Salmonella* spp., *Actinobacillus equuli*, *Listeria monocytogenes*). Other causes of foal diarrhea include equine rotavirus, equine coronavirus, *Rhodococcus equi*, *Clostridium perfringens*, *Clostridium difficile*, *Lawsonia intracellularis*, and *Cryptosporidium* spp.

Contributing Institution:
National Institutes of Health Comparative Biomedical Scientist Training Program (CBSTP) in collaboration with
Colorado State University Veterinary Diagnostic Laboratory
https://nih-cbstp.nci.nih.gov/
https://vetmedbiosci.colostate.edu/vdl/

JPC Diagnosis:
Liver: Hepatitis, necrotizing, multifocal to coalescing, random, with occasional intracytoplasmic bacilli.

JPC Comment:
The contributor provides an excellent, thorough review of Tyzzer disease, a well-known and well-described clostridial disease of wide veterinary importance. Less well-known, sadly, is the fascinating biography of Dr. Ernest Edward Tyzzer, the scientific Renaissance man for whom Tyzzer disease is named.

Dr. Tyzzer was born in a suburb of Boston in 1875 and paid his way through college and Harvard Medical School by trapping muskrat, fox, mink, skunk, and weasels.[10] His pathology and parasitology-steeped career began in his second year of medical school when, during one of his trapping trips, he discovered parasites in a fox carcass and brought the carcass to class, endearing himself to the pathology faculty (though perhaps less so to his fellow students).

His curiosity about the natural world led to a broad study of infectious agents in a variety of species. Immediately after graduating from medical school, Tyzzer began studying the histologic lesions of varicella virus and determined that the intranuclear inclusions generated in the disease were likely viral

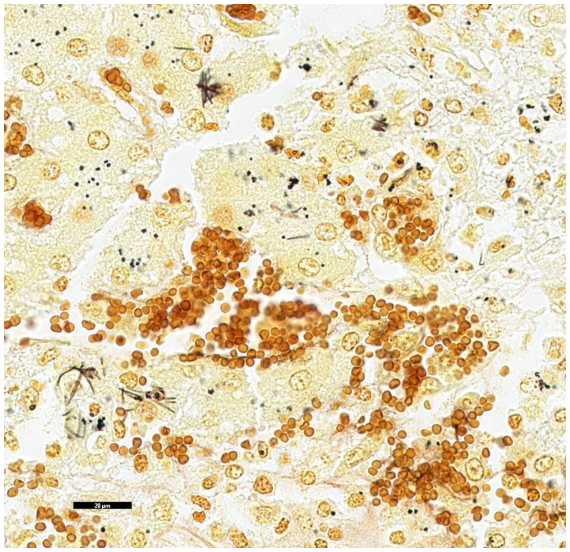

Figure 1-6. Liver, foal. A silver stain demonstrates haphazardly arranged stacks of filamentous bacilli consistent with Clostridium piliforme. (Steiners, 400X) (Photo courtesy of: National Institutes of Health Comparative Biomedical Scientist Training Program (CBSTP) in collaboration with Colorado State University Veterinary Diagnostic Laboratory).

in nature and not morphologic stages of an unknown protozoal parasite, the accepted theory at the time.[10] Tyzzer spent the next eleven years as the Director of Research for the Harvard Cancer Commission, where he investigated the occurrence of spontaneous murine tumors, host responses to transplantable tumors, and the biology of metastasis. During this time, three mice supplied by Tyzzer to one of his research collaborators became the founding members of the DBA inbred strain of mice now commonly used in medical research.[10]

Dr. Tyzzer also discovered and described the first *Cryptosporidium* species, *C. muris*, in the gastric glands of laboratory mice in 1907. Two follow-on publications in 1910 and 1912 contained further descriptions, gleaned solely from light microscope observations, about the life cycle and biology of *C. parvum* and *C. muris* that are still largely accepted today.[9]

Tyzzer was also responsible for correctly classifying the causative agent of blackhead in turkeys, naming it *Histomonas meleagridis,* and identifying that ingestion of contaminated nematode eggs was the connection between *Histomonas meleagridis* and *Heterakis* spp.[10] Having worked out the pathogenesis of the disease, Tyzzer then set up an experimental turkey farm where he worked out effective management practices to produce healthy turkeys and outlined these practices in pamphlets distributed to farmers by the Massachusetts Department of Agriculture. Tyzzer received a citation from the Governor of Massachusetts for this work, crediting him for saving the Massachusetts turkey industry.[10]

Dr. Tyzzer was involved in the discovery and elucidation of many other conditions not described above, including Tyzzer's disease, the causative agent of which he first described in a colony of Japanese waltzing mice in 1917. Tyzzer was also known for his humor, which was on display after he published a case report on his very own bleeding rectal polyp and accompanying *Entamoeba coli* infection. Though never identifying the patient, his manuscript stated that no systematic follow up study could be arranged "owing to the non-cooperative attitude of the patient".[10]

This week's slide conference, moderated by MAJ Kelsey Fiddes, Chief of Resident Training at the Joint Pathology Center, kicked off with this classic presentation of Dr. Tyzzer's eponymous disease. As is typical of this disease, the characteristic filamentous bacilli are covertly stacked within the cytoplasm of hepatocytes near the outside edge of necrotic lesions and require some imagination to appreciate with H&E staining alone. Silver stains such as Steiner's and Warthin-Starry allowed visualization of these bacteria and their classic "haystack" appearance.

Conference discussion focused mainly on differences in clinical presentations among various species. The classic "heart, intestine, liver" triad of lesions repeated breathlessly by countless pathology residents is seen mainly in laboratory animals, while horses manifest their Tyzzer mainly through necrotizing hepatitis, as in our case. *Clostridium piliforme* infection in rats is associated with megaloileitis and with encephalitis in gerbils. Finally, a 2023 retrospective study found that the most common manifestation of Tyzzer disease in kittens is ulcerative colitis, followed by hepatitis and perianal dermatitis.[2]

References:

1. Barthold SW, Griffey SM, Percy DH. Mouse. Pathology of laboratory rodents and rabbits. 4th ed. Wiley;2016:1–118.
2. Fingerhood S, Mendonca FS, Uzal FA. Tyzzer disease in 19 preweaned orphaned kittens. *J Vet Diagn Invest*. 2023;35(2): 212-216.
3. García JA, Navarro MA, Fresneda K, Uzal FA. Clostridium piliforme infection (Tyzzer disease) in horses: retrospective study of 25 cases and literature review. *J Vet Diagn Invest*. 2022;34(3):421–428.
4. Hasel KM, Summers BA, De Lahunta A. Encephalopathy with idiopathic hyperammonaemia and Alzheimer type II astrocytes in Equidae. *Equine Vet J*. 1999; 31(6):478–482.
5. Jubb KVF, Kennedy PC, Palmer N. Pathology of domestic animals. Academic press; 2012.
6. Navarro MA, Uzal FA. Pathobiology and diagnosis of clostridial hepatitis in animals. *J Vet Diagn Invest*. 2020;32(2): 192–202.
7. Smith KJ, Skelton HG, Hilyard EJ, et al. Bacillus piliformis infection (Tyzzer's disease) in a patient infected with HIV-1:
8. confirmation with 16S ribosomal RNA sequence analysis. *J Am Acad Dermatol*. 1996;34(2):343–348.
9. Swerczek TW. Tyzzer's disease in foals: retrospective studies from 1969 to 2010. *Can Vet J*. 2013;54(9):876.
10. Tzipori S, Widmer G. A hundred-year retrospective on cryptosporidiosis. *Trends Parasitol*. 2008;24(4):184-189.
11. Weller TH. *Ernest Edward Tyzzer 1875-1965*. National Academy of Sciences; 1978.

CASE II:

Signalment:
3-month-old, female Speckled Sussex, avian (*Gallus gallus*)

History:
Four birds from a backyard flock were losing weight and anorexic. This bird died suddenly.

Gross Pathology:
Necropsy was performed on a 3-month-old intact female chicken. It was dark brown with light speckling. The keel bone was prominent and pectoral muscles were atrophied. The eyes were sunken in the orbits. Loose white to green fecal material was present around the

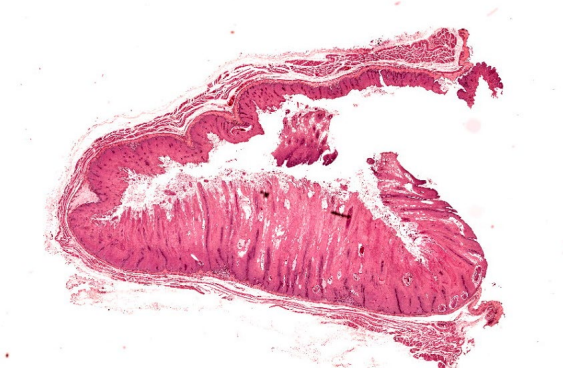

Figure 2-1. Crop, chicken. There is segmental and marked hyperplasia of the squamous mucosa of the crop (center bottom). (HE, 5X)

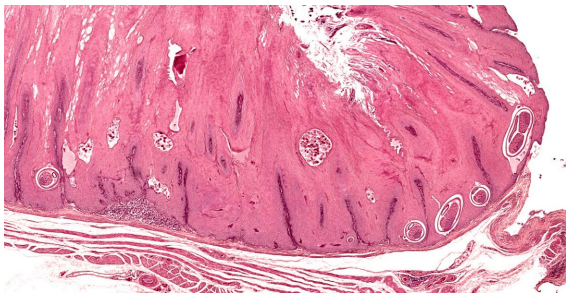

Figure 2-2. Crop, chicken. There are few cross and tangential sections of nematodes and eggs in tunnels within the hyperplastic mucosa. (HE, 37X)

cloaca and ventral tail feathers. Diffusely the skin was bright red.

Laboratory Results:
Fecal examination revealed few eggs (0-3/10X) of *Capillaria* sp., *Ascaridia* sp. and *Heterakis gallinarum*.

Microscopic Description:
Crop: Numerous cross-sections of nematodes are embedded within the markedly thickened mucosa. The parasites are 160-350 micrometers in diameter and contain an approximately 6 micrometer smooth cuticle, coelmyarian musculature, hypodermal bacillary bands, a body cavity, a multinucleated intestine and ovaries. There are bioperculated, oval embryonated eggs (*Capillaria* sp.) within the space occupied by the nematode, or in spaces by themselves within the mucosal epithelium.

Contributor's Morphologic Diagnosis:
Crop: Ingluvitis, proliferative, multifocal, moderate with intralesional nematode parasites, *Capillaria* sp.

Contributor's Comment:
The crop and esophageal mucosa is markedly thickened and contain numerous cross sections of parasites and eggs. The cuticle, digestive tract and body cavity qualifies these as nematode parasites. The bioperculated, oval embryonated eggs present in the parasite, within and on the surface of the mucosa are consistent with *Capillaria* sp. Similar nematode parasites and eggs were also present in the small intestine and double operculated eggs (*Capillaria* sp.) and eggs consistent with *Ascaridia* and *Heterakis gallinae* infections are present on fecal flotation. Although chickens can harbor many species of *Capillaria*, only *Capillaria annulatus* and *contortus* are present in both the crop and small intestine. As nematode parasites were not recovered during necropsy, definitive identification was not possible.

This chicken had a severely thickened crop mucosa and was malnourished as evidenced by the prominent keel bone and pectoral muscle atrophy. The loose fecal material around the cloaca and ventral tail feathers are consistent with diarrhea, which is often associated with heavy intestinal *Capillaria* infections. *Capillaria* infections are usually of little consequence in most chickens; however, when there are heavy infections in the crop

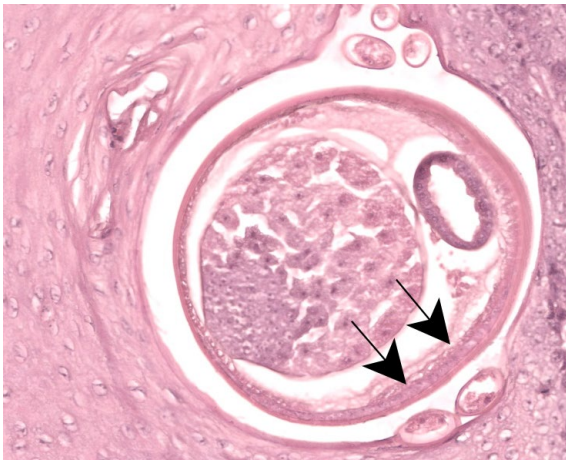

Figure 2-3. Crop, chicken. Cross section of an adult female aphasmid nematode with a smooth cuticle, pseudocoelom, bacillary band (arrows), a small intestine lined by uninucleate cells, and a cross section of an ovary. There are few eggs in the space surrounding the nematode. (HE, 382X)

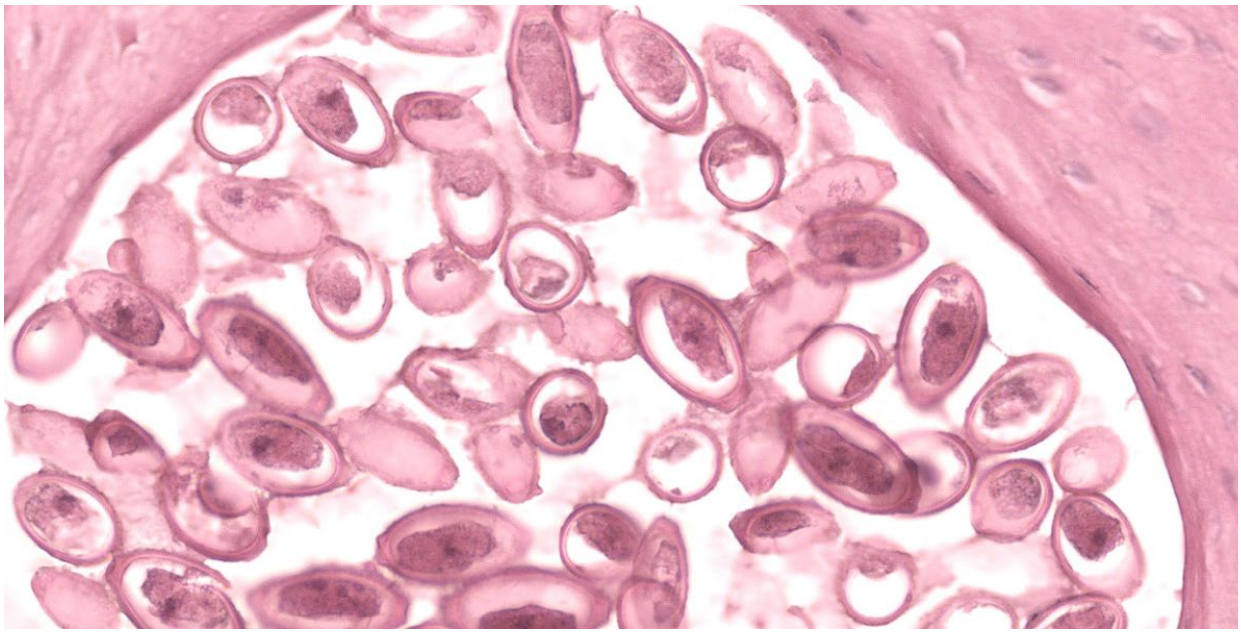

Figure 2-4. Crop, chicken. Numerous asymmetrically bioperculated eggs are present within tunnels in the hyperplastic mucosa. (HE, 680X)

and esophagus by *C. annulatus* or *contortus* (the most pathogenic *Capillaria* species) the mucosa can become so thickened that swallowing can be difficult to impossible. This can result in high mortality as was present in this case. All 4 chickens in this backyard flock died and had lesions similar to those in this chicken. As both *C. annulatus* and *contortus* are transmitted by earthworms, backyard flocks in humid environments are extremely susceptible. Death of this chicken was subsequent to heavy Capillaria infections of the crop, esophagus and small intestine.

Contributing Institution:
Tuskegee University
Pathobiology Department
https://www.tuskegee.edu/programs-courses/colleges-schools/cvm

JPC Diagnosis:
Crop, mucosa: Hyperplasia, segmental, marked, with few adult female aphasmid nematodes and numerous eggs.

JPC Comment:
Capillariasis is a common disease of free-range chickens. Some species within the *Capillaria* genus infect the upper digestive system, particularly the crop and esophagus, while others cause intestinal or cecal disease.[1] The adult worms are thin and filamentous, giving them the monikers "thread worms" or "hairworms," and produce characteristic bi-operculate eggs. These worms burrow into their preferred mucosal lining, typically without causing clinical signs, but with increasing numbers, severe inflammation, diarrhea, wasting, and death may occur.

The life cycles vary among the *Capillaria* species; some have a direct life cycle, while other require an intermediate host, most commonly an invertebrate such as an earthworm.[1] The most common *Capillaria* species that cause disease in chickens are *C. contorta*, *C. annulata*, *C. anatis*, *C. bursata*, *C. caudinflata*, and *C. obsignata*. As the contributor notes, of these, *C. annulata* and *C. contorta*

are found in the crop and esophagus, *C. anatis* occurs in the ceca, and the balance inhabit the small intestine. Depending on the species and life cycle, chickens are infected by ingesting litter containing worm eggs or by ingesting an infected intermediate host.

As in this case, the typical histologic lesion of crop capillariosis is thickening and inflammation of the mucosa, while intestinal and cecal-focused *Capillaria* species may cause inflammation, hemorrhage, and erosion of the gastrointestinal linings.

Conference discussion centered around histologic differences between the avian crop and esophagus and the difficulty in differentiating between the two locations. While differences appear to be species-specific, in general the crop has no or few glands compared to the esophagus, where glands are typically more numerous. Conference participants felt it was quite difficult to determine if examined section was esophagus or crop, and many participants felt they would have had to wing it were it not for the contributor's gross necropsy findings.

Conference participants also noted the very mild nature of the inflammatory infiltrate and postulated that this might be because larvae are typically restricted to the mucosa in crop capillariasis. Participants felt that the striking hyperplasia was the more important histologic finding and deserved pride of place in the JPC morphologic diagnosis.

References:

1. Swayne DE, Glisson JR, McDougald LR, et.al. *Diseases of Poultry*. 13th ed. Blackwell Publishing Ltd;2008:1162-1164.

CASE III:

Signalment:
4-year-old Holstein cow, bovine (*Bos taurus*)

History:
The cow developed progressive weight loss, recumbency, weakness, depression, and mild hyperthermia. Despite treatment with an antibiotic (ceftiofur), a non-steroidal anti-inflammatory drug (flunixin), a corticosteroid (dexamethasone), and a parenteral solution containing vitamins, glucose and amino acids, her clinical condition deteriorated over a few weeks, necessitating euthanasia due to poor prognosis. The cow was pregnant at approximately 5 months of gestation.

Gross Pathology:
Multifocally infiltrating/expanding the myocardium and extending into the adjacent endocardium and epicardium in the atria, interventricular septum and the left ventricular free wall, there were multiple, yellowish/tan, irregular mass-like foci of approximately 1 to 8 cm, with poorly defined borders. In the myocardium, some of the larger foci exhibited a central 1–2 cm area of necrosis, demarcated by an incomplete thin (1–2 mm) red rim/halo (hemorrhage). The mesenteric lymph nodes were markedly enlarged, measuring up to 15 x 8 cm.

Laboratory Results:
In 2019, the cow had tested positive for serum antibodies against bovine leukemia virus (BLV) by ELISA and for BLV by qPCR on a sample of whole blood (6.45 x 104 BLV genome copies/µL), and a complete blood count revealed leukocytosis (white blood cells: 14.7 x 109/L; reference range: 5.8–12.6 x 109/L) with lymphocytosis (10.07 x 109/L; reference range: 1.7–5.6 x 109/L). A sample of heart obtained during the autopsy also tested positive for BLV by qPCR (1 x 106 genome copies/mg).

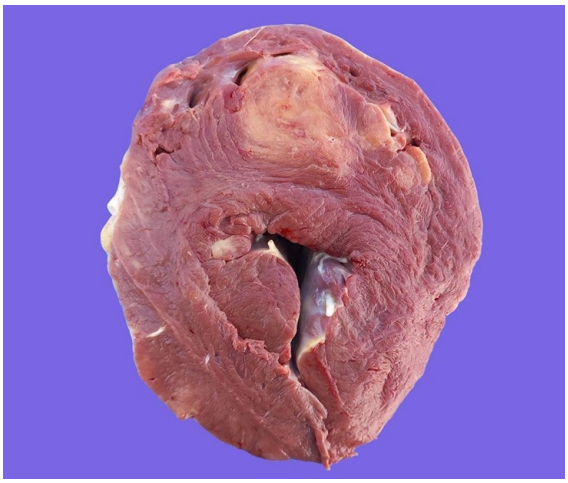

Figure 3-1. Heart, ox. Multiple, yellowish/tan, irregularly shaped, foci with indistinct borders ranging from 1 to 8 cm infiltrate the myocardium. The larger focus (upper center) involve the right aspect of the interventricular septum. (Photo courtesy of: Plataforma de Investigación en Salud Animal, Instituto Nacional de Investigación Agropecuaria (INIA), La Estanzuela, Uruguay).

Microscopic Description:
Two sections of heart are examined. Extensively infiltrating the myocardial interstitium including the endomysium, separating myocardial fibers, and multifocally infiltrating the endocardium and the conduction system, is an unencapsulated, densely cellular neoplasm composed of round cells arranged in sheets. Neoplastic cells are round to oval, with distinct borders, scarce cytoplasm, and high nuclear-to-cytoplasmic ratio. Nuclei are large, round, oval or reniform (moderate anisokaryosis), with coarse or clumped chromatin and 1 to 3 magenta nucleoli. The mitotic count is up to 8 per 400x field. Additionally, in one of the sections there is a fairly well-demarcated central area of necrosis that involves both the cardiomyocytes and the entrapped infiltrating neoplastic cells. In this area cardiomyocytes have hypereosinophilic and coagulated sarcoplasm, with loss of transverse striations (necrosis), neoplastic cells exhibit karyolysis, and there are occasional foci of erythrocyte extravasation (hemorrhages). Occasional intact, thick-walled, oval, basophilic, protozoal cysts morphologically resembling *Sarcocystis* spp. cysts are present within the sarcoplasm of cardiomyocytes.

Contributor's Morphologic Diagnosis:
Heart: Lymphoma, holstein, bovine.

Contributor's Comment:
The diagnosis of Enzootic Bovine Leukosis (EBL) in this cow was based on gross, microscopic, molecular (qPCR), and serological (ELISA) findings. The seroprevalence of BLV was high in the herd.

EBL is caused by BLV, genus *Deltaretrovirus*, family *Retroviridae*, which is closely related to primate T-lymphotropic viruses including human T-cell lymphotropic virus 1 and 2 and simian T-cell lymphotropic virus.[14,16] EBL is a contagious lymphoproliferative condition, and the most frequent neoplastic disease of cattle.[14] The disease has been reported in almost every cattle raising country during the last century.[12,14,16] However, to date, BLV has been successfully eradicated from over 20 countries, including several European countries, New Zealand, and Australia.[6,14,20] Thus, it is mainly present in eastern Europe, the Americas, and some Asian and middle eastern countries.[6,14] Most of these countries have reported continuous increases in BLV prevalence in beef and dairy cattle herds, although prevalence is usually higher in dairy farms.[1,14,16] While BLV has been linked to breast cancer in women, the eventual causal relationship between this virus and breast cancer is still a matter of debate in the scientific community.[2,5,8]

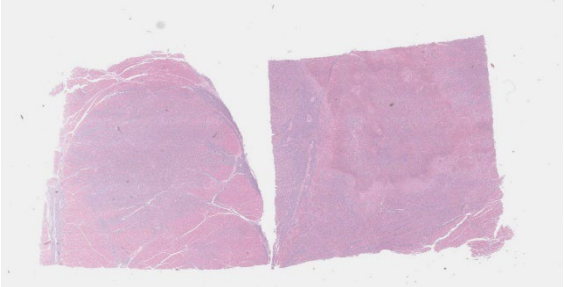

Figure 3-2. Heart, ox. Two sections of myocardium are submitted for examination.

BLV is harbored in circulating lymphocytes of infected cattle and can be transmitted horizontally and vertically; the former being the main form of transmission.[6,13,14,20] As fresh blood, semen, saliva, milk, and nasal secretions are known sources of BLV proviral DNA, direct contact, iatrogenic procedures, blood-sucking insects, and natural breeding are potential transmission routes.[6,14,20] Vertical transmission via transplacental infection or ingestion of colostrum/milk is thought to account for a small proportion of BLV infections.[13,14] Intrauterine transmission has been reported to occur in 4–8% of calves born to BLV seropositive dams.[6] Transmission through the ingestion of colostrum or milk from BLV-infected cows is possible since they both can contain provirus or free viral particles. However, colostrum and milk can also contain high titers of protective antibodies that could prevent infection in calves.[12,13] Quantification of the risk of this transmission route is currently lacking.[13]

BLV is capable of infecting different immune cells, with a predilection for B-lymphocytes.[16] Viral infection occurs after destabilization of the cell membrane by a transmembrane glycoprotein. Afterwards, the virus integrates into the host cell genome, interfering with gene expression and altering proliferative and apoptotic processes.[14] These mechanisms are responsible for the different clinical features of the disease in cattle. On one hand, animals suffering from benign persistent lymphocytosis develop a massive proliferation of B-lymphocytes as a consequence of a blockage of their apoptosis along with an increase of proliferation.[11] In contrast, neoplastic transformation is associated with inactivation of the tumor suppressor gene p53.[14]

Exposure to BLV may lead to four different outcomes: a) failure in establishing an infection, probably due to genetic resistance of the host and/or other factors; b) asymptomatic infection, which is characterized by detectable antibodies and no clinical or hematological abnormalities; c) persistent lymphocytosis with detectable antibody titers; and d) development of malignant lymphocytic neoplasia (lymphoma) in seropositive individuals.[6,12,14] In the case described herein, the cow had evidence of persistent lymphocytosis in 2019, and while she was asymptomatic at that time, whether she concurrently had subclinical lymphoma was not determined. It is believed that the outcome of the infection may be influenced by the genetic constitution of the individual, its immune status, and the infective dose.[6] The majority of infected cattle (70%) remain asymptomatic, while approximately 30% develop a benign lymphocytosis that persists for years, and it is not associated with the tumoral disease.[6,14,17] The development of lymphoma occurs in 1–5% of BLV-infected individuals usually after a latency period of 1–8 years, regardless of the development of persistent lymphocytosis, and is considered the only symptomatic form of EBL.[6,12,17] While lymphoma occurs regardless of the development of persistent lymphocytosis, it is estimated that approximately two thirds of the cows that develop

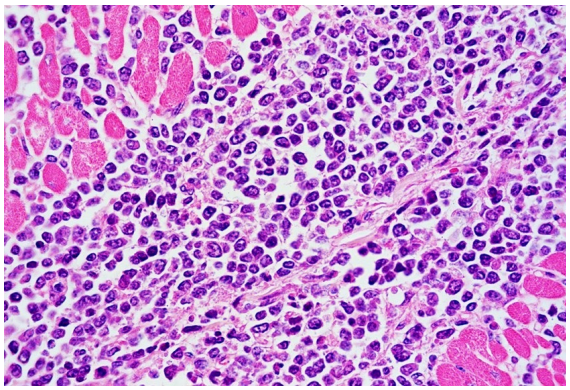

Figure 3-3. Heart, ox. Infiltrating the myocardial interstitium and separating individual cardiomyocytes is a densely cellular neoplasm of neoplastic lymphocytes arranged in sheets. (Photo courtesy of: Plataforma de Investigación en Salud Animal, Instituto Nacional de Investigación Agropecuaria (INIA), La Estanzuela, Uruguay). (HE, 200X)

BLV-induced lymphoma previously undergo persistent lymphocytosis.[11]

BLV-induced lymphoma may be located in any organ, but the abomasum, kidney, heart, spinal cord, uterus, and lymph nodes are the most commonly affected ones.[4,6] Therefore, the clinical presentation may vary depending on the organ or tissue involved.[4,6] Overall, the most frequent clinical findings consist of inappetence, weight loss, drop in milk production, pallor, weakness, recumbency, etc.[6,15] In the case presented here, although the heart was extensively affected, no signs or lesions compatible with congestive heart failure were detected, suggesting that extensive cardiac involvement may have occurred somewhat recently prior to clinical disease, or without significantly affecting cardiac function until a few weeks before euthanasia, when the cow became symptomatic. Additionally, because there was histologic evidence of neoplastic infiltration in the conduction system of the heart, as well as evidence of acute myocardial necrosis, we speculate that either one or both pathological findings could have precipitated the clinical deterioration in this case. Besides the heart and mesenteric lymph nodes, no other tissues were affected in this cow.

Macroscopically, lymphomas are yellowish, white to tan, bulging masses with a homogeneous aspect. Neoplastic tissue is usually firmer than normal lymphoid tissue, and it can be found surrounding bright yellow necrotic foci.[3,6] In the heart, two gross presentations, consisting of nodular and diffuse forms, have been described.[3] In the former, bulging lesions are located in the atrium and blend into the epicardial fat, thus making them hard to differentiate. Diffuse cardiac lesions are usually disseminated in the ventricular myocardium. Interestingly, our findings include both patterns since nodular lesions were present in the right atrium but there were also extensive lesions in the myocardium of the interventricular septum and left ventricular free wall.

Histological findings typically include dense cellular masses or diffusely infiltrative neoplasms composed of sheets of neoplastic round cells (B cells) that infiltrate, disrupt and/or replace the parenchyma of the affected organs.[10] Neoplastic cells exhibit scarce cytoplasm and distinct cell borders, and large, irregularly round nuclei. The mitotic index is usually high.[6]

Clinically, differential diagnoses for the cardiac presentation include various conditions including bacterial infections (*Histophilus somni*, abscess, and bacterial endocarditis), parasitic diseases (i.e. sarcocystiosis, cysticercosis), traumatic reticulopericarditis, and

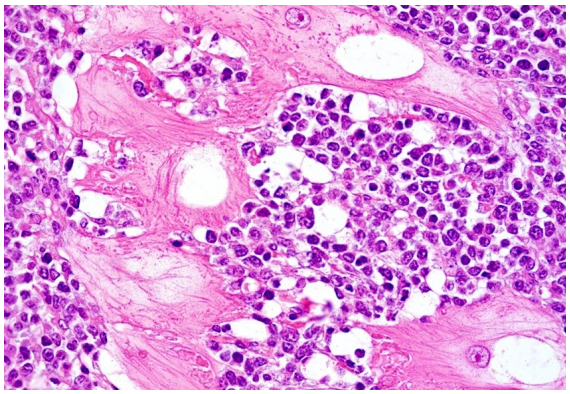

Figure 3-4. Heart, ox. Neoplastic cells infiltrate the cardiac conduction system, surrounding and separating Purkinje fibers. (Photo courtesy of: Plataforma de Investigación en Salud Animal, Instituto Nacional de Investigación Agropecuaria (INIA), La Estanzuela, Uruguay). (HE, 400X)

primary cardiac tumors (rhabdomyoma, rhabdomyosarcoma, peripheral nerve sheath tumors [neurofibroma, schwannoma, neurinoma, neurofibroma], hemangioma, hemangiosarcoma, fibroma, fibrosarcoma, angiolipoma, angioleiomyoma, leiomyoma, leiomyosarcoma, adenomatoid tumor, hamartoma, mesothelioma, and myxoma).[6,19] Enlargement of lymph nodes can be found in many other conditions, such as tuberculosis, paratuberculosis, caseous lymphadenitis, actinobacillosis, sporadic bovine leukosis (which is not caused by BLV), zygomycotic lymphadenitis, theileriosis, brucellosis, etc.[6] Histologically, lymphoma is readily distinguishable from all these conditions, except for sporadic bovine leukosis, which usually occurs in young calves instead of adult cattle.

Antemortem diagnosis of BLV exposure and infection can be accomplished by serologic (ELISA, agar gel immunodiffusion) and molecular methods (PCR, qPCR). Lymphoma can be diagnosed by cytology and/or histopathology coupled with viral detection by PCR in postmortem specimens.[6,14,20]

Contributing Institution:
Plataforma de Investigación en Salud Animal
Instituto Nacional de Investigación Agropecuaria (INIA)
La Estanzuela, Uruguay
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JPC Diagnoses:
1. Myocardium: Lymphoma.
2. Myocardium: Sarcocysts, few.

JPC Comment:
As the contributor details, aggressive efforts have led to the eradication of EBL in many countries; however, this fact belies the difficulty of eradication, largely due to the limited ability to identify "aleukemic" animals who are infected but subclinical.[7] To that end, much of the current research surrounding EBL is focused on developing effective diagnostics that can be used to identify and remove asymptomatic animals from herds.

Among these new diagnostics are droplet digital PCR (ddPCR) assays that can be used to identify and quantify BLV provirus (viral DNA that has been integrated into the host genome) in animals with recent infection or low proviral load.[7,15] ddPCR is a relatively recent nucleic acid quantification modality that has several benefits over PCR and quantitative PCR, including 1) the ability to provide quantitative counts without the need for a reference curve, and 2) the ability to provide reproducible quantitative data in unpurified samples that contain minute amounts of target DNA.[7] Recent research shows that ddPCR can detect proviral DNA as early as 2 days post-infection, much earlier than the 13 days post infection with traditional PCR-based methods.[7] ddPCR is also able to detect and quantify provirus in individual and bulk

tank milk, an important diagnostic advantage allowing testing of easily accessible samples that have far lower levels of provirus and far higher levels of contaminates than required or allowed with legacy PCR techniques.[7]

The key innovation of the ddPCR technique is the fractioning of DNA samples into tens of thousands of individual droplets using an oil and water technique. Once the sample is fractionated, the PCR reaction is carried out in each individual droplet. Once the PCR reaction has terminated, the fluorescent properties of each individual droplet are analyzed in a method similar to flow cytometry, and the number of droplets that contain sample is used to quantify the original amount of proviral DNA.[9]

It is hoped that ddPCR quantification of proviral DNA levels in whole blood or milk may be a good indicator of disease and disease progression in the field.[18] The quantification of proviral DNA can also inform herd management decisions as animals with low proviral loads are less effective virus transmitters and thus can be segregated, but need not necessarily be culled, to prevent disease transmission to naïve animals.[7]

The most recent research into BLV diagnostics has innovated on the ddPCR proviral assay. Previous research has identified an association between the bovine MHC-DRB3 gene and proviral load in certain species of cattle, with one allele associated with a high proviral load (a BLV susceptibility gene) and another allele associated with a low proviral load (a BLV resistant gene). This year, researchers developed a single-well assay that combines the ddPCR proviral quantitative assay with allele typing, including identification of homo- and heterozygotes, to provide a more holistic view of which animals are at risk of spreading disease.[15] These diagnostic innovations could soon provide a pathway for economically viable eradication efforts that minimize culling, even in areas of high disease prevalence.

This case presents a classic demographic, clinical, gross, and histologic case of BLV-induced lymphoma. In a heart with this gross appearance, lymphoma should be at the top of the differential list, though neurofibroma and granulomatous disease can't be definitely ruled out prior to histologic evaluation. Conference participants discussed the curious subgross appearance of the examined tissue section, which contains a large confluent, well-demarcated area of pallor reminiscent of an infarct; however, histologically, while neoplastic cells in this area are often necrotic, the cardiomyocytes appear viable. No conclusions were reached as to the origin of this unique appearance.

The scattered sarcocysts are a common, incidental finding in cattle and are most commonly tissue cysts of *Sarcocystis cruzi*.

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CASE IV:

Signalment:
3-year-old, Holstein bull (*Bos taurus*)

History:
A Holstein bull with a history of chronic weight loss and wasting was slaughtered on-farm for human consumption. Since multiple

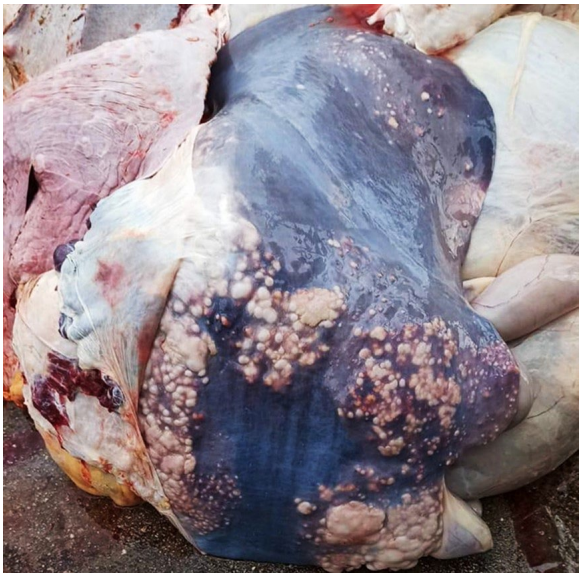

Figure 4-1. Liver, ox. Multiple coalescing, irregularly round, 0.5 to 5 cm granulomas protrude from beneath the hepatic capsule. (Photo courtesy of: Nacional de Investigación Agropecuaria (INIA), Route 50, Kilometer 11, La Estanzuela, Colonia 70006, Uruguay).

lesions were found in the liver, lung, bronchial and hepatic lymph nodes, the practitioner decided to submit these samples for diagnostic workup.

Gross Pathology:
The liver, lungs, and hepatic and bronchial lymph nodes had multiple granulomas. In the liver, these granulomas were multifocal to coalescing, roughly round, nodular, firm, and were readily visible protruding from the diaphragmatic surface of the capsule. The granulomas ranged from 0.5 to 5 cm in diameter and were frequently surrounded by a pearly white capsule of connective tissue (fibrosis). On cut surface, the granulomas frequently contained a yellowish central area of caseous material (necrosis) and crepitated when sliced with the blade of the knife, suggesting mineralization.

Laboratory Results:
Mycobacterium bovis was isolated from fresh samples of lung and bronchial lymph node inoculated in egg yolk agar. *Mycobacterium bovis* DNA was amplified by qPCR from frozen samples of hepatic and bronchiolar lymph nodes.

Microscopic Description:
Liver: approximately 50% of the parenchyma is replaced by multiple granulomas. The granulomas are composed of a pale eosinophilic center with cellular debris (necrosis) and scattered deposits of coarse basophilic or amphophilic amorphous to crystalline material (mineralization). These necrotic and mineralized centers are surrounded and infiltrated by inflammatory cells, notably epithelioid macrophages, occasionally forming multinucleated giant cells with peripherally located nuclei (Langhans type), and fewer lymphocytes and plasma cells. The granulomas are frequently surrounded by a thick capsule of collagenous fibrous connective tissue. The hepatic parenchyma in adjacent areas is displaced and partially compressed by the granulomas. In these regions there is distortion/disruption of the hepatic cord histoarchitecture, hepatocytes are atrophic, the sinusoids are expanded by connective tissue (fibrosis), with variable degrees of portal, bridging, dissecting and perivenular fibrosis. In these areas there are also scattered infiltrates of lymphocytes, macrophages, and multinucleated giant cells. Portal areas also exhibit moderate bile duct hyperplasia. Intracellular, 2-µm long, acid fast bacilli were detected in multinucleated giant cells in histologic sections of liver stained with Ziehl-Neelsen.

Contributor's Morphologic Diagnosis:
Liver: Hepatitis, granulomatous, multifocal to coalescing, chronic, severe with caseous necrosis and mineralization, multinucleated

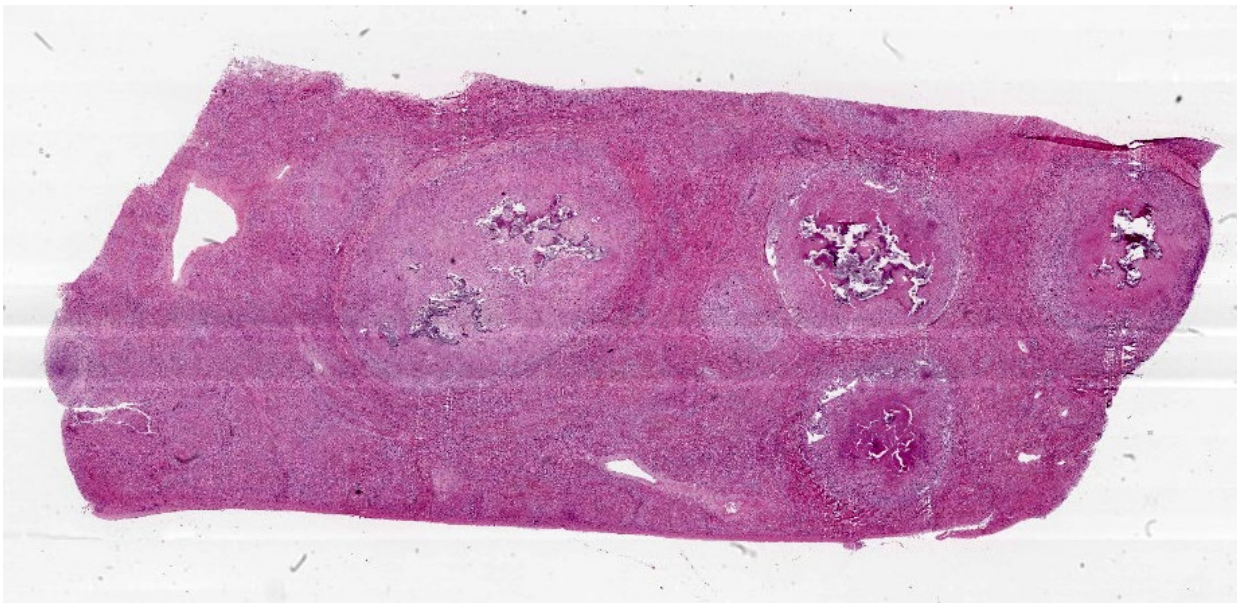

Figure 4-2. Liver, ox. One section of liver is submitted for examination. There are several discrete granulomas effacing approximately 50% of the section. (HE, 5X)

giant cells, intra-histiocytic acid-fast bacilli, and fibrosis, holstein bull, bovine.

Contributor's Comment:
The diagnosis of bovine tuberculosis in this case was mainly based on pathological findings coupled with the identification of acid-fast bacilli by Ziehl-Neelsen, and isolation and molecular identification of *Mycobacterium bovis*.

Mycobacteria are facultative intracellular, aerobic, non-motile, pleomorphic bacilli.[8,16] They resist decolorization by acids during staining procedures because of the high content of lipids of their cell walls. Hence, they are considered acid-fast bacteria and can be stained with Ziehl-Neelsen or Fite-Faraco stains, but not with Gram stains.[17] The mycobacteria that cause tuberculosis are grouped within the Mycobacterium tuberculosis complex (MTBC), which includes *M. tuberculosis*, *M. bovis*, *M. caprae*, *M. africanum*, *M. microti*, *M. canettii*, *M. pinnipedii*, *M. orygis*, *M. suricattae*, *M. mungi*, and the Dassie and Chimpanzee bacilli.[3,11] Within this extensive group, only two members are confirmed causes of bovine tuberculosis: *M. bovis* and, to a lesser extent, *M. caprae*.[6,12]

Although bovine tuberculosis has a worldwide distribution, its prevalence has declined significantly in many regions, and approximately 20 countries (mainly from Europe) are considered tuberculosis-free as a result of rigorous eradication programs.[2,5] Similar eradication programs are currently being implemented in other countries, such as the US, Mexico, New Zealand, and Japan.[5] The disease is still widely spread in Africa, Asia, and Central and South America.[2,5]

Mycobacteria are very resistant to environmental conditions, which facilitates indirect infection from contaminated sources.[9] However, direct transmission from infected animals is the main route of infection.[9] Inhalation of aerosols containing mycobacteria is the primary route of transmission in cattle.[6,9] *Mycobacterium bovis* can also be excreted in feces, urine, and colostrum/milk; hence, contaminated feedstuff and water are potential sources of infection. Oral transmission requires a large infective dose.[6,9] In young

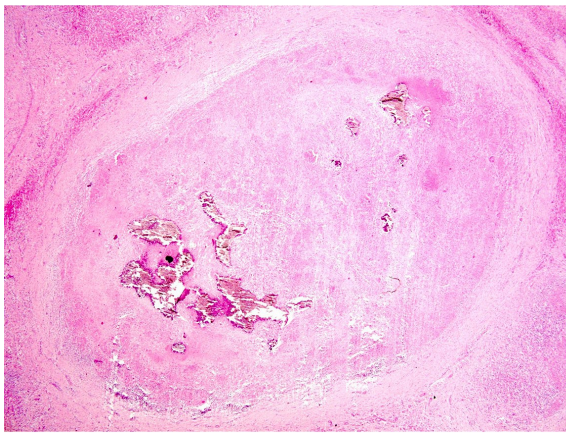

Figure 4-3. Liver, ox. Replacing the hepatic parenchyma is a granuloma with a central area of caseous necrosis and mineralization surrounded by a thick capsule of connective tissue. (Photo courtesy of: Nacional de Investigación Agropecuaria (INIA), Route 50, Kilometer 11, La Estanzuela, Colonia 70006, Uruguay). (HE, 100X)

calves, infection through ingestion of unpasteurized colostrum/milk from infected cows is possible.[9] Other uncommon infection sources and routes include cutaneous exposure, congenital infection, or transmission through semen, vaginal and uterine discharges, among others.[6]

Bovine tuberculosis spreads throughout the organism in two stages: i) the primary complex and ii) post-primary dissemination. After lodging in the lesion inflicted at the entry site, mycobacteria are transported to the draining lymph node(s) where they establish a secondary infectious focus, generating the primary complex.[7] When both lesions (at the entry point and the local lymph node) are present, the primary complex is classified as complete, and when the lesion at the site of entry is absent (as in most cases occurring after digestive transmission) the primary complex is referred to as incomplete.[7] Depending on the immune status of the host, dissemination from the primary complex (post-primary dissemination) may occur either via lymphatic or hematogenous spread, or through pre-existing anatomical routes in the organs. Each of these forms of spread will determine different post-primary presentations: late generalization or chronic organ tuberculosis.[6,7] Another presentation is called early generalization, which occurs due to the infection's spread during the early stages as a consequence of a poor immune response.[7]

The clinical presentation usually varies with the localization of the infection. Overall, clinical signs are nonspecific (i.e., progressive weight loss, fluctuating fever, weakness, inappetence), thus the diagnosis should not be based on clinical examination alone. It should be considered that cattle with extensive miliary lesions may be clinically normal.[5,6]

The characteristic gross lesion is the tubercle, a circumscribed, often encapsulated, tan to yellow granuloma, often with central caseous necrosis and/or mineralization, which has been referred to as a "caseocalcareous granuloma".[4] There is large variation in the size of the tubercles; they can be small enough to be overlooked during autopsy, or involve a major part of an organ.[18] Tubercles are most frequently seen in bronchial, retropharyngeal, and mediastinal lymph nodes.[4] Other sites commonly affected include the lung, liver, spleen, and the serosal surfaces of body cavities.[18] Other locations, including the brain and meninges, have also been described.[14]

Histologically, small granulomas consist of epithelioid macrophages and few Langhans-type multinucleated giant cells and neutrophils.[4,7,11] As the lesion progresses, a central area of caseous necrosis, consisting of eosinophilic homogeneous material with necrotic cell debris and mineralization develops.[4,7,11] This necrotic core is surrounded by macrophages, multinucleated giant cells, lymphocytes, and occasional neutrophils.[4,7,11] Over time, a fibrous connective tissue capsule is

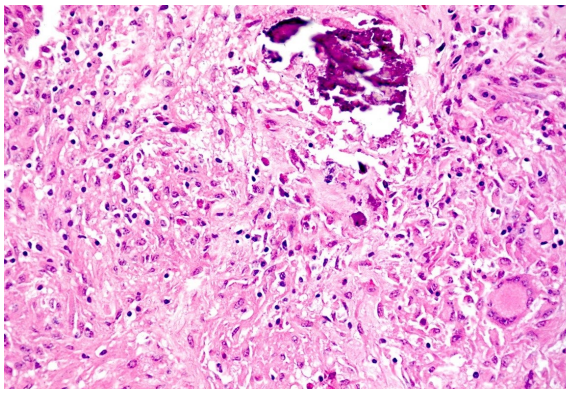

Figure 4-4. Liver, ox. There is infiltration by abundant macrophages that occasionally form multinucleated giant cells with peripherally located nuclei, and fewer lymphocytes, scattered mineralization, and fibrosis (Photo courtesy of: Nacional de Investigación Agropecuaria (INIA), Route 50, Kilometer 11, La Estanzuela, Colonia 70006, Uruguay). (HE, 400X)

formed. Acid-fast bacilli may be found extracellularly in the necrotic core or within the cytoplasm of macrophages and giant cells.[7,11] The absence of acid-fast bacilli on histopathological examination does not rule out tuberculosis.[7,18]

Differential diagnoses on clinical or gross pathology grounds include many conditions such as mycobacteriosis associated with the M. avium-intracellulare complex, atypical mycobacteria, bacterial pneumonia and lung abscesses (i.e. *Mycoplasma bovis*, *Pasteurella multocida*, *Trueprella pyogenes*, aspiration pneumonia), actinobacillosis, fungal diseases (zygomycosis, blastomycosis, aspergillosis, histoplasmosis, coccidioidomycosis), foreign body granuloma, traumatic pericarditis, bovine leukemia virus, and cestode cysts.[5,6,10] However, histologically, tuberculoid granulomas are usually easily distinguishable from all these conditions.

Diagnostic assays include direct tests for the identification of the agent, generally in postmortem specimens, such as microscopic examination, culture, immunohistochemistry, and molecular techniques.[6,18] Indirect tests are performed on specimens from live animals to detect infected individuals, and can be based on the cellular (intradermal tuberculin test, gamma-interferon test, and lymphocyte proliferation test) or humoral immunity (ELISA).[6.18]

Contributing Institution:
Plataforma de Investigación en Salud Animal
Instituto Nacional de Investigación Agropecuaria (INIA)
La Estanzuela, Uruguay
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JPC Diagnosis:
Liver: Granulomas, multifocal to coalescing, with bridging fibrosis.

JPC Comment:
As noted by the contributor, the classic lesion of tuberculosis is the tubercle, or tuberculous granuloma. Though a seemingly simple structure, the presence of a granuloma is the result of a complex sequence of inflammatory events requiring 1) the presence of an inciting agent with indigestible, poorly degradable, and persistent antigens, 2) a Th1 host immune response, and 3) an interplay between cytokines, chemokines, and other inflammatory mediators within the chronic inflammatory lesion.[1]

Granulomas are classically described as forming via a Type IV, cell-mediated hypersensitivity reaction. Sensitization occurs upon initial exposure to the antigen, which forms antigen-specific T memory lymphocytes. With chronic exposure, here enabled by the virulence factors that allow *Mycobacterium bovis* to live within macrophages indefinitely, memory T lymphocytes recognize these persistent antigens presented in MHC II complexes by macrophages, and initiate a

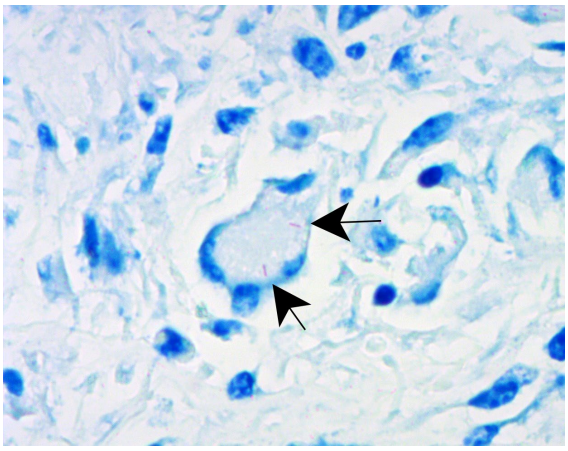

Figure 4-5. Liver, ox. Two ~2-µm long acid-fast bacilli (arrows) compatible with Mycobacterium bovis are present within the cytoplasm of a multinucleated giant cell. (Photo courtesy of: Nacional de Investigación Agropecuaria (INIA), Route 50, Kilometer 11, La Estanzuela, Colonia 70006, Uruguay). (Ziehl-Nielsen, 400X)

complex inflammatory sequence. Th1 lymphocytes secrete cytokines, such as TNF and IL-17, which recruit monocytes from the circulation to the site of inflammation. Once in the tissue, Th1-lymphocyte derived IFN-ɣ converts the monocytes to activated macrophages. The activated macrophages then secrete cytokines such as IL-12 which facilitate further development of Th1 lymphocytes.[15] Activated macrophages also elaborate IL-1 and TNF-α, both of which act to increase local expression of adhesion molecules on endothelial cells, thus recruiting more leukocytes to the inflammatory party. Meanwhile, Th1 lymphocytes secrete IL-2, which acts in an autocrine fashion to induce Th1 lymphocyte proliferation.[15]

The result of this persistent, Th1 lymphocyte-driven inflammation is the granuloma, which functions to wall off persistent antigenic stimuli from the rest of the body and is typically described as paucibacillary due to the few identifiable acid-fast bacteria. There are only a few conditions that are associated with the formation of granulomas, so their presence provides the astute pathologist with a radically pruned differential list.

In contrast to this nodular granulomatous morphology, certain mycobacterial conditions are associated with diffuse/lepromatous inflammation which is paradigmatically associated with a Th2 lymphocyte-predominant response. Lepromatous responses are poorly delineated, have a widespread distribution, abundant bacteria, relatively fewer lymphocytes, many macrophages, and lack a distinct capsule.[1]

In lepromatous immunological response, Th2 lymphocytes elaborate IL-4, IL-10, and TGF-β which inhibit the Th1 response and inactivate the microbicidal responses of macrophages, most notably iNOS. This facilitates the survival of the bacilli and shuts down the interplay between lymphocytes and macrophages that lead to granuloma formation.[13] The resulting diffuse granulomatous inflammation is seen in *Mycobacterium leprae* infection in humans and *Mycobacterium avium* subsp. *paratuberculosis* infection (Johne's disease) in cattle, sheep, and goats.[1]

This case rounds out this week's tour of classic entities with excellent examples of paradigmatic, Th1-driven tubercle formation. Conference participants felt the tubercles were so paradigmatic that a morphologic diagnosis of "granulomas" encapsulated a host of implied histologic features, including granulomatous inflammation, mineralization, necrosis, and concentric fibrosis. Conference participants discussed the striking bridging fibrosis and thought the consequent ischemia could account for the diffuse hepatocellular atrophy observed in less affected areas of the hepatic parenchyma.

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