Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2011-2012

Conference 15

01 February 2012

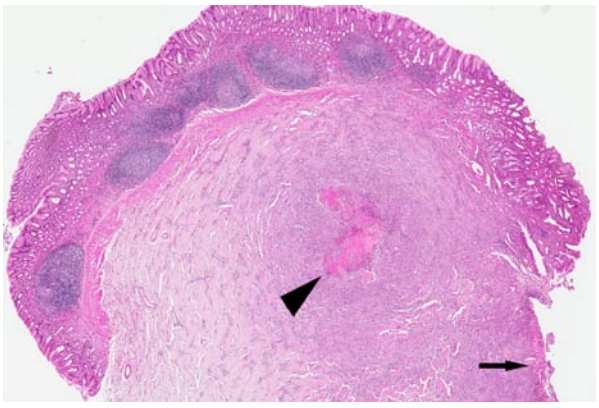
CASE I: 11180095 (JPC 4003099).

Signalment: Cat (*Felis silvetris catus*), 12-year-old spayed female mix breed.

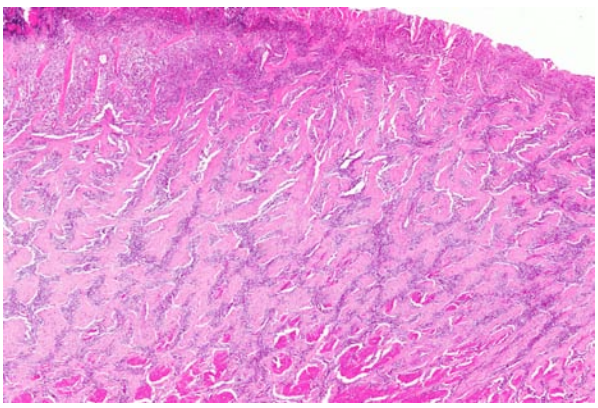
History: A 12-year-old mix breed cat was referred with a two-month history of persistent vomiting and loss of weight. At palpation an abdominal mass was detected. Ultrasound showed a heterogeneous and thickened gastric wall. Laparotomy revealed an intramural ulcerated mass close to the pyloric sphincter that was completely removed. The cat was still alive 5 months after surgical resection.

Gross Pathology: Gross examination of surgical biopsy showed a severely ulcerated mucosa with a diffuse and severe thickening of the gastric wall that appeared grayish.

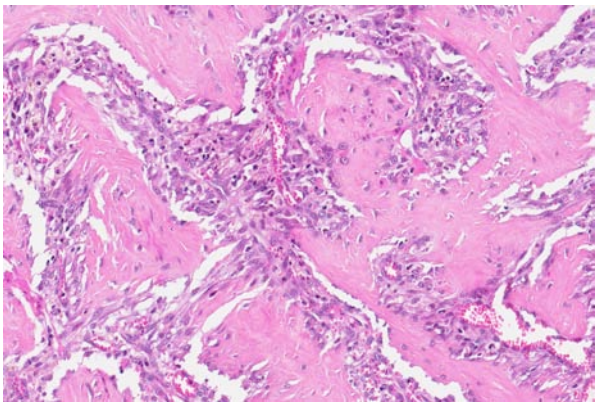
Contributor's Histopathologic Description: The histological appearance of the gastric lesion consisted of an ulcerated and fibrinous exudative mass expanding and replacing the wall at the pyloric/fundic junction. The lesion affected the inner layers (*mucosa* and *tunica muscularis)* of the gastric wall and consisted of branching and anastomosing trabeculae of dense collagen separated by a densely cellular population of spindle-shaped cells (sclerosing fibroplasia). The trabecular collagen merged into



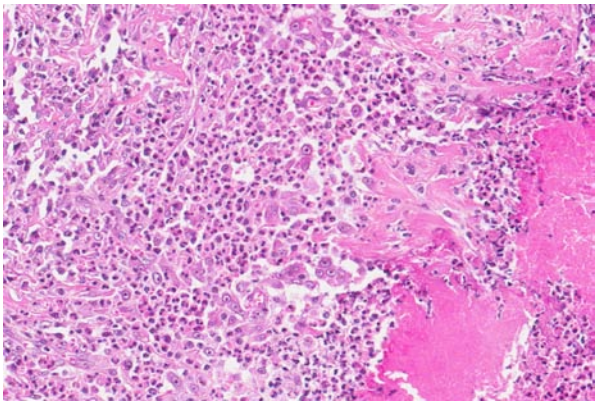
1-1. Stomach, cat. Section of stomach with lymphoid follicles in mucosa, lage area of mucosal ulceration (arrow), and large focus of Splendore-Hoeppli material in the muscular tunic. (HE 6.3X)



1-2. Stomach, cat. Retiform pattern of dense sclerosing fibroplasia effacing the ulcerated mucosa (top), submucosa, and muscular tunic (bottom). (HE 24X)



1-3. Stomach, cat. Higher magnifiacation of dense fibrous connective tissue separated by highly vascular granulation tissue. (HE 196X)



1-4. Stomach, cat. Scattered throughout the dense fibrous connective tissue are foci of large numbers of eosinophils and aggregates of brightly eosinophilic Splendore-Hoeppli material (at right). These areas also contain moderate numbers of macrophages and mast cells. (HE 6.3X)

typical granulation tissue at the periphery of the lesion. The fibroplasia contained variably dense infiltrates of eosinophils, mast cells, and fewer neutrophils, lymphocytes, and plasma cells. Multifocally there were necrotic foci with intralesional bacterial colonies and prominent eosinophils.

Contributor's Morphologic Diagnosis: Stomach: gastric eosinophilic sclerosing fibroplasia.

Contributor's Comment: We report a lesion in which eosinophilic inflammation is the predominant feature and which appears to be limited to the gastrointestinal tract. The presentation is typical of a feline gastrointestinal eosinophilic sclerosing fibroplasia as previously described.[1] Grossly, the lesion is an ulcerated mass at the pyloric sphincter or ileocecocolic junction which, due to the presence of dense sclerotic fibroplasias, can be clinically misdiagnosed as a neoplasm. Microscopically, the characteristic trabecular and anastomosing pattern of dense collagen which resembles osteoid, and the presence of numerous mast cells may lead to the incorrect diagnoses of osteosarcoma or sclerosing mast cell tumor. In the present case, bacteria were detected histologically at the center of necrotic foci enclosed in the submucosa. In a previous study of 25 cats[1] with similar eosinophilic sclerosing lesions, 15 had intralesional bacteria; Gram-negative rods were present in most of the lesions. On the contrary in Ozaki's *et al* study[6], gram-positive cocci (specifically, methicillin-resistant *Staphylococcus aureus*) were the most frequent. The significance is unknown. One hypothesis is to consider that bacterial organisms could initiate these lesions. However, the pyloric sphincter and ileocecocolic junction locations suggest that bacterial action could be combined with physical forces, such as mucosal foreign-body penetration. The reasons for the fibroplasia and eosinophilic response are not clear. Activated eosinophils can produce numerous mediators such as TGF-ß and IL-1 that can play a role in fibrosis by increasing extracellular matrix and fibroblast proliferation. The prominent eosinophilic infiltration in cats occurs as part of the feline eosinophilic granuloma complex or in response to a variety of stimuli, including parasites, viruses, bacteria, and fungi. *Toxoplasma gondii* was reported to cause eosinophilic fibrosing gastritis in cats.[5] An inherited eosinophilic dysregulation was also suspected in cats with eosinophilic granuloma complex, and in a recent study[1], hypereosinophilia was reported in 58% of tested cats, suggesting that genetically predisposed cats could develop eosinophilic inflammation in response to the penetration of bacteria into the gastro-intestinal wall secondary to mucosal foreign-body penetration.

JPC Diagnosis: Stomach: Necrosis, focally extensive, with marked fibrosis and eosinophilic and histiocytic inflammation.

Conference Comment: The etiopathogenesis of feline gastrointestinal eosinophilic sclerosing fibroplasia (ESF) remains controversial, with various hypotheses including sclerosing mast cell tumor (sMCT) and abcess-forming inflammatory granulation tissue with eosinophilic infiltration. It is also possible that ESF represents the common end point of any of a number of neoplastic or inflammatory processes; thus it is important to be aware of the potential differential diagnosis and diagnostic dilemmas presented by ESF.[1,2,3,4,7]

Conference participants agree with the contributor in this case and favor the diagnosis of feline gastrointestinal eosinophilic sclerosing fibroplasias (ESF), likely due to some previous inflammatory condition in the gastrointestinal tract. There is some significant slide variation, and some sections may not contain identifiable pylorus.

sMCT and ESF have several areas of major difference, including location, clinical pathology, clinical findings, survival time, and the presence of bacteria within the lesions. In sMCT, 76% of the lesions were located in the small intestine compared to only 16% for ESF. The majority of ESF cases are located mainly at the pyloric sphincter of the stomach as in this case (48%) or the ileocecocolic junction (24%). 58% of ESF had a peripheral eosinophilia, which was not reported in sMCT, and 100% of ESF had a palpable mass, compared to only 17% with sMCT. While both conditions showed spread to local lymph nodes, this only occurred in 28% of ESF cases as compared to 66% with sMCT, and there was metastasis to the liver in 66% of sMCT cases and no report of hepatic spread with ESF. Grossly, ESF presented as an ulcerated mass expanding the wall of the stomach or intestine and microabscesses or necrotic foci with cultured bacteria were present in 56% of cases and in all ileocecocolic and colonic cases, while sMCT presented as a marked transmural expansion of the small intestine by a firm, homogenous tan mass with abrupt mucosal ulceration in 58% of cases with no intralesional bacterial colonies and occasional surface colonies in ulcerated areas. The survival time with sMCT was much lower with an average of 92% of patients dead within 2 months of diagnosis compared to a variable survival time with ESF, depending on different circumstances, such as intestinal perforation or euthanasia, although cats treated with prednisone had a significantly higher survival time of up to 2 years.[1,2,3,4,7]

There are areas of similarity with these two conditions which gives rise to much of the controversy with these entities. In both conditions, there is a marked trabecular pattern of dense collagen, a heavy infiltrate of eosinophils, and the presence of mast cells (either the neoplastic component or an infiltrate of physiologic mast cells). Also, there is spread to local lymph nodes in both cases, which present as diffuse effacement of the lymph node architecture by dense collagenous trabeculae and multifocal microabscesses with ESF, and replacement of nodal architecture by neoplastic mast cells in sMCT. Both conditions have a fairly low mitotic rate, with 1 per 20 400x high power fields with sMCT and a variable number in ESF. Clinical signs were similar between each condition, which is expected, including similar rates of presentation for vomiting and weight loss.[1,2,3,4,7]

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CASE II: 09-126 (JPC 3134341).

Signalment: Eight-year-old male golden retriever, *Canis familiaris.*

History: This dog presented in respiratory distress with a left sided systolic heart murmur and a lifelong history of generalized weakness, difficulty eating, and abnormal stance with hyperextended carpi, hock flexion, and abduction of all 4 paws. Frequent multiform ventricular premature complexes were noted during cardiology exam, as were periods of non-sustained ventricular tachycardia. This animal had survived previous bouts of both aspiration pneumonia and congestive heart failure and was treated for both based on echocardiographic and radiographic findings. The animal died during hospitalization.

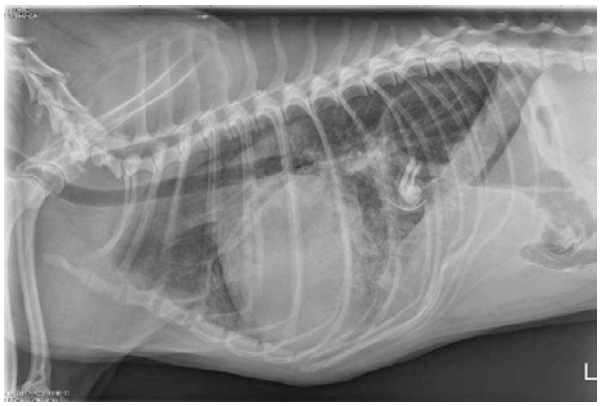
Gross Pathology: The animal exhibited marked symmetrical atrophy of the masticatory, paravertebral, and proximal limb muscles resulting in pronounced bony prominences. The right middle and ventral aspects of both caudal lung lobes were red-purple, firm, and rubbery. Sections from the right middle lobe sank in 10% formalin. The heart exhibited marked dilation of both ventricles and atria and multifocal, white-tan streaks within the myocardium, especially within the interventricular septum near the apex. The muscular portions of the diaphragm were markedly thickened up to 1 cm in cross section. The proximal third of the stomach was displaced cranially into the thorax through a thick, fibrous, lax, tendinous portion of the diaphragm. The liver was enlarged and dark red with rounded margins.

Contributor's Histopathologic Description: Heart: The myocardium contains broad swaths of degenerating myofibers that contain abundant granular, basophilic material (mineral) and is multifocally replaced by coalescing bands of fibrous connective tissue with variable numbers of admixed fibroblasts and lesser amounts of mature adipose tissue. Myofibers in areas of mineralization are typically hypereosinophilic with flocculent to fragmented sarcoplasm and pyknotic nuclei (degeneration and necrosis). Small numbers of neutrophils and macrophages are occasionally present at the periphery of these mineralized areas.

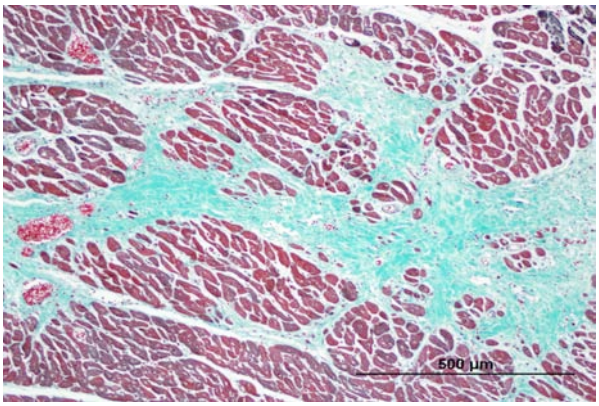
Diaphragm: The diaphragm is markedly thickened. Myofiber numbers are diffusely and markedly reduced while remaining myofibers are individualized by marked fibrosis and adipose tissue deposition. Myofibers vary markedly in diameter and frequently exhibit loss of cross striations, hyalinization, and fragmentation (degeneration). Scattered myofibers contain abundant granular basophilic material (mineral) within the sarcoplasm.

Contributor's Morphologic Diagnosis: 1. Heart: myocardial mineralization and necrosis, multifocal to coalescing, moderate to marked, with moderate fibrosis.
2. Diaphragm: myocyte necrosis and loss, multifocal to coalescing, marked with marked fibrosis and fat replacement.

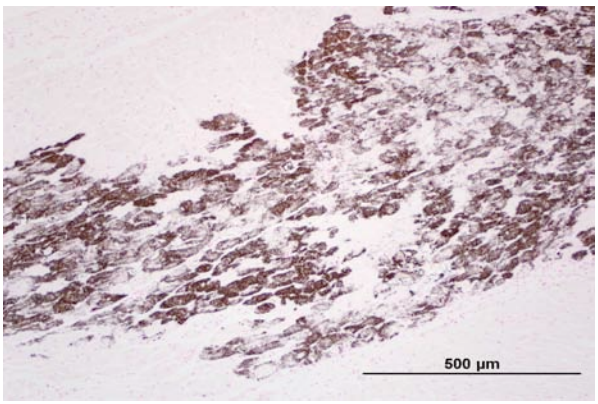
Contributor's Comment: The patient's widespread muscle necrosis with replacement by adipose tissue and fibrous connective tissue is consistent with Golden Retriever Muscular Dystrophy (GRMD), an animal



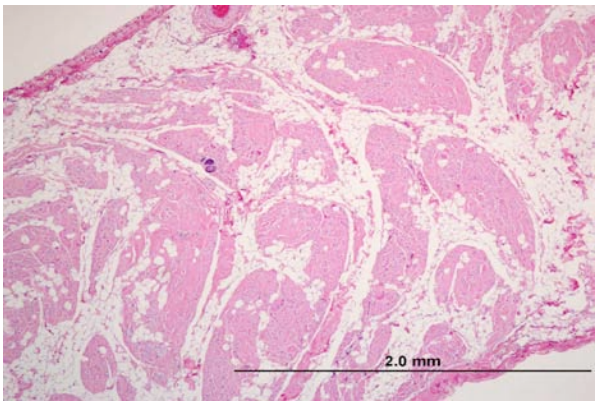
2-1. Lateral radiograph indicates generalized cardiomegaly with left atrial enlargment and an unstructured interstitial pattern that coalesces into a patchy alveolar pattern in caudoventral lung fields that is consistent with cardiogenic edema and/or pneumonia. The cranial half of the stomach is present within the thorax consistent with a hiatal hernia. Gas and a small amount of barium are present within the stomach. Photograph courtesy of the Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough Street, Raleigh, NC 27606 http://cvm.ncsu.edu/dphp/index.html



2-2. Heart, dog. Coalescing bands of fibrous connective tissue separate the cardiac myofibers. (Masson's Trichrome) Photograph courtesy of the Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough Street, Raleigh, NC 27606 http://cvm.ncsu.edu/dphp/index.html



2-3. Heart, dog. Calcium deposition within broad swaths of myofibers is highlighted. (Von Kossa) Photograph courtesy of the Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough Street, Raleigh, NC 27606 http://cvm.ncsu.edu/dphp/index.html



2-4. Diaphragm, dog. Myofiber numbers are markedly reduced. Remaining myofibers are individualized by marked fibrosis and adipose tissue deposition. A few myofibers contain abundant granular basophilic material (mineral) within the sarcoplasm. (HE) Photograph courtesy of the Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough Street, Raleigh, NC 27606 http://cvm.ncsu.edu/dphp/index.html

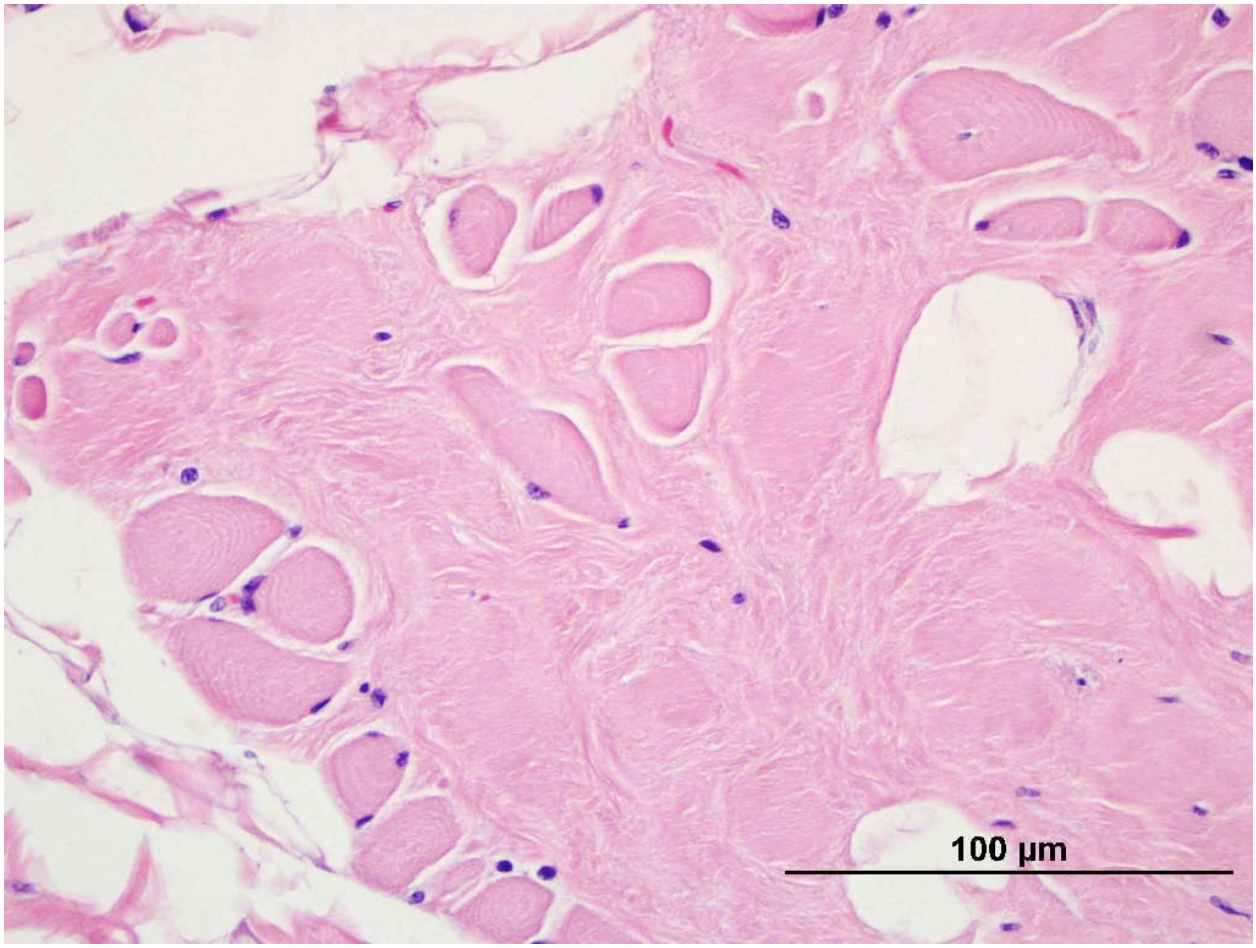
model for the human condition, Duchenne Muscular Dystrophy (DMD).[10-12] Both DMD and GRMD are the result of X-linked deficiencies in the cytoplasmic protein dystrophin. This protein is responsible for linking cytoplasmic actin to the transmembrane complexes of the sarcoglycans and dystroglycans, which interact with extracellular matrix components. Absence or dysfunction of dystrophin results in necrosis and regeneration of myocytes with progressive replacement by fibrous and adipose tissue. In young pups, death is thought to occur from respiratory failure following necrosis of diaphragmatic myofibers, while in older animals death frequently follows dysphagia and subsequent aspiration pneumonia or congestive heart failure.[11] This dog had evidence of aspiration pneumonia and congestive heart failure at necropsy. The role of the recent onset of ventricular arrhythmias in the animal's death was also considered. In young men with DMD, death from a combination of pneumonia and cardiovascular decompensation is common.[3]

X-linked dystrophin deficiencies have been identified in cats and a variety of dog breeds, including Irish Terrier, Samoyed, Rottweiler, Dalmation, Shetland Sheepdog, Labrador Retriever, Brittany Spaniel, Rat Terrier, Belgian Groenendael Shepherd, Schnauzer, and Spitz, but are best studied in the *mdx* mouse, German Shorthair Pointer, and Golden Retriever.[11] This particular patient was a member of the breeding colony at the National Center for Canine Models of Duchenne muscular dystrophy (NCDMD) at the University of North Carolina-Chapel Hill. As the murine models fail to show severe clinical signs or cardiac lesions comparable to those observed in humans, these canine models fill an important niche in therapeutic studies.[5,12]

Muscular dystrophies have been identified in people resulting from deficiencies or alterations of more than 30 different proteins[10] and may be X-linked, autosomal recessive, or autosomal dominant.[8] Many of these deficiencies have not yet been observed in veterinary species outside of the laboratory. Examples of spontaneous muscular dystrophies that have been identified in animals are summarized in Table 1. Other references contain a more extensive list of murine musculodystrophy models.[2,10]

Table 1. Spontaneous muscular dystrophies in veterinary species[modified from 8]

Deficient Protein (Human Disease Name)	Protein Location	Species
Laminin α_2 (Congential muscular dystrophy type 1A) (RC)	Extracellular Matrix	Dog[8] Cat[4,6,8] *dy/dy* mouse[8,9]
α-, β-, γ-, or δ-sarcoglycan (Limb-girdle muscular dystrophy types 2C-F)	Transmembrane	Dog[1,10] Cat[7] BIO14.6 hamster[10]
Dysferlin (Limb-girdle muscular dystrophy type 2B)	Transmembrane	SJL-*Dysf* mouse[9]
Dystrophin (Duchenne muscular dystrophy, Becker muscular dystrophy)	Cytoskeleton	Dog Cat *mdx* mouse[8,11]



2-5. Diaphragm, dog. Higher magnification shows marked fibrosis separating myocytes of variable diameter. (HE) Photograph courtesy of the Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough Street, Raleigh, NC 27606 http://cvm.ncsu.edu/dphp/index.html

JPC Diagnosis: 1. Heart, myocardium: Degeneration, necrosis, and loss, multifocal, with myocardial fibrosis and mineralization.
2. Diaphragm: Myofiber atrophy and loss, diffuse, moderate with myocyte atrophy and marked fatty infiltration.

Conference Comment: Dystrophin-dependent muscular dystrophy is confirmed immunohistochemically by the absence of dystrophin staining, or by Western blot analysis. Although muscle atrophy is the usual course in dystrophin-dependent muscular dystrophy, marked muscular hypertrophy of unknown cause is seen in mice, cats, and rat terrier dogs. Clinical pathology changes include a marked increase in serum creatinine kinase (CK) and aspartate aminotransferase (AST) in neonates; these continue to rise until 6 months of age. Serum CK and AST levels in older dogs are elevated to a lesser extent. Clinical signs of neuromuscular weakness progress until 8-12 months of age before stabilizing. Affected dogs exhibit a stiff-legged gait, thickened muscles at the base of the tongue, excessive drooling, abdominal breathing, and ribcage deformities due to contracture of the diaphragm. In some breeds, such as the rat terrier, there is paradoxical muscle hypertrophy of the thighs, neck, and shoulders. Female carriers are clinically normal but have elevated serum CK and AST.[13]

Gross lesions include severe degeneration indicated by pale streaks in the diaphragm, trapezius and sartorius muscles. In chronic cases, the diaphragm is thickened, contracted, and fibrotic. In the heart, the left ventricular wall and right side of the interventricular septum are most severely affected by necrosis, mineralization, and fibrosis. Common histologic findings not seen in this case are the presence of swollen and dark-staining fibers ("large dark fibers"), which represent the earliest stages of degeneration. Muscle fiber necrosis is due to the influx of calcium through defects in the sarcolemma. The basal lamina of necrotic fibers is preserved, leaving an empty sarcolemmal tube capable of fully regenerating the myofibers.[13]

Other common examples of muscular dystrophy in veterinary species include X-linked muscular dystrophy in mixed-breed cats, which have similar clinical, gross, and histologic findings to those seen in dogs. Cats with muscular dystrophy are also prone to develop malignant hyperthermia associated with restraint or general anesthesia. Unlike the canine condition, cardiac involvement in cats is not common. Merino sheep have an autosomal recessive muscular dystrophy, and skeletal muscle of affected sheep expresses normal dystrophin. The major gross feature is the replacement of the intermedius, soleus, anconeus, and medial head of the triceps with mature adipose tissue. This disorder only affects type 1 muscle fibers, with initial lesions of type 1 fiber hypertrophy followed by myofibril loss and formation of sarcoplasmic masses at the center or periphery of the cell. Some fibers develop peripheral annular fibrils known as "ringbinden". This condition is a result of loss of alpha-actinin and desmin proliferation, which forms the sarcoplasmic masses. It is important to distinguish true muscular dystrophies, which are inherited progressive myopathies, from other muscle disorders such as secondary nutritional degenerative myopathies.[13]

There are four categories of reaction to muscle injury. Focal monophasic reactions result from an isolated single event; multifocal monophasic reactions result from a single injurious event that causes widespread lesions in the same phase of injury; focal polyphasic reactions are due to repeated mechanical injury at the same location; and multifocal polyphasic reactions are the result of continuous injury over a prolonged period of time such that lesions are widespread and exhibit various pathologic changes ranging from degeneration to necrosis to regeneration.[13] This case is an example of a multifocal polyphasic (multiphasic) muscle lesion.

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CASE III: 09-565-3 (JPC 3136038).

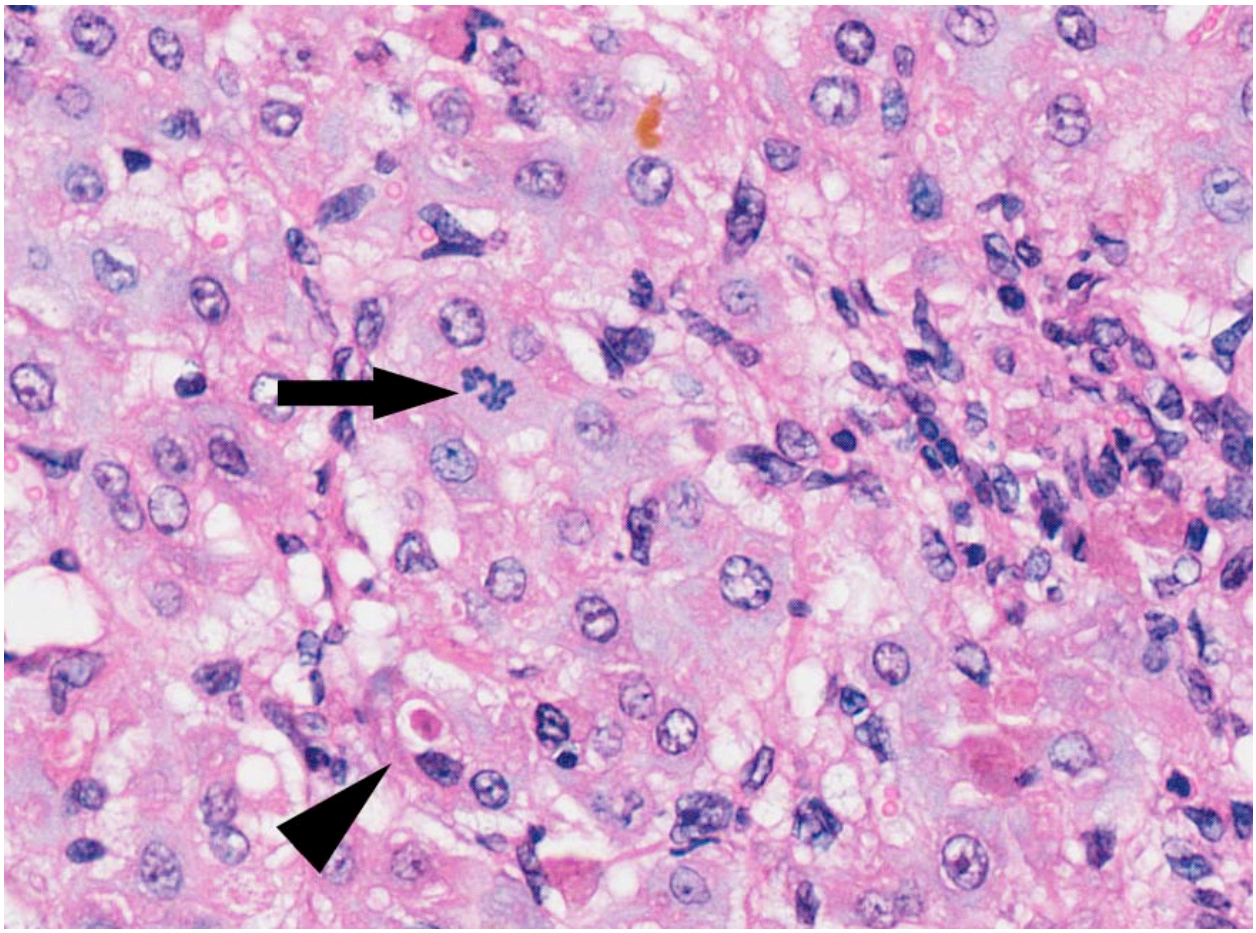
Signalment: Adult merino sheep of unidentified gender, (*Ovis orientalis aries*) ovine.

History: This animal was one of multiple affected animals from a flock in Narrogin, Western Australia, submitted in March 2009. The animals had been turned out onto a lupin and wheat paddock (presumptive stubble) approximately six weeks previously (January 2009). Multiple animals were displaying signs of marked lethargy and weakness. Ten sheep were recumbent and unable to rise and were subsequently euthanized.

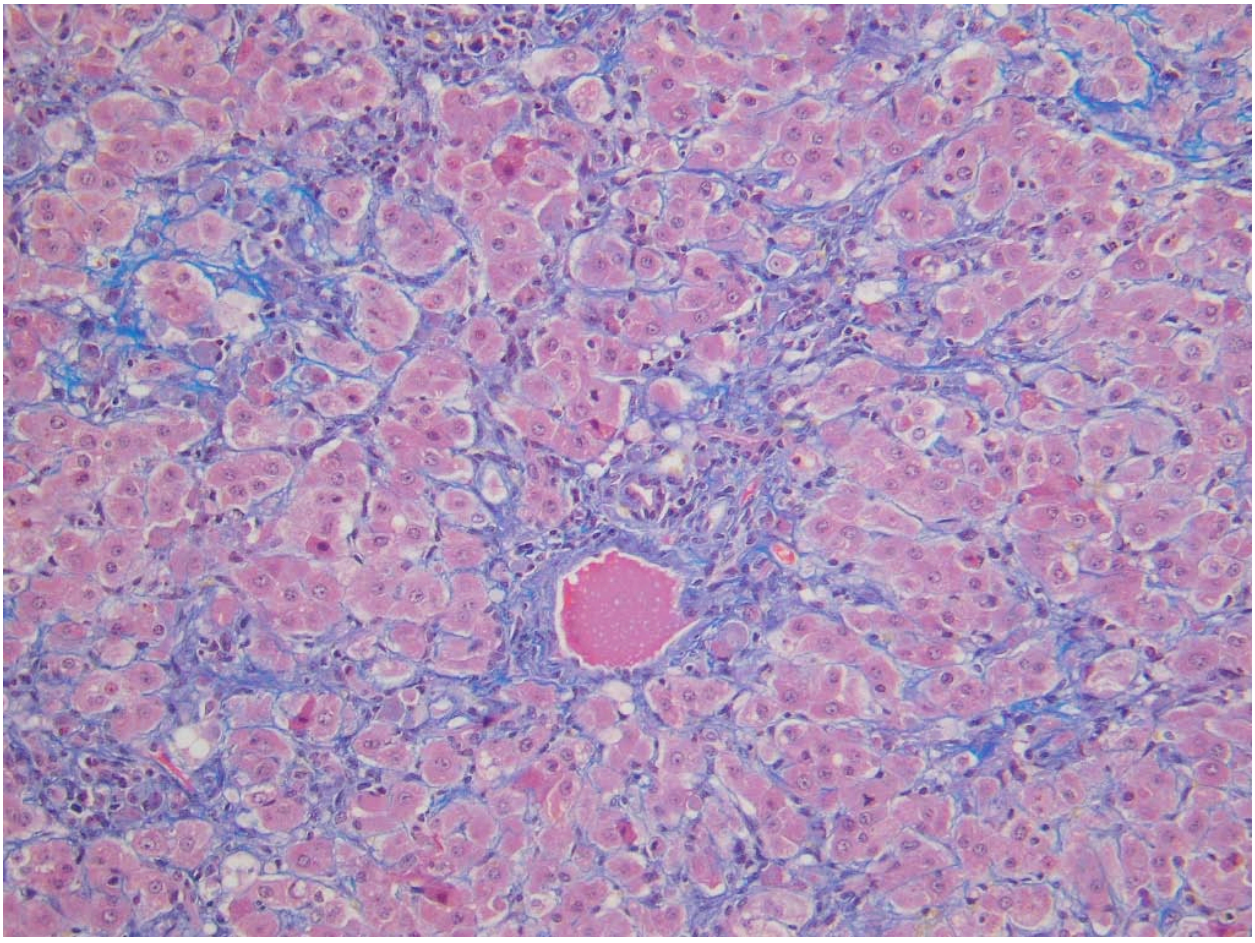
Gross Pathology: A post mortem examination was conducted on one of the affected animals in the field by the local veterinarian, who subsequently submitted both fresh liver and formalin fixed tissues to the Western Australian Department of Agriculture and Food for examination. The veterinarian reported extremely dry ruminal contents (suggesting dehydration) and the presence of peritoneal and pleural "proteinaceous" effusions. The liver was reportedly small with a yellow tinge and the kidneys appeared very dark.

Laboratory Results: Aerobic and anaerobic culture performed on the samples of fresh liver yielded no growth.

Contributor's Histopathologic Description: The hepatic architecture is diffusely disrupted by increased fibrous tissue surrounding portal triads, with fine septa ramifying outwards, distorting the lobular architecture and dissecting the hepatic plates resulting in isolation of small clusters of hepatocytes with occasional individualisation. Numerous hepatocytes appear swollen with moderate to marked anisocytosis and there are numerous mitotic figures, many of which appear abnormal, often arrested in metaphase. There are occasional scattered shrunken hypereosinophilic hepatocytes with condensed nuclei, consistent with apoptosis. Mild, diffuse bile duct proliferation is also apparent. Low numbers of lymphocytes, macrophages and neutrophils are present, particularly periportally. Modest numbers of canaliculi contain bile plugs. Also scattered throughout the hepatic parenchyma are



3-1. Liver, sheep. Marked disruption of hepatocellular architecture with nodulear regeneration, bile plugs, necrotic, rounded up hepatocytes (arrowhead), and mitotic figure (arrow), changes consistent with phomopsin toxicosis in the small ruminant. (HE, 350X)



3.2. Liver, sheep. A Masson's trichrome stain demonstrates the amount of fibrous connective tissue within the section, which is difficult to appreciate on the HE stain. Photograph courtesy of the Department of Anatomic Pathology, Murdoch University School of Veterinary and Biomedical Sciences, Faculty of Health Sciences. South Street, Western Australia, Australia, 6150. http://www.vetbiomed.murdoch.edu.au/ in conjunction with the Department of Agriculture and Food, Government of Western Australia, South Perth, Western Australia, Australia, 6951. http://www.agric.wa.gov.au/PC_92812.html

occasional cells (hepatocytes and Kupffer cells +/- macrophages) with faint intracytoplasmic yellow-golden brown granular pigment. Shikata's Orcein and Schmorl's stains demonstrated this pigment to be a mix of copper and lipofuscin (+/- ceroid), respectively. A Perl's Prussian blue also demonstrated the presence of a minimal amount of iron.

Contributor's Morphologic Diagnosis: Liver: Moderate, diffuse, chronic hepatocellular dysplasia and dissecting portal fibrosis, with mild cholestasis, biliary hyperplasia and intracytoplasmic pigment accumulation.

Contributor's Comment: The history, clinical signs, reported necropsy findings and histopathological changes observed in the liver of this sheep are consistent with the syndrome of chronic phomopsin poisoning, which is also widely referred to as lupinosis. Lupinosis is a mycotoxicosis seen in animals ingesting lupin stubble or seed (*Lupinus* spp.) infected with the fungus *Diaporthe toxica* (anamorph *Phomopsis* sp.). *Diaporthe woodii* (anamorph *Phomopsis leptostromiformis*) was originally (and incorrectly) thought to be the source of the causative toxins.[6,11] *Diaporthe toxica* is parasitic on green lupins, and grows saprophytically on the dead host,[2,9] and produces a number of different toxins, the best known of which are the phomopsins A, B & C.[8] These compounds are hexapeptides, and appear to act predominately on the hepatic parenchyma, where they bind to tubulin and inhibit microtubule polymerization leading to progressive loss of microtubules. This in turn disrupts mitosis, resulting in mitotic arrest in metaphase and commonly fatty infiltration.[1] It is important to note that this disease is a distinct entity from the disease known as lupine poisoning, which causes acute neurological signs and results from the ingestion of lupines containing quinolizidine alkaloids.[7,9] Lupinosis most commonly occurs in sheep, but also occurs in cattle, and, rarely, in horses and pigs.[4,7] Outbreaks of the disease are most common in countries where lupins are eaten as a dead standing crop or grazed as stubble, a situation most often seen in

Australia. Lupinosis occurs occasionally, but not commonly, in South Africa and New Zealand.[4]

Clinically, a variety of clinical syndromes are observed, dependent on the species involved, the dose of toxin and the duration of exposure.[2,6,8] In sheep, acute lupinosis occurs when animals graze highly toxic stubbles over a short period. Clinical signs include anorexia, lethargy, weakness and jaundice. Rarely, hepatic encephalopathy or photosensitization is observed. Clinical signs are typically seen within two days of introduction to stubble. Most animals within a flock are affected and deaths occur within three or four days.[7] Chronic lupinosis occurs following the ingestion of low doses of toxins over a prolonged period. Variable numbers of sheep within a flock are affected and animals are typically weak and in poor body condition. Jaundice may or may not be apparent, and those sheep that are jaundiced are commonly anemic. Mortality rates are low. The disease course may also be subacute and intermediate in severity, and it is this form of the disease that is most commonly observed in Australia.[8] A toxin-related nutritional myopathy has also been reported in association with lupinosis, which may or may not respond to supplementation with vitamin E or selenium.[8] Experimental phomopsin toxicity has been shown to reduce reproductive performance also.[7]

On gross examination, livers from acutely affected sheep are enlarged and frequently discolored yellow or tan, with histological examination revealing variable degrees of hydropic change and an increased rate of hepatocyte loss (from widespread apoptosis). The degree of fatty change is variable and depends largely on the nutritional status of the animal. As the disease course progresses, increased, but ineffective mitotic activity becomes evident with the presence of numerous arrested mitoses. Progressive hepatic fibrosis occurs and there is accumulation of complex granular pigment within macrophages in the affected tissue, and may include any combination of copper, ferric iron, ceroid or lipofuscin. Variable degrees of portal hyperplasia may also be present.[6,9] Some of these changes, including hepatic fibrosis and biliary hyperplasia, can also be observed in other hepatotoxicities including pyrrolizidine alkaloid and aflatoxicosis. As in phomopsin toxicity, these toxins inhibit normal mitosis, and DNA replication frequently continues resulting in the production of greatly enlarged hepatocytes (megalocytosis). In Australia, sheep grazing lupin stubble may also be concurrently exposed to plants containing pyrrolizidine alkaloids such as heliotrope (*Heliotropium europaeum*), which may result in additive or synergistic hepatotoxity.[2]

Lupinosis has been largely eliminated in Australia by the breeding of *Phomopsis*-resistant lupins, and is seen now in years with late finishes and/or increased summer rainfall.

JPC Diagnosis: Liver: Hepatocellular degeneration and necrosis, diffuse, moderate, with fibrosis, intracanalicular cholestasis, Ito cell hyperplasia, and prominent hepatocellular mitotic activity.

Conference Comment: A few other noteworthy histologic features in this case include a decrease in the lobule size and a decrease in the distance separating central and portal areas, reflecting hepatocyte necrosis and atrophy. Other potential toxic causes for this lesion would include aflatoxin, which may present with more hepatic regeneration, bridging portal fibrosis, biliary hyperplasia and hemorrhagic necrosis, with maintenance of hepatic trabeculae; sporidesmin, a mycotoxin which targets biliary epithelium and causes necrosis; microcystin, a toxin of blue-green algae, which causes disassociation of centrilobular hepatocytes and submassive hepatic necrosis and hemorrhage; and pyrrolizidine alkaloids, which also cause bridging fibrosis, biliary hyperplasia, and megalocytosis.[9] Sheep appear to have increased resistance to pyrrolizidine alkaloid intoxication compared with other species.

The contributor mentioned the possibility of photosensitization in acute phomopsin toxicosis. Photosensitization appears as erythema, dermal edema, vesicle or bullae formation, exudation and extensive epidermal necrosis. There are three types of photosensitization. Type I, or primary photosensitization, occurs from ingestion of a plant or drug containing photoreactive substances, which are deposited in the skin. Type II photosensitization occurs in animals with a genetic inability to metabolize heme pigments, which results in a buildup of the photoreactive hematoporphyrin pigments, such as uroporphyrin I, coproporphyrin I, and protoporphyrin III. Type III, or hepatogenous photosensitization, occurs with an abnormal buildup of phylloerythrin, which is a degradation product of chlorophyll, because of a damaged or immature liver. The photosensitization in this case is type III and is secondary to cholestasis.[3] In cattle, phomopsin toxicity causes anorexia and ketosis in pregnant or lactating cows, and chronic exposure leads to fibrotic hepatitis with nodular regeneration (cirrhosis).[9]

The toxic principles of true lupinosis (not to be confused with phomopsin toxicosis) are quinolizidine alkaloids, which causes nicotinic effects such as salivation, ataxia, seizures, and dyspnea, and the alkaloid anagyrine, which causes arthrogryposis in calves and lambs following *in utero* exposure.[5,10]

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CASE IV: NCAH 2011-1 (JPC 4003053).

Signalment: Adult female beef ox, (*Bos taurus*).

History: Gross lesions were identified during the postmortem inspection of the carcass at slaughter. Lymph node samples were submitted for laboratory evaluation through the United States Department of Agriculture (USDA), Bovine Tuberculosis Eradication Program. Due to the gross lesions, the carcass was condemned (not used for human consumption).

Gross Pathology: The carcass was in normal body condition. The left parotid lymph node, and to a lesser degree the adjacent lymph nodes of the head, contained irregularly shaped areas which were discolored green. On cut surface, patchy areas of green extended into both the cortex and medulla. The facial musculature adjacent to the parotid lymph node, the tracheobronchial lymph nodes, and mediastinal lymph nodes were also multifocally green.

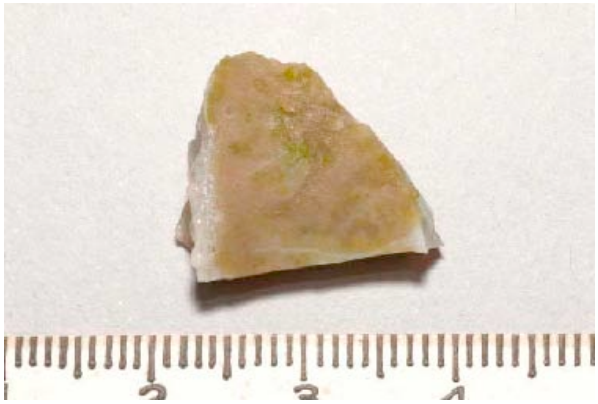
Contributor's Histopathologic Description: Lymph node: Large coalescing aggregates of macrophages and epitheloid macrophages with abundant eosinophilic cytoplasm expand the cortical and medullary sinuses. Numerous algal cells with low numbers of eosinophils, multinucleate giant cells, lymphocytes and plasma cells are intermixed with the macrophages. The algal cells are round, 7 to 15 microns in diameter, have a thin refractile cell wall, single basophilic round nucleus and amphophilic to eosinophilic granular cytoplasm. Low numbers of larger algal cells (sporangia) are filled with 2 to 6 daughter cells (sporangiospores or endospores). Intact algal cells and empty cell walls with no internal structures (degenerate algal cells) are both free in the sinuses and within macrophages. Within the cortex, there are few lymphoid follicles.

Contributor's Morphologic Diagnosis: Lymph Node: Lymphadenitis, granulomatous, severe, diffuse, chronic, with large numbers of algal organisms consistent with green algae.

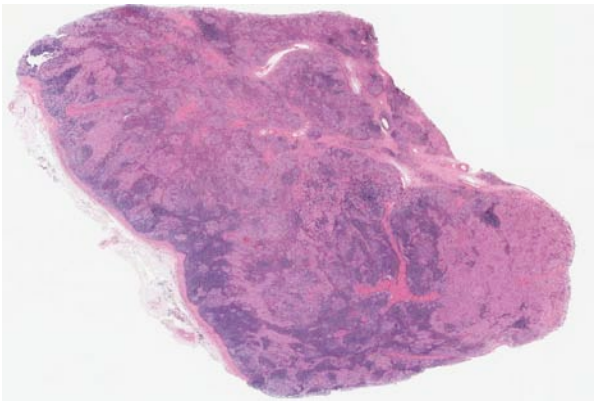
Contributor's Comment: Algal infections are uncommon opportunistic pathogens of domestic and wild mammals and humans.[1] Culture is frequently required to make a definitive etiologic diagnosis, but even with culture, the precise genus and species of green algal infections have not always been clearly identified.[1] *Chlorella* spp. are commonly cited as the etiology of green algal infections, but it is important to note that not all green algal infections are due to *Chlorella* spp.[3,6]

Green algal infections have been described most commonly in sheep and in cattle, with individual case reports in a dog, man and several species of wild mammals.[1,4,5] The algae are found commonly throughout the world and infections have been associated with stagnant water or pasture irrigated with raw sewage.[5] Disease varies from subclinical with a localized lymphadenitis to severe clinical disease and systemic lesions.[1,2,5] Underlying immunosuppression or an overwhelming infectious dose are possible predisposing factors to causing disease by this ubiquitous organism.[5] Our case is consistent with one of the largest reports (a group of 8 cattle cases) in which green algae induced lymphadenitis was identified through postmortem slaughter inspection.[6] Green algal infections are also identified several times a year in cattle by the USDA Food Safety Inspection Service pathology laboratory, which receives samples from federally inspected slaughter facilities (S Hafner, personal communication).

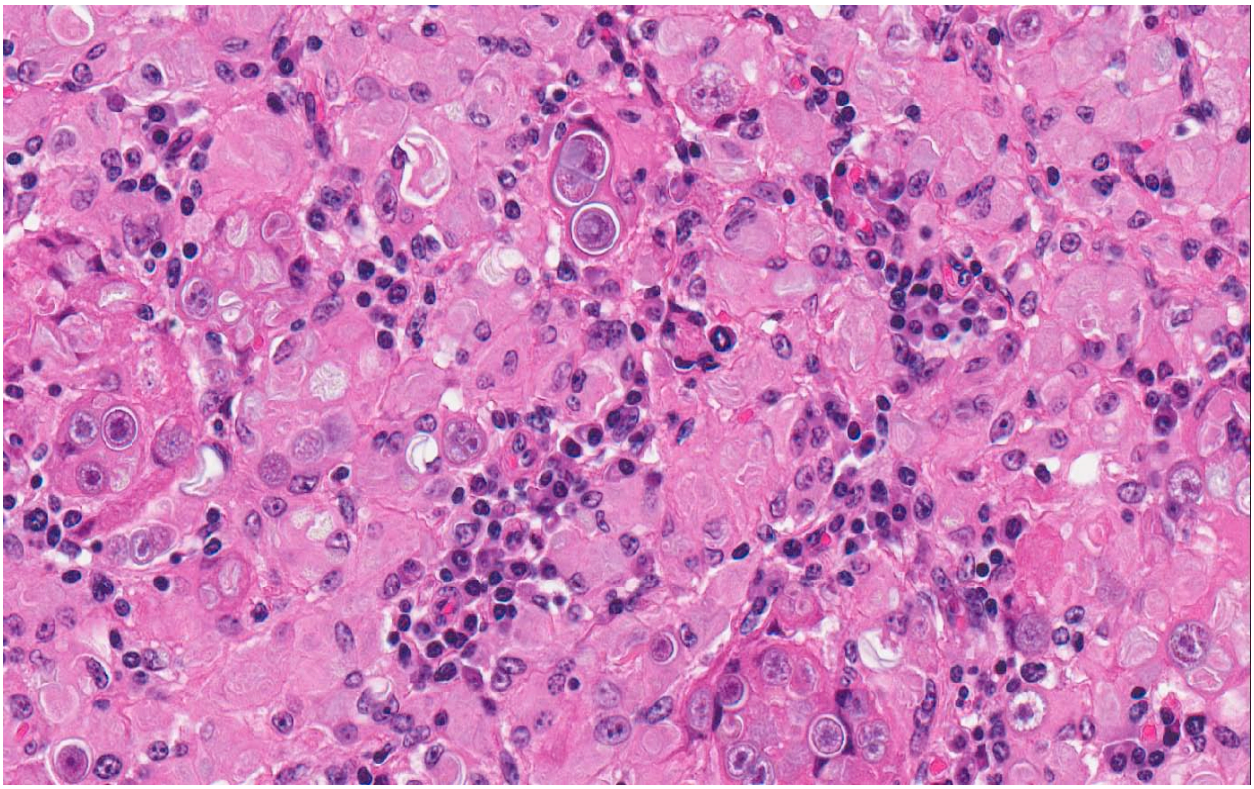
Based on the lesion and organism morphology on HE stained slides, the primary differential diagnoses



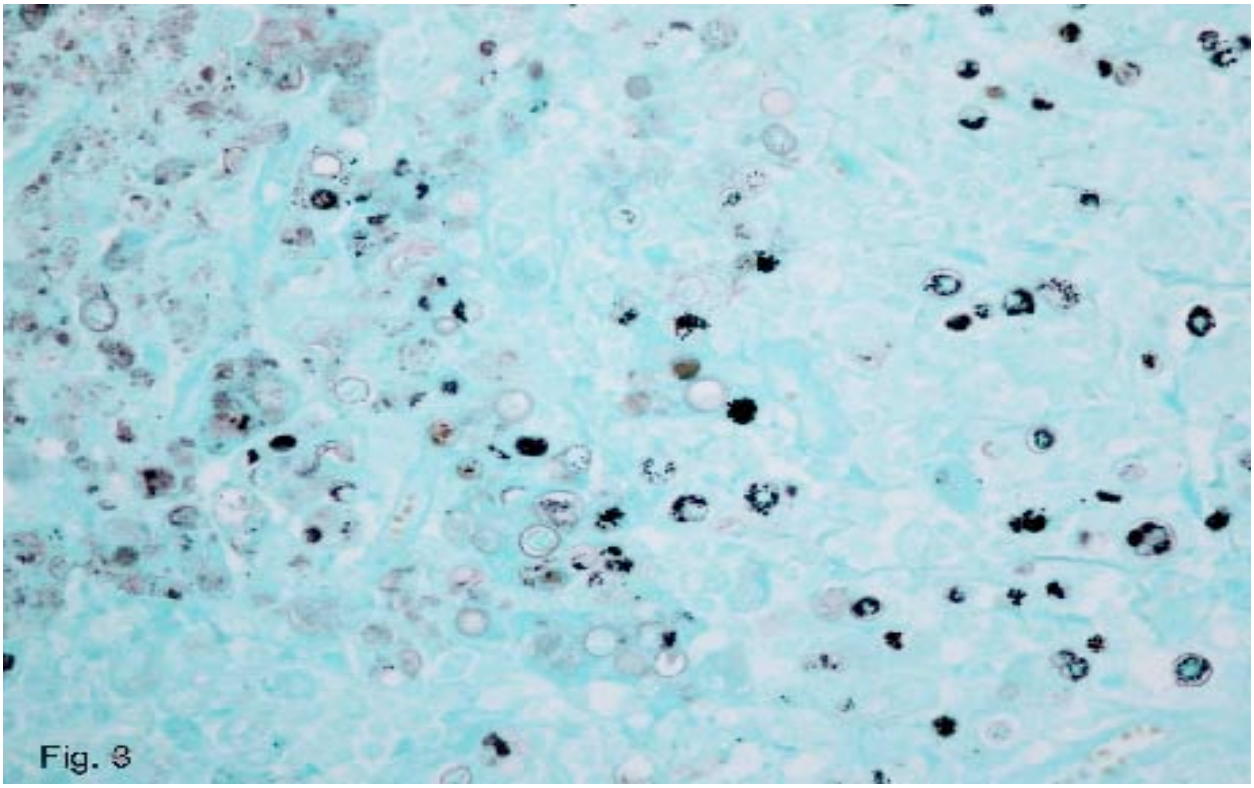
4-1. Lymph node, ox. The left parotid lymph node, and to a lesser degree the adjacent lymph nodes of the head contained irregularly shaped areas which were discolored green. Photograph courtesy of the National Centers for Animal Health, 1920 Dayton Avenue, Ames, IA 50010. http://www.aphis.usda.gov/animal_health/lab_info_services/about_nvsl.shtml



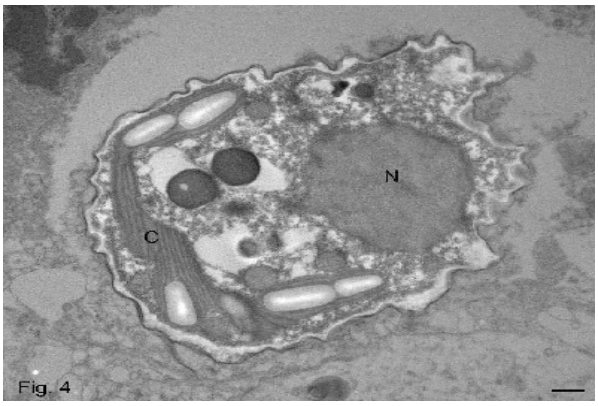
4-2. Lymph node, ox. The normal nodal architecture is effaced by multifocal to coalescing areas of granulomatous inflammation. (HE, 6X)



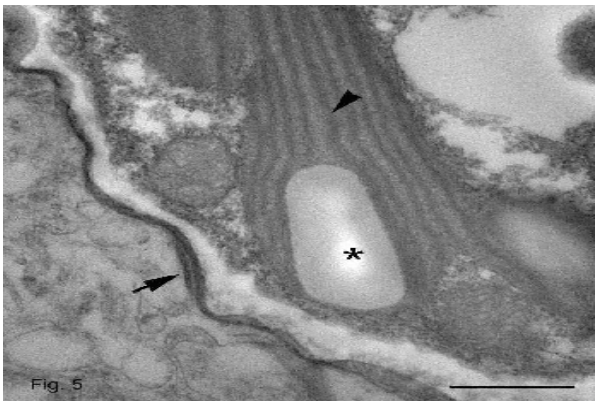
4-3. Lymph node, ox. Epithelioid macrophages and multinucleate giant cell macrophages contain numerous Chlorella algal cells, as well as multinucleated endosporulating sporangia (small arrows), and degenerate forms consisting primarily of a collapsed cell wall (large arrow). (HE, 400X)



4-4. Lymph node, ox. The large silver-positive granules are helpful in differentiating Chlorella from Prototheca, according to Chandler. (Gomori Methenamine Silver). Photograph courtesy of the National Centers for Animal Health, 1920 Dayton Avenue, Ames, IA 50010.



4-5. Lymph node, ox. Transmission electron micrograph. Single algal cell with round nucleus (N) and a large chloroplast (C). Lead citrate and uranyl acetate. Bar = 500 nm. Photograph courtesy of the National Centers for Animal Health, 1920 Dayton Avenue, Ames, IA 50010.



4-6. Lymph node, ox. Transmission electron micrograph. Within the chloroplast is a starch granule (asterisk) and thylakoids (arrowhead). The thylakoids form parallel stacks termed grana. Cell wall (arrow). Lead citrate and uranyl acetate. Bar = 500 nm. Photograph courtesy of the National Centers for Animal Health, 1920 Dayton Avenue, Ames, IA 50010. http://www.aphis.usda.gov/animal_health/lab_info_services/about_nvsl.shtml

include various types of green algae and *Prototheca* spp. Protothecosis occurs more commonly than disease by green algae and *Prototheca* spp. are achlorous, which is a key criteria for differentiating green algae from *Prototheca* spp.[1,5] Chandler et. al.[1] uses three criteria for differentiating *Chlorella* (a type of green algae) from *Prototheca* spp. and for making a presumptive diagnosis. In chlorellosis there are: 1) grossly visible green discoloration of the tissues due to the presence of chlorophyll in the algae, 2) spherical algal cells, with an average diameter of 9 microns that exhibit endosporulation, and 3) algal cells that have abundant large cytoplasmic granules that are strongly positive with PAS, GF, and GMS stains.[1] Other methods used to differentiate the two include impression smears of fresh tissue and transmission electron microscopy, both of which are based on the presence of numerous chlorophyll/chloroplasts.[1,5] It is important to remember that the green color of the *Chlorella* algae is lost during tissue fixation and processing.[1]

JPC Diagnosis: Lymph node: Lymphadenitis, granulomatous, diffuse, moderate to marked with numerous intrahistiocytic and extracellular endosporulating algae and moderate plasmacytosis.

Conference Comment: The contributor provided an excellent discussion of *Chlorella* spp. and the associated pathology. Conference participants discussed the differences between *Chlorella* and *Prototheca*, and the difficulties in differentiating the two entities by standard histologic methods.

In addition to *Chlorella* and *Prototheca,* other pathogens that reproduce asexually by endosporulation include *Rhinosporidium seeberi* and *Coccidioides immitis*.

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