Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2018-2019

C o n f e r e n c e 24

10 April 2019

Conference Moderator:

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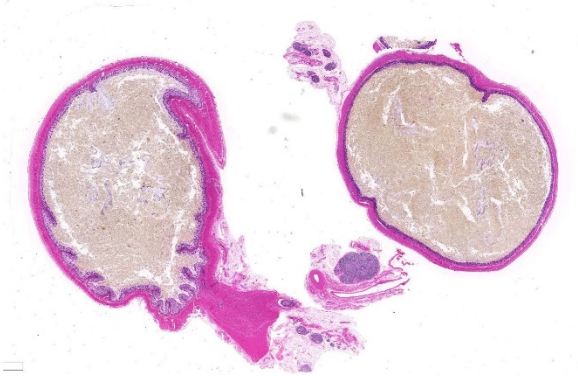
Univ. of Pennsylvania School of Veterinary Medicine

CASE I: W837-12 (JPC 4088206).

Signalment1 day old, female, Quarterhorse, equine (*Equus caballus*)

History: The foal was presented for development of colic several hours after birth. The foal was white with small areas of grey/black coloration on the tail base, lip and ear; the mare had a solid bay coat with a small area of white flecking on the left shoulder and the stallion was paint. The foal was suckling but passage of meconium was not observed. Reduced gastrointestinal sounds were noted on auscultation, but the examination was otherwise unremarkable. No meconium was palpated within the rectum. A moderate amount of intestinal gas was noted on abdominal radiographs. The colic failed to resolve with supportive treatment and the abdomen became progressive distended and tympanic over the next 12 hours. Due to continued deterioration, the foal was euthanized and submitted for post-mortem examination.



Colon, day-old foal. The colonic lumen is distended with meconium and the wall appears diffusely and transmurally thinned. It is unclear if the distention is due to dilation proximal to a more affected segment). (HE, 4X)

Gross Pathology: The rectum and small colon appeared to be diffusely small and pale. The large colon was filled with meconium and also appeared to be segmentally mildly stenotic along the right dorsal section, though the entire gastrointestinal tract was patent.

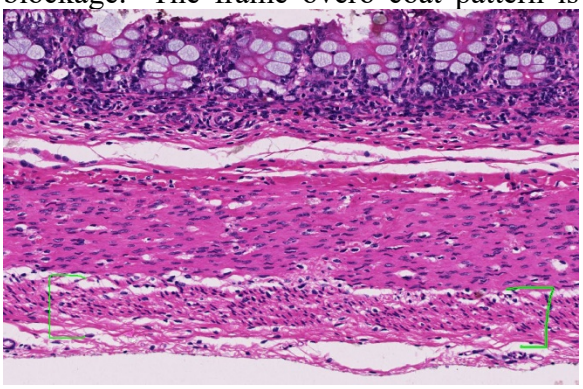
The small intestine was distended with gas and liquid meconium distally, while the stomach and proximal duodenum contained ingested milk. All other body systems were unremarkable.

Laboratory results: NA

Microscopic Description:
Within the colon, there is complete absence of ganglion cells within the gut wall, together with a severe paucity of myenteric (Auerbach's) ganglia and submucosal nerves (Meissner's). The submucosa was reduced, but in the tissue that remained there was increased prominence of stromal cells.

Contributor's Morphologic Diagnoses: Ileocolonic aganglionosis, severe, diffuse, chronic (consistent with overo lethal foal syndrome)

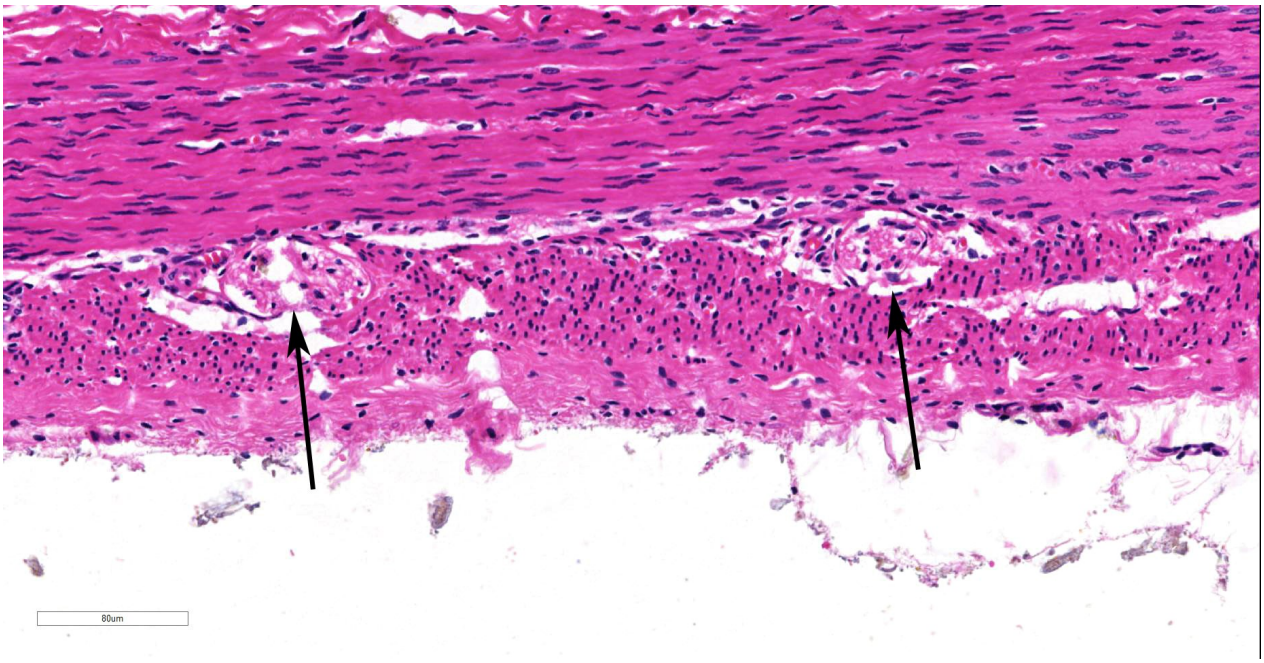
Contributor's Comment: Lethal White Foal Syndrome (LWFS) is a disease associated with horse breeds that register white coat spotting patterns. Breeding between particular spotted horses, generally described as 'frame overo', produce some foals that, in contrast to their parents, are all white or nearly all white and die shortly after birth of severe intestinal blockage.[1] The frame overo coat pattern is described as white markings on the lateral and ventral aspects of the neck and torso, whereas a pattern with more white on the dorsal cervical and lumbar regions and the legs is called 'tobiano'.[3].



Colon, day-old foal. The muscularis is markedly thinned, the outer longitudinal layer (green brackets) in particular. (HE, 238X)

Overo lethal white syndrome occurs in newborn foals is associated with the 'overo' coat pattern that receives a copy of the mutated OLW gene (Ile118Lys EDNR-B) from each parent.[8] Two heterozygote carriers of the mutated gene must be mated to produce a homozygous lethal white foal. Color patterns with highest incidence (> 94%) of heterozygotes were frame overo, highly white calico overo, and frame blend overo. White-patterned bloodlines with lowest incidence of heterozygotes (< 21 %) were tobiano, sabino, minimally white calico overo, splashed white overo, non-frame blend overo, and breeding-stock solid. The mutation was not detected in solid-colored horses from breeds without white patterning.[4,6,8]

Phenotypically, the altered gene causes lack of skin pigmentation and white coat colour which was strongly associated with endothelin receptor B (EDNR-B) genotype. Endothelin receptors are important in neural crest cell migration. Neural crest cells are precursor cells to a variety of cell types including melanocytes, neural and glial cells of the peripheral, including the enteric, nervous systems.[9] A consequence of a foal carrying two copies of the mutated EDNR-B gene is that neural crest cells do not migrate to the skin or the gut appropriately, thus resulting in foals being born with a white hair coat and aganglionosis of the submucosal and myenteric ganglia of the distal part of the small intestine and of the large intestine, resulting in intestinal immotility and colic, similar to human Hirschsprung's disease.[3,4]



Colon, day-old foal. A cross section through the myenteric plexus shows a single hypocellular nerve fiber without any adjacent ganglion cells. (HE, 313X)

The condition Hirschsprung's disease in human children, seen in 1 in 5000 live births, and can be familial or sporadic. In these children often only a segment of the colon or the distal rectum is involved and leads to congenital megacolon. Familial Hirschsprung's disease has been associated with one or more mutations in three separate genes: 1) the RET proto-oncogene, 2) the endothelin-3 (EDN-3) gene, and 3) the endothelin-B receptor (EDNR-B) gene.[2] Endothelin-3 knockout mice have congenital megacolon due to colonic aganglionosis and coat color spotting due to regional lack of epidermal melanocytes. Endothelin-B receptor knockout mice have the same phenotype.[4] Mutation of this receptor gene has also been shown to be responsible for familial Hirschsprung's disease in Mennonite families.[6] Affected humans also occasionally exhibit abnormalities in skin pigmentation.

White patterning in horses has been strongly associated with EDNR-B genotype. Determination of EDNR-B genotype by use of a DNA-based test is the only way to determine with certainty whether white-patterned horses can produce a foal affected with OLWS.[3] (3).

DNA-based test that identifies horses that are heterozygous for the Overo lethal white gene has been developed. The allele-specific polymerase chain reaction test locates and amplifies the specific mutated site in the endothelin receptor B gene (EDNR-B gene).[3] The test for this mutant allele can be utilized in all breeds where heterozygous animals may be unknowingly bred to each other including the Paint Horse, Pinto horse, Quarter Horse, Miniature Horse, and Thoroughbred.

Contributing Institution:
Veterinary Pathology, Faculty of Veterinary and Agricultural Sciences, University of Melbourne http://www.fvas.unimelb.edu.au

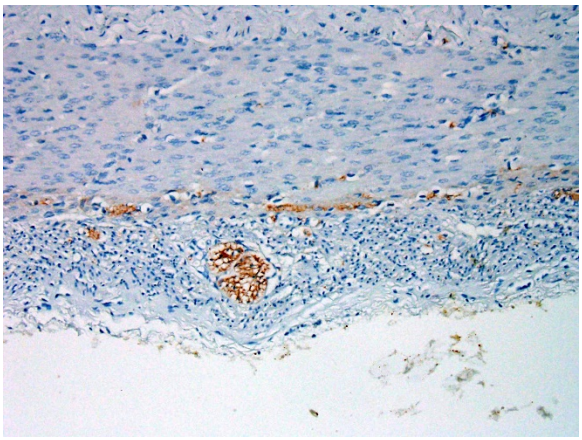
JPC Diagnosis: Colon: Aganglionosis,

diffuse, severe, with marked hypoplasia of the muscular tunics.

JPC Comment: The contributor has done an excellent job in describing the molecular basis of lethal white foal syndrome and Hirschsprung's disease, its human equivalent. Hirschsprung's disease was named after Danish pediatrician Harald Hirschsprung, who first described the condition in two infants in 1886.

Today, a number of mouse models are available for colonic aganglionosis, which exploit mutations in the endothelin 3 receptor, as well as glial cell line-derived neurotrophic factor (GDNF) and its receptor, tyrosine kinase.[5] In addition to colonic aganglionosis and lack of appropriate colonic contractions, these mutations also result in white spotting of the coat, giving rise to the term "lethal spotting mice". According to the Jackson Laboratory, lethal white spotting mice and similar homozygous "piebald mice" usually die within the third week of life.[7]

Another "lethal white" syndrome is seen in guinea pigs. While the genetic basis of this disease has not been worked out, the overall picture strongly suggests defective migration of neural crest cells during embryogenesis. Similar to the syndrome seen in overo foals, the condition arises in approximately 25% of guinea pigs born to parents carrying the red "roan" allele. While aganglionosis has not been documented in affected guinea pigs, affected individuals manifest albinism, microopthalmia, cataracts, hypodontia and malocclusion, intestinal malabsorption, and diminished immune responses.[10] They are, however, reputed to have excellent personalities.



Colon, day-old foal. A cross section through the myenteric plexus demonstrates the absence of neurons. (anti –NSE, 400X)

The thickness of the muscular tunics was a subject of spirited discussion, with some of the attendees favoring a hypoplastic change due to lack of innervation during development, and some favoring a thinning due to distention from accumulated waste and sampling proximal to the a more profound obstruction.

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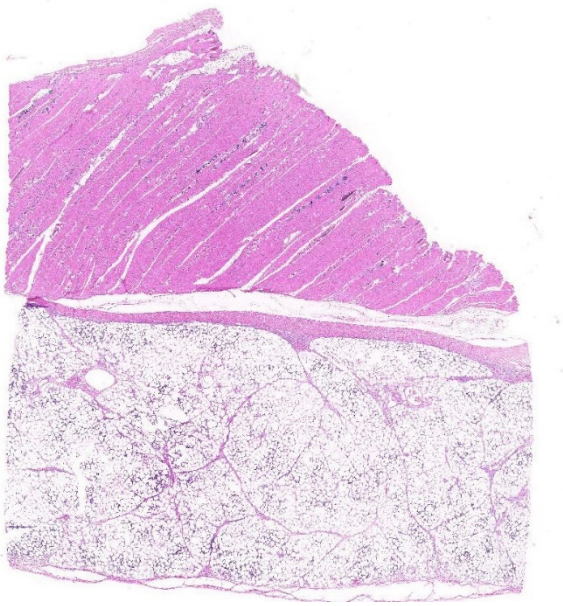
CASE II: P/2014-95 (JPC 4066219).

Signalment: 7-week-old, female Cob-X, horse, *Equus caballus.*

History: The filly was presented with lethargy and unwillingness to suckle over the last few days. On clinical examination she exhibited tachycardia, tachypnea, fever, increased capillary refill time, pink mucous membranes, loud and harsh respiratory sounds, and had a pasty fecal consistency. She was treated with antibiotics and nonsteroidal anti-inflammatory drugs (NSAIDs).

The following day, the foal was much the same, yet drinking milk from bucket. Additionally, she exhibited a stiff gait, which was more obvious on the hindlimbs when compared to the forelimbs. Her treatment with antibiotics and NSAIDs was continued and supplemented by intravenous fluid therapy.

The next day the foal became recumbent and died.
In the previous weeks, a number of foals, five of which died, had deteriorated with similar symptoms at the yard down the road, which belonged to the same owner. During the days following submission of the filly for post



Abdominal wall, foal. A section containing skeletal muscle and abdominal fat is submitted for examination. (HE, 5X)

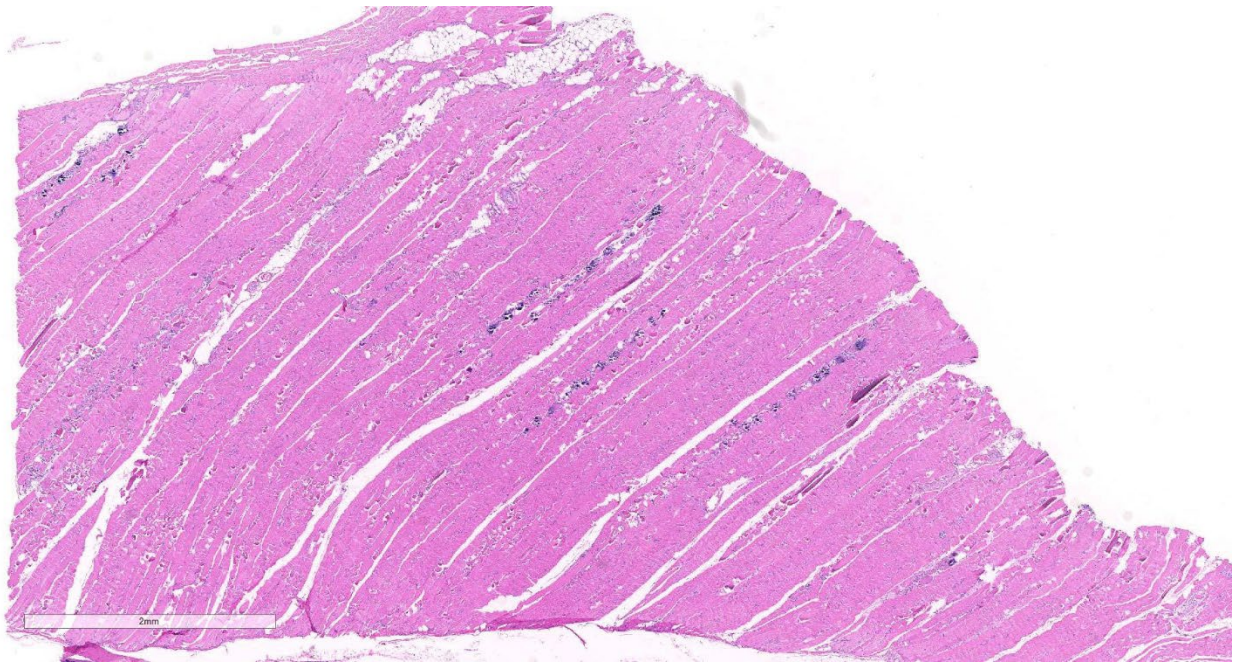
mortem examination, also other foals at the same yard started to show symptoms.

Gross Pathology: Upon gross examination, the cause of death in this foal was not apparent. The adipose tissue in this foal diffusely appeared yellow and, where associated with the mesentery, slightly nodular. Additionally, there was a mild to moderate catarrhal enteritis and very mild ascites.

Laboratory results: NA

Microscopic Description:
In a multifocal distribution, the striated (skeletal) myofibers are homogenously eosinophilic, devoid of striations and frequently fragmented, exhibiting irregular contours (hyaline degeneration). Some of these fibers stain strongly eosinophilic (Zenker's degeneration), whilst others exhibit a punctate intracytoplasmic basophilic stain (mineralization). Occasional degenerative myofibers are being infiltrated by macrophages.
In a multifocal to coalescing distribution, the interstitium of the overlying adipose tissue contains moderate numbers of neutrophils admixed with lesser numbers of macrophages and small to moderate amounts of granular basophilic material (mineralization). Numerous adipocytes, mainly in close association with the inflammatory infiltrates exhibit a blurring of their cytoplasmic vacuoles with pale eosinophilic or basophilic staining (necrosis), whilst in others small clusters of neutrophils are present within lipid vacuoles. Multifocally, the stromal tissue supporting the sheets of mature adipocytes is mildly to moderately expanded by small numbers of plump spindle cells (activated fibroblasts).

Contributor's Morphologic Diagnoses: Myofiber degeneration, segmental, acute to subacute, multifocal to coalescing, moderate to marked; abdominal wall with



Abdominal wall, foal. Myofiber necrosis and mineralization are visible in skeletal muscle even at low magnification (HE, 18X)

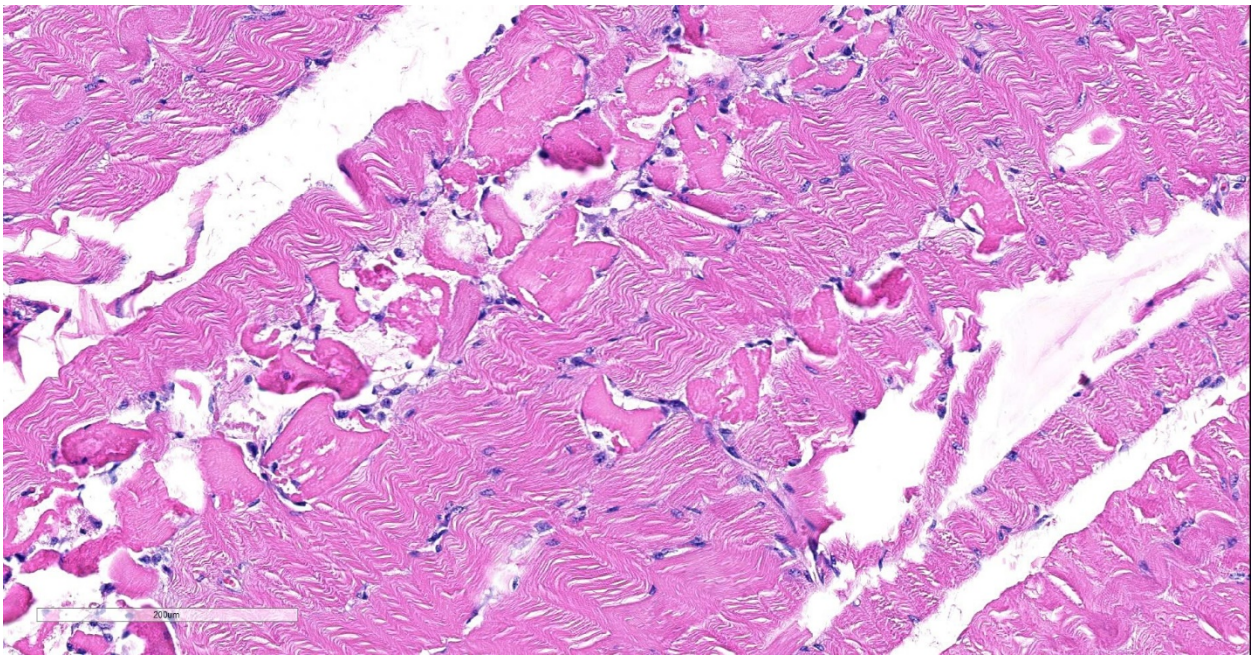
a) Myofiber mineralization, multifocal, moderate; and
b) Myositis, histiocytic, subacute, multifocal, mild.

1.) Adipocyte necrosis, acute to subacute, multifocal to coalescing, moderate; abdominal wall with
a) Steatitis, neutrophilic and histiocytic, subacute, multifocal to coalescing, moderate to marked; and
a) Mineralization, multifocal, mild to moderate; and
b) Fibrosis, subacute, multifocal, mild.

Contributor's Comment: The combination of muscle degeneration and fat necrosis with prominent inflammatory changes in the fat (also termed "yellow fat disease") is a disorder seen in equine and donkey foals ranging from one day to 12 weeks [2,7] and young equids up 3 years [1,5], and is thought to be the results of a deficiency of vitamin E and/or selenium, or a diet high in unsaturated fatty acids [1]. However, possibly also a more complex aetiology may need to be considered, since vitamin E/selenium supplementation in these patients often is only of very little or no curative value and these foals may also exhibit normal tissue selenium or gluthathione peroxidase concentrations [6].

Whilst the metabolism of vitamin E and selenium is incompletely understood, the pathogenesis for the muscular changes observed in these foals is considered to be based on lipid peroxidation and damage to proteins of mitochondria, endoplasmic reticulum and the cytosol by radicals due to a relative lack of radical-quenching systems. Both vitamin E and selenium play a role in radical scavenging via the gluthatione peroxidase/gluthathione reductase system.[7]

Following injury to the cellular membranes by free radicals, these membranes are unable to upkeep the homeostasis. Mitochondria take up the surplus of calcium following the



Abdominal wall, foal. There are multifocal areas of skeletal muscle necrosis, with vacuolation and fragmentation. (HE, 194X)

intracellular influx of this, which negatively affects their ability to produce energy. Energy is required for the recapture of calcium-ions following muscle contracture, with the increase in calcium further resulting in a hypercontractile state which in turn results in coagulative destruction of the myofilaments.[7]

Similarly, the changes to the adipose tissue are considered to be the result of progressive peroxidation [1]. The relative distribution of necrosis and mineralization, however, in comparison to the extent of steatitis may vary between the different sites examined.[6]

Affected foals are considered to likely be born to selenium-deficient mares or possibly also vitamin E-deficient mares.[1,6] Interestingly "yellow fat disease" has a relatively high incidence in Shetland ponies and cold-blooded horses indicating a possibly hereditary predisposition of these regarding for example transplacental passage and liver storage of selenium or colostral vitamin E, or that these horse groups may be relatively over-represented amongst suboptimally fed equids.[1]

Clinically, affected foals and horses exhibit depression, fever, tachycardia and variable tachypnea. [1,2,5]. Additionally, mild to moderate abdominal discomfort and tenderness, painful swelling of the nuchal ligament, groin and axillary regions, stiffness and muscular weakness are present, which may affect their ability to suckle.[3,5,6,7] Their feces are of abnormal consistency.[1,2]

In biochemistry, lactate dehydrogenase, creatine kinase and aspartate aminotransferase are increased,[1,2,3] whilst serum vitamin E or selenium is decreased.[1,3,5]

Upon gross examination, affected foals/horses exhibit hardened, multinodular and focally hemorrhagic adipose tissues of the nuchal ligament, the coronary sulci, the subcutis and throughout the abdominal cavity with or without yellow-brown discoloration and which may be chalky on cross-section.[1,2,3,5] Subcutaneous oedema, and discoloration and striation of the skeletal muscles may also be seen.[1,3,6] Some horses exhibit evidence of a chronic enteritis and bronchopneumonia.[1]

The histological changes comprise necrosis of fat cells combined with a steatitis and mineralisation, and degeneration and mineralisation of muscle fibres combined with inflammatory infiltrates.[1,5,7] Severely affected foals exhibit a monophasic myopathy, whilst subacute cases exhibit polyphasic changes.[5]

Interestingly, the myocardial changes are variable and range from no changes to loss of striations, mineralization and small foci of myocardial necrosis.[1,5] Degenerative changes to the adrenal cortex with necrosis, calcification and replacement fibrosis of the zona fasciculata have also been reported.[4]

The moderator reviewed features of fat necrosis in histologic section (in the absence of saponification, which was minimal in the adipose tissue on this slide., to include loss of adipocyte nuclei, color change from basophilic to eosinophilic cell membranes, and extension of inflammation into adipocyte cytoplasm.

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JPC Diagnosis: 1. Skeletal muscle: Degeneration and necrosis, polyphasic, multifocal to coalescing, with mineralization.

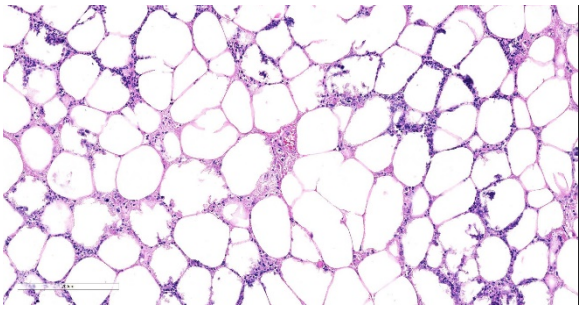
2. Fat: Steatitis, necrotizing, multifocal to coalescing, marked.

JPC Comment: The contributor has done an outstanding job of reviewing the syndrome of Vitamin E/selenium deficiency in the foal, and this section is an outstanding example of the damage that may be seen in multiple tissues.

Vitamin E/selenium imbalance may have one of the most diverse repertoire of damage in many animal species, with some animals (such as pigs) manifesting in a multitude of syndromes. Most species will demonstrated muscle necrosis, often both skeletal and cardiac, in the face of Vitamin E deficiency. Decreased reproductive efficiency is also a common finding across species. Panniculitis may be seen as a result of dietary Vit E deficiency in cats, mink, foals, swine as a result of feeding high-fish diets, but is also seen in certain waterbirds, such as herons. In dogs, a vitamin E-deficient diet may result in ceroidosis of intestinal smooth muscle, so-called "brown dog gut".

Vascular damage may be seen in animals with Vitamin E deficiency, to include cerebellar hemorrhage and necrosis in turkey poults, as well as exudative diathesis in poultry and swine, in which fluid exudes from blood vessels yielding a soft edematous overlying tissue. Vitamin E dieficiency may also result in hemolytic anemia in a number of species, including humans and non-human primates. Vitamin E deficiency in guinea pigs may result leukoencephalomalacia in feti. Cutaneous manifestations of Vitamin E deficiency include alopecia and seborrhea dermatitis in goats, as well as alopecia in calves on Vitamin E-deficient milk replacer.[4]

Growing pigs may suffer a number of manifestations of Vit E/Selenium imbalance, including massive hepatic necrosis (hepatosis



Abdominal wall, foal. There is multifocal dystrophic calcification of necrotic myofibers. (HE, 194X)

dietetica), arteriolar degeneration and thrombosis (mulberry heart disease), exudative diathesis, and white muscle disease. These syndromes rarely occur concurrently in the same animal.

Vitamin E was discovered in 1922 by Drs. Herbert Evans and Katharine Bishop, who identified it as a dietary fertility factor for laboratory rats. In fact, the name "tocopherol" is derived from the Greek meaning "to carry a pregnancy". It is obviously a necessary dietary factor for all animal species. As a widely used mendicant on the human market, it has enjoyed great popularity as a dietary supplement and ingredient in topical products for the skin in humans, largely without documented benefits. The benefits of megadosing on Vitamin E, begun in the 1930's and 1940's by the Shupe brothers (who extolled its virtues in treating heart disease) have yet to be proven beneficial in treating cardiovascular disease or a wide range of other diseases for which it has been purposed.

Participants remarked on the lack of saponification in the areas of necrotic fat on this slide. In the absence of obvious saponification, a lack of vital staining, loss of adipocytes nuclei, and extension of inflammatory cells within the cytoplasmic border of affected adipocytes are all clues to the potential viability of damaged adipocytes.

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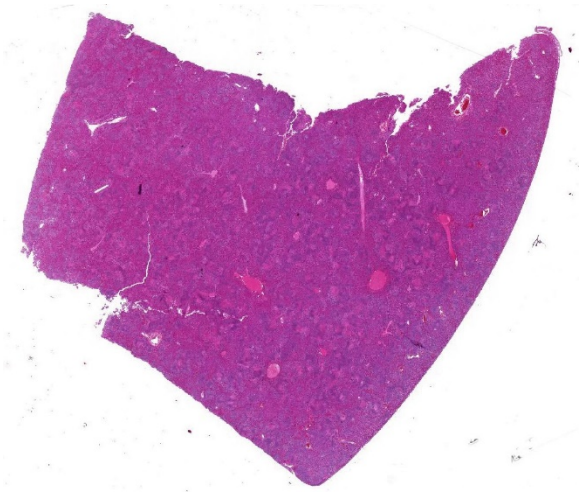
CASE III: S643/17 (JPC 4101752).

Signalment: Foal, 3 weeks of age, female, crossbred horse, *Equus caballus*



Liver, foal. A section of liver is presented for examination. (HE, 4X)

History: This foal was born without any complication in March 2017 and developed normal the following days. At the third week of life, the animal was put with other horses and foals on the pasture. The following morning, the foal was lethargic and dyspneic with a body temperature of 40 °C. A veterinarian was called in immediately. The foal was treated with NSAIDs. The body temperature declined within 30 minutes. Another 30 minutes later, the foal showed tachycardia (100/min), reduced breathing rate (3/min) with cyanosis of mucous membranes and died spontaneously. All other horses and foals in the flock were healthy the entire time.

Gross Pathology: Postmortem examination was performed immediately the same morning. The foal was in good body condition and developmental stage. A marked jaundice was visible with intensely yellowish staining of mucous membranes, connective tissue and fasciae as well as the intima of large vessels. The liver was severely enlarged, mottled reddish brown with a friable consistency. White spots were not visible within the parenchyma. The spleen was mildly enlarged. Beside alveolar edema and emphysema of the lungs, other

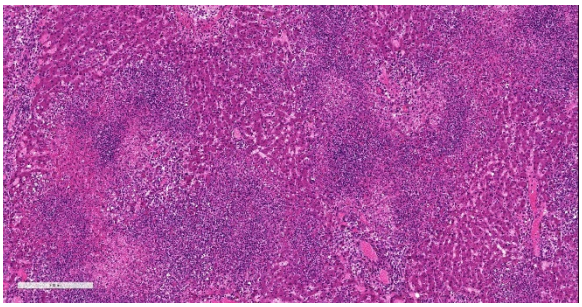
organs and tissues including heart, brain, thymus, umbilicus, and joints were unremarkable macroscopically.

Laboratory results: Virology (detection of cytopathogenic viruses in cell culture): negative

Bacteriology (aerobic and anaerobic culture): numerous colonies of E. coli and Pseudomonas sp. in all organs, few colonies Clostridium perfringens in the small intestine, and unspecific bacteria (e.g. *Acinetobacter johnsonii,* aerobic Bacillus sp., *Enterobacter ludwigii, Providencia rettgeri*) in liver, lung, intestine, spleen and brain

PCR/liver: Amplification of a 633 bp fragment of *Clostridium piliforme* (GenBank accession number L07416) (Fig.1)

Microscopic Description:
Liver: Affecting 80% of hepatic parenchyma are multifocally to coalescing and randomly distributed areas of lytic necrosis characterized by loss of cellular detail and replacement of hepatocytes by nuclear and cellular debris admixed with fibrillar to beaded eosinophilic material (fibrin) and erythrocytes (hemorrhage). Foci measure up to 1.2 mm in diameter and are variably infiltrated by intact and degenerated neutrophils and fewer macrophages. Hepatocytes adjacent to the necrotic areas exhibit either hypereosinophilia with maintenance of cell border and karyorrhectic or pyknotic nuclei (coagulative necrosis). Other hepatocytes are swollen with pale eosinophilic and often finely vacuolated cytoplasm (degeneration). In some hepatocytes at the periphery of the necrosis numerous barely visible filamentous, pale basophilic bacilli (0.5 x 5 □m) can be seen. Portal areas are moderately infiltrated by lymphocytes, plasma cells, macrophages and fewer neutrophils.



Liver, foal. Multifocal to coalescing areas of lytic necrosis occupy about half of the section. (HE, 81X)

Using Warthin-Starry stain argyrophilic filamentous bacteria are visualized within hepatocytes at the periphery of necrotic areas and within the debris (Fig. 2).
Contributor's Morphologic Diagnoses: Liver: Hepatitis, random, necrotizing and suppurative, acute, multifocal to coalescing, severe with intracellular argyrophilic filamentous bacteria. Tyzzer´s disease.

Contributor's Comment: A fatality in a young foal with a rapid course of disease, fever and jaundice, focused the postmortem examination on infectious diseases with highly virulent pathogens. Differentials after necropsy were infection with EHV-1 and bacterial sepsis (e.g. Enterobacteriaceae, *Actinobacillus equuli*). The typical histomorphology with random areas of necrosis and detection of argyrophilic bacteria were highly suggestive of Tyzzer´s disease. This was confirmed by PCR detecting a 633 bp fragment of *Clostridium piliforme* in a liver sample of this foal.

Clostridium piliforme, formerly known as *Bacillus piliformis,* is a gram-negative filamentous bacillus that is not cultivable in cell free media. In domestic animals, young foals are most often affected. Often, they are found dead or die after short illness.[3] Wild rodents are believed to be the reservoir of the bacterium.

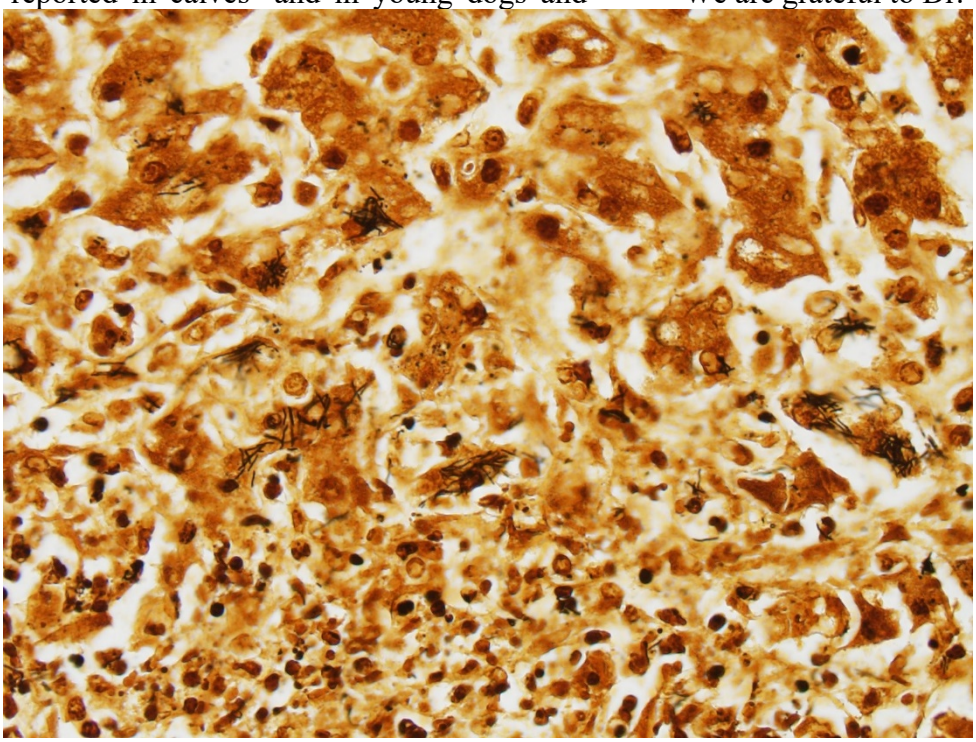
In 2013 Swerczek[11] reviewed 148 cases of Tyzzer´s disease from central Kentucky and highlighted the predispositions, risk factors, and the mode of transmission. Foals in the first month of age are most frequently affected and at the highest risk for Tyzzer´s disease. Adult horses are believed to be resistant. There are more cases in spring in association with heavy rainfall. Interestingly, on 18th of March, two days before this foal came to the pasture, heavy rainfall started in the region of the herd in the western part of Germany (35 l/m^2 rain in 48 hours). It is suggested that foals are infected by consuming feed contaminated with environmental spores of Clostridium piliforme or by ingestion of feces of the mares. Foals show coprophagy only from the second to the fifth week of life.[10]

Some cases of Tyzzer´s disease were reported in calves[4] and in young dogs and cats, some of them suffering from immunosuppression.[3,6,12]

Mongolian gerbils are exquisitely susceptible to fatal Tyzzer´s disease, and many other laboratory animals (e.g. mice, rats, lagomorphs, hamsters, guinea pigs) are at risk for the infection with Clostridium piliforme.[1] Cases were reported in marsupials[2] and birds.[5,7] The infection is extremely rare in nonhuman primates and humans.[9] Recently, a case of Tyzzer´s disease was reported in a captive born cotton-top tamarin. A necrotizing typhlocolitis, accompanied by myocarditis was found additionally to the hepatic lesions .[8]

In the following weeks, no other cases of Tyzzer´s disease occurred in the herd of the affected foal.

We are grateful to Dr. W. Herbst, Institute of Hygiene and Infectious Diseases of Animals, University of Giessen, for performing the PCR.



Liver, foal. Numerous sheaves of 1x4 intracytoplasmic bacilli consistent with Clostridium piliforme are present within the cytoplasm of intact hepatocytes at the periphery of areas of necrosis. (Warthin Starry 4.0, 400X)

Contributing Institution:
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http://www.uni-giessen.de/cms/fbz/fb10/institute_klinikum/institute/pathologie

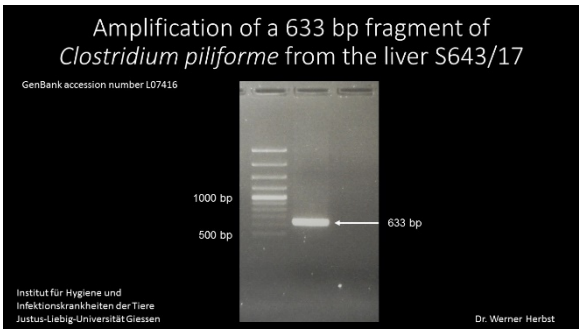
JPC Diagnosis: Liver: Hepatitis, necrotizing, multifocal to coalescing, severe, with intracytoplasmic filamentous bacilli.

JPC Comment: The contributor has provided an excellent review of Tyzzer's disease in a variety of species, and the previous case of Tyzzer's disease in this year's WSC Conference (Conf. 4, Case 1) in the colon of a cat should be reviewed for additional information on this often fatal disease.

In the foal, the disease is considered highly fatal but not contagious. Disease is seen between 1 and 6 weeks, with an average of 20 days.[11] Outbreaks, first identified in Kentucky in 1964, may claim multiple animals on a farm, and are likely the result of soil contamination by these resistant bacilli and ingestion by foals. Another postulated route of infection is directly from the mare's feces. It is thought that increased nutrition of nursing mares may result in a bloom of *C. piliforme* within the intestine. *C. piliforme* infection is extremely rare in adult healthy horse, but will result in an increased numbers within feces, and coprophagic foals may receive a large oral inoculum in this fashion.[11]

As opposed to the diseases in laboratory species, most cases of Tyzzer's disease in the horse are primarily hepatic in nature. Enteric lesions may be present, but diarrhea is rare, and myocardial lesions are also very rare.

The moderator and attendees discussed the use of terms subacute and acute when describing this lesion. While a consensus was not reached, and both sides provided viable arguments, the moderator stressed the need for precision in the usage of such terms in reporting out equine cases, especially in insurance cases.



A 633bp fragment of C. piliforme was idenfied by PCR from the liver of this foal. (Photo courtesy of Dr. Werner Herbst, Institute fur Hygiene und Infektionsksrakheiten der Tiere Justus-Liebig-Universitat Giessen)

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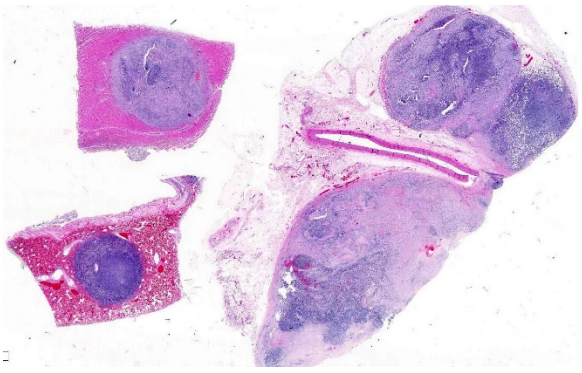
CASE IV: 16L-2013 (JPC 4085442).

Signalment: 1 year 5 month old female neutered Newfoundland (*Canis lupus familiaris*) dog.

History: The animal presented to the Department of Veterinary Neurology at the University of Liverpool's small animal teaching hospital with a history of chronic right pelvic limb monoparesis and lameness that had significantly worsened in the last 2-3 weeks prior to presentation along with severe weight loss. The referring veterinary surgeon had performed CBC and biochemistry which were unremarkable; gracillis muscle biopsy that revealed muscle atrophy of unknown etiology. On clinical examination patella reflex was absent in the right hind limb with mildly reduced withdrawal reflex. Magnetic resonance imaging (MRI) was performed and revealed an infiltrative paravertebral mass and due to the guarded prognosis euthanasia was performed and a full post mortem completed.

Gross Pathology: There was a large lumbar to sacral paraverterbral multi-lobulated tan firm infiltrative mass within the paravertebral right muscles extending from the level of L4 to the sacrum and expanding into the retroperitoneal space measuring 18 x 6 x 9

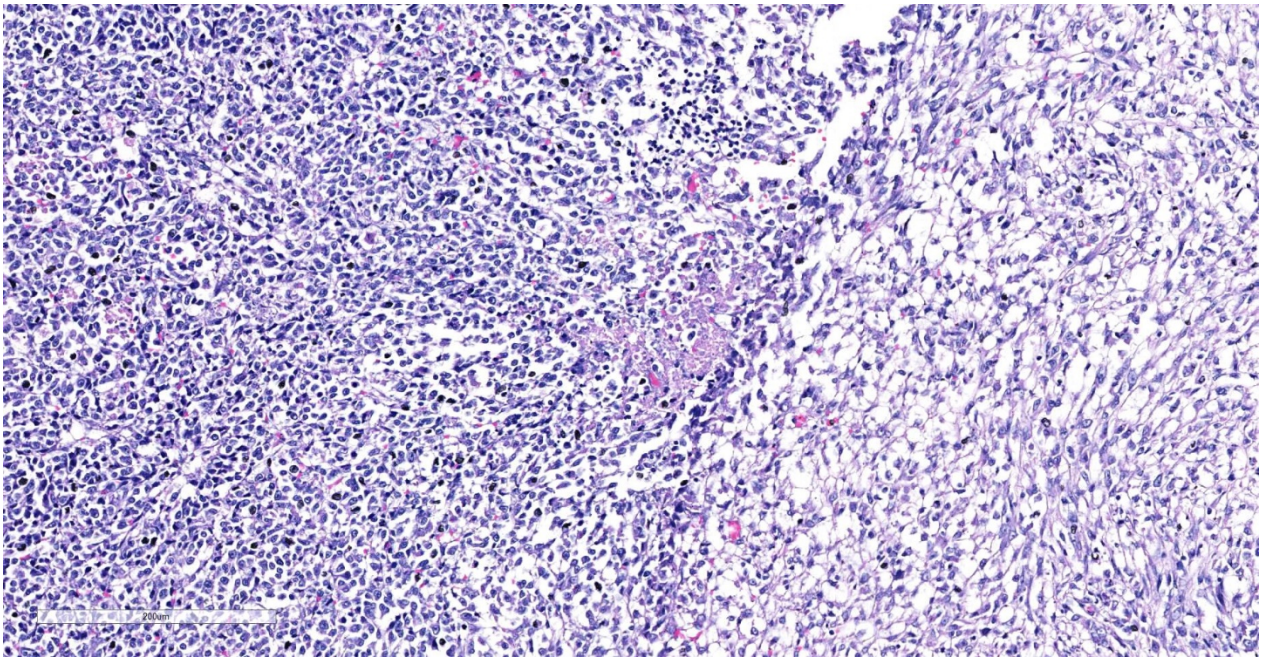


Spinal musculature (per contributor), heart, lung. dog: Sections from multiple tissues, each with a neoplasm in it, are presented for evaluation.

cm. The mass invaded the right side of L5-L7 vertebrae. Similar tan masses up to 2 cm in diameter were observed randomly scattered in the liver, pancreas, omentum, lung and myocardium. There was also enlargement of the right ovary by a tan firm mass measuring 7 x 4.5 x 2 cm. Multifocal masses with full thickness ulcerations were observed in the gastric mucosa of the fundic region.

Laboratory results: MRI of lumbar spine: there was a large (18 x 6 x 9 cm) irregularly shaped and multilobulated complex soft tissue mass located in the paraspinal soft tissue ventral to the caudal lumbar spine extending from the level of L4 to the sacrum. The mass filled the right side of the epidural space and wrapped around the cauda equine and the caudal spinal cord. The mass invaded the right side of L5-L& vertebrae. The mass caused ventral displacement of the abdominal organs and compression of the aorta and caudal vena cava.

Cytology: large numbers of neoplastic cells arranged in loose aggregates, moderately cohesive sheets or individualized amid a finely stippled pink background. Occasional macrophages with erythrophagia and intracytoplasmic hemosiderin are scattered throughout. Neoplastic cells are pleomorphic spanning in morphology from small round cells with a high nuclear to cytoplasmic ratio, round nucleus, granular chromatin and scant blue cytoplasm to polygonal cells with more abundant and often vacuolated cytoplasm to clearly fusate cells with oval to reniform nuclei sometimes embedded amid scant pink extracellular matrix and occasionally surrounded by capillaries. Nucleoli are consistently large and pale blue, in number of one to three. Mitoses are occasionally seen. Rarely, small variably sized round dark blue granules (possible hemosiderin) are noted within the cytoplasm. Occasionally, erythrocytes are closely associated with these cells or a single erythrocyte is seen intracellularly. Anisocytosis and anisokaryosis are moderate. Rare elongated multinucleated cells are noted (either neoplastic cells or ('strap cells'). In one slide small fragments of skeletal muscle myocytes



Paravertebral muscle tumor: Neoplastic skeletal muscle cells assume two morphologies in this field - a alveolar pattern (left) and more typical spindle at right. (HE, 178X)

with occasional centralized nuclei are noted scattered amid the neoplastic cells.

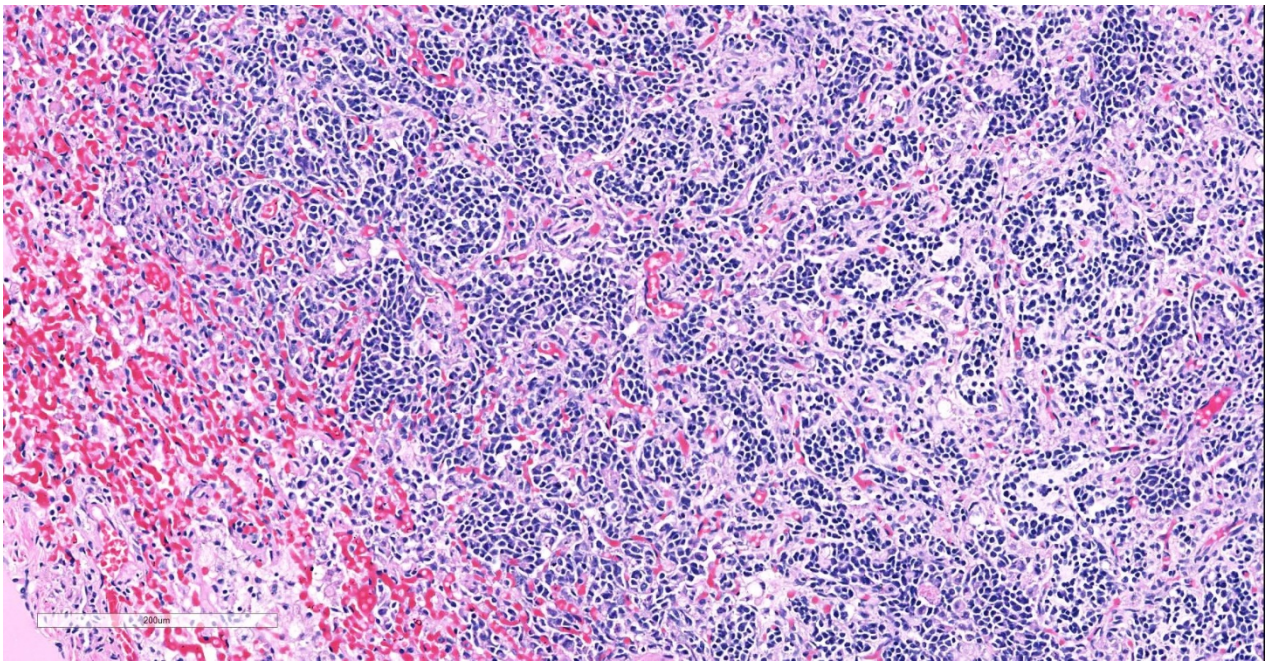
Cytological findings are most suggestive of a sarcoma possible rhabdomyosarcoma.

Trucut biopsy: Focally within the section, cells arranged in linear streams within a moderate amount of collagenous stroma, are elongated with indistinct cell borders, variably sized from 40 x 20 µm up to 300 x 200 µm with abundant pale to brightly eosinophilic cytoplasm, sometimes containing striations, and multiple, up to 7, central to para-central nuclei. Chromatin is finely stippled to coarsely clumped, often with a central prominent nucleolus. Adjacent to this there are extensive to polygonal, with indistinct cell borders, approximately 30 x 10 µm with abundant eosinophilic cytoplasm and a central elongated vesiculated nucleus. Chromatin is finely stippled and nucleoli are often indistinct. There is scant fibrovascular stroma and scattered necrotic cells. Mitotic figures are rare. There is mild to moderate multifocal mixed (neutrophils-dominated) inflammation.

Immunohistochemistry:
Immunohistochemistry for pancytokeratin, vimentin and myoglobin was performed on sections of the skeletal muscle mass, the mass in the myocardium and on the mass in the lung. The neoplasm was strongly positive for vimentin and negative for pancytokeratin. There were approximately 40% of neoplastic cells that were positive for myoglobin.

Microscopic Description:
Skeletal muscle: Completely effacing the skeletal muscle there is a non-encapsulated, poorly demarcated, densely-cellular and infiltrative neoplasm. The neoplasm is biphasic with one population of cells arranged haphazardly into streams and the second population arranged into sheets. Cells of the first population are spindled with indistinct cell borders, approximately 15-40 µm with abundant pale eosinophilic cytoplasm and usually a single centrally placed nucleus. Occasionally these cells are large and contain many up to 6 nuclei arranged in a row (strap cell, fig. 4, variable between slides). Chromatin is finely stippled to coarsely clumped, and nucleoli vary from indistinct to a prominent single nucleolus to up to 3 nucleoli. Anisocytosis and anisokaryosis are marked and mitotic index is 1 per 10HPF. The second population is interwoven with the first and is composed of sometimes densely packed sheets and other times with a looser arranged alveolar-like structure (fig. 5) with cells sloughed into a lumen both with a fine fibrovascular stroma. Cells are round with mostly distinct cell borders, approximately 20-30 µm diameter with a moderate amount of eosinophilic cytoplasm and a large round to oval central to para-centrally placed nucleus (rhabdomyoblasts). Occasionally cells are multinucleated and several nuclei are aligned in a row. Chromatin is finely stippled and dense to coarsely clumped and open with nucleoli variable from indistinct up to 3 per nucleus. Anisocytosis and anisokaryosis are marked and mitotic index is 6 per 10 HPF. Multifocally throughout the neoplasm there are large areas of necrosis and smaller areas of hemorrhage.

Heart: Within the myocardium there is an approximately 6 mm diameter well demarcated, non-encapsulated densely cellular neoplasm. The neoplastic cells are arranged haphazardly in streams and with a smaller population arranged in sheets with a moderate quantity of fibrovascular stroma. Cells arranged in streams are spindled with indistinct borders, approximately 5 x 30 µm with abundant eosinophilic cytoplasm and an elongated centrally placed nucleus. While cells arranged in sheets are round to



Lung, dog. Within the lung, neoplastic cells assume a very definitive alveolar pattern. (HE, 176X)

polygonal with distinct cell borders, approximately 10-20 µm in diameter with a moderate amount of eosinophilic cytoplasm and a large round to polygonal central to para-centrally placed nucleus. Occasionally in both populations there are large elongated multinucleated cells with nuclei arranged in a row (strap cells, variable between slides). Chromatin is finely stippled to coarsely clumped with from indistinct up to 3 nucleoli per nucleus. Anisocytosis and anisokaryosis are marked and mitotic index is 9 per 10 HPF which are often bizarre.

Multifocally scattered throughout the neoplasm there are areas of lytic necrosis admixed with small amounts of haemorrhage. Also scattered throughout the neoplasm there are areas of deeply basophilic irregular vacuolated material.

Lung: In the lung there is and approximately 5 mm diameter non-encapsulated, reasonably well demarcated but infiltrative, densely cellular neoplasm. The neoplasm is tri-phasic with cells arranged in sheets, streams, cords and packets and sometimes reproducing alveolar-like structures divided by abundant fibrovascular stroma. Cells are hyperchromatic, round to polygonal to spindled with mostly distinct cell borders, approximately 10 µm diameter up to 15 x 30 µm and 10 x 40 µm with a moderate amount of eosinophilic cytoplasm and a central to para centrally placed, round to polygonal to elongated hyperbasophilic nucleus. Chromatin is finely stippled to coarsely clumped and there are indistinct up to 3 nucleoli per nucleus. Cells are highly pleomorphic and mitotic index is 23 per 10 HPF often with bizarre mitotic figures. There are large areas of coagulative necrosis multifocally within the neoplasm and smaller areas of hemorrhage. The lung is diffusely moderately hyperemic.

Contributor's Morphologic Diagnoses:
Skeletal muscle rhabdomyosarcoma, alveolar type
Heart metastatic rhabdomyosarcoma
Lung metastatic rhabdomyosarcoma

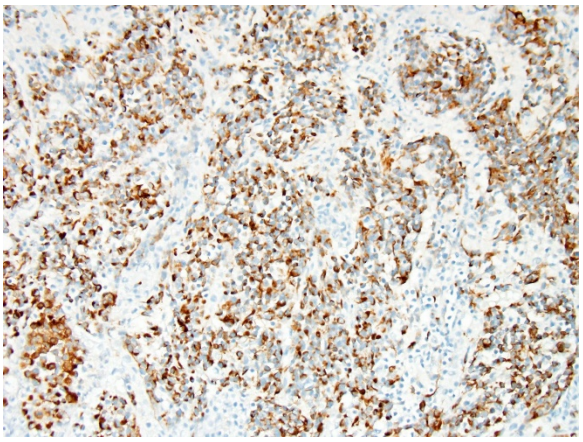
Contributor's Comment: Rhabdomyosarcoma (RMS) is the malignant neoplasm derived from skeletal muscle. They are highly variable in-terms of age of onset, location, gross and histological appearance[1,2,3,4,5,10]. RMS are common in human children and juvenile dogs under 2 years old[2]. Both the human and veterinary RMS can be separated into different categories depending on the various degrees of differentiation towards skeletal muscle.
The veterinary RMS can be subclassified as follows[2,4,5,6,7]:

- **Embryonal RMS**: embryonal RMS can be further divided due to the cell morphology into Myotubular, Rhabdomyoblastic and Spindle cell. Another embryonal RMS is the Botryoid embryonal RMS. All embryonal RMS are most frequently encountered in juvenile animals (less than 2 years old).
    - Myotubular – this is a RMS that is dominated by classical multinucleated strap cells with frequently observable cross-striations.
    - Rhabdomyoblastic – is composed of mostly small round to polygonal cells with abundant eosinophilic cytoplasm and only occasionally cells with cross striations. This may be confused with the solid variant of the alveolar RMS.
    - Spindle cell – The RMS is composed of mostly spindle shaped cell in low cellularity often arranged in a storiform pattern.
    - Botryoid RMS – RMS that is usually found in the trigone region of the urinary bladder with predominance in the female population and large breed dogs. The term botryoid is used due to the macroscopic grape like appearance. The tumor must be contained in the submucosa.

- **Alveolar RMS**- the alveolar pattern is determined by the presence of an area of neoplastic cells that are arranged in a glandular like pattern to give "alveolar" spaces. There is also a solid variant where the cells are in dense sheets. Often in the alveolar RMS there will only be a small area displaying the "alveolar" pattern and the rest of the neoplasm is in sheets or anaplastic. Neoplastic cells tend to be round with small to moderate amounts of eosinophilic cytoplasm and multinucleated giant cells (strap cells) that are common in the embryonal RMS are uncommon in the alveolar variant.

- **Pleomorphic RMS**- rare as only RMS that do not display any features of embryonal or alveolar RMS can be termed truly pleomorphic. The histological features are of a mesenchymal neoplasm with marked anisocytosis and anisokaryosis and bizarre mitotic figures. The pleomorphic RMS tends to be observed in adult rather than juvenile animals.

RMS is one of the most common neoplasms in human children under 15 years old with there being a difference in survival time between embryonal and alveolar variants[1,6,7,8,9]. It is therefore important to be able to differentiate between the subtypes. The prognostic impact of dividing RMS into subclasses has not yet been determined for



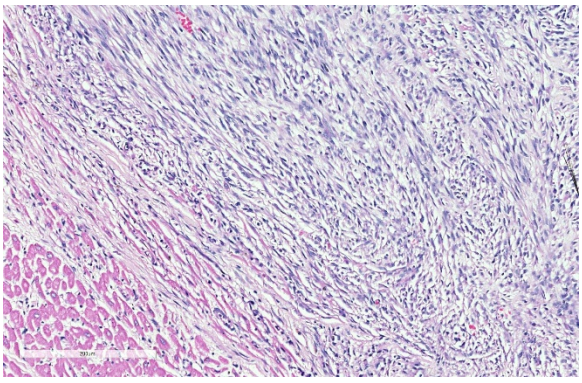
Lung, dog. Neoplastic cells within the lung stain strongly immunopositive with desmin. (anti-desmin, 200X)

veterinary species however in human medicine the pleomorphic and the alveolar variants have the worse prognosis with the embryonal variants having a more favorable outcome[6].

Differential diagnosis for RMS are nephroblastoma, germ cell tumors, malignant peripheral nerve sheath tumors and anaplastic sarcomas[2,6]. It can be extremely difficult to differentiate some RMS on histological cell morphology alone especially if there are low numbers of strap cells. Therefore further diagnostic techniques such as immunohistochemistry and electron microscopy are often required[2,4]. Due to RMS being a mesenchymal proliferation neoplastic cells label strongly for vimentin. Desmin may be useful but labelling can be uneven in both human and veterinary species. Smooth muscle actin can be used to rule out leimyosarcomas[2]. Myoglobin expression may be decreased or lost on undifferentiated rhabdomyosarcomas.
Immunohistochemistry was performed on our case which was strongly positive for vimentin and many neoplastic cells were positive for myoglobin and all neoplastic cells were negative for pan-cytokeratin.

The case presented here is most likely the alveolar variant of a rhabdomyosarcoma due to the areas within the primary and the metastatic neoplasms having alveoli-like structures. All rhabdomyosarcomas have a degree of pleomorphism and only if there is no evidence of embryonal or alveolar differentiation then the pleomorphic variant can be diagnosed[6]. A variant described in human medicine but not in veterinary medicine is the spindle cell/sclerosing rhabdomyosarcoma[11]. The spindle cell/sclerosing RMS is described as being composed fascicles of spindle cells or primitive round cells embedded in sclerotic matrix with varying numbers of rhabdomyoblasts[11] which can often mimic the classic alveolar pattern[9] and is consistent with some areas in the case presented here.

Clinical features of all RMS vary depending on the location of the neoplasm. Embryonal RMS (except botryoid RMS) are usually observed in the head and neck regions[2]. Alveolar RMS can be found in a wide range of regions and pleomorphic RMS tend to be within the skeletal muscle[2,4].



Heart dog. Neoplastic cells within the heart assume a more traditional spindle cell "myotube appearance. (HE, 176X)

Contributing Institution:
University of Liverpool, Leahurst Campus, Chester High Road, Neston, Wirral, UK; https://www.liverpool.ac.uk/vetpathology/

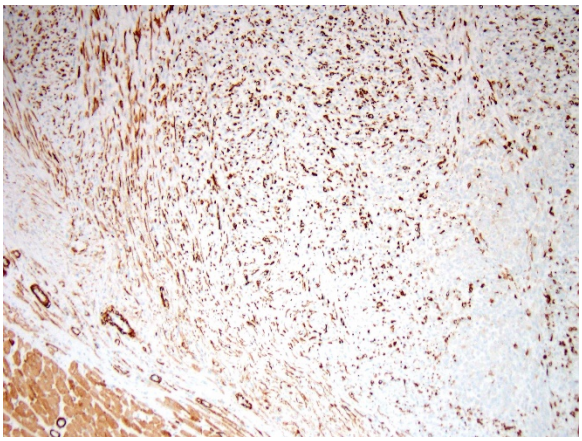
JPC Diagnosis: Paraspinal fibrovascular tissue (per contributor): Rhabdomyosarcoma, alveolar type.

Heart, lung: Rhabdomyosarcoma, alveolar type, metastatic

JPC Comment: The contributor has provided an excellent review of the subclassification of rhabdomyosarcoma in human and veterinary species, and in doing so, has illustrated the difficulty so often encountered in trying to classify them. This is also a process that, as mentioned by the contributor, is not of as much importance in veterinary medicine as it is in human medicine, where prognostic information is based on precise classification. .

The neoplasm in this case shows two definitive phenotypic morphologies – that of a spindle cell tumor (myotubular) with few classic strap cells, as well as the nesting and packeting of cells with a polygonal appearance, the so-called alveolar pattern (presence in the lung here notwithstanding. The primary neoplasm of the paraspinal musculature demonstrates both patterns, while in the metastatic nodule in the lung the alveolar pattern predominates, and in the heart, the spindle cell pattern predominates (likely attesting to different microenvironments in various metastatic sites.

A number of immunohistochemical markers were applied on this section, although the morphology of the neoplasm is strongly suggestive of a skeletal muscle, and the metastatic nodules meets the most critical



Heart dog. Neoplastic cells within the heart stain strongly immunopositive with muscle specific actin. (anti-MSA, 200X)

determinant of malignancy. Vimentin was not used in this case due to the obvious spindle cell morphology – quite a number of human pathologists at the JPC eschew the use of vimentin in many subspecialties. The appropriate choice of immunostains in muscle neoplasms may not only indicate a diagnosis, but also yield clues as to the degree of differentiation. Desmin and muscle-specific actin are non-specific muscle markers that will stain smooth and cardiac muscle as well as myofibroblasts. Smooth muscle actin should not stain skeletal muscle but may be an important part of an immunopanel to rule out smooth muscle tumors. Myoglobin is expressed in skeletal and cardiac muscle, but not smooth muscle, and may or may not be expressed in skeletal muscle malignancies, especially poorly differentiated tumors. Myogenin and myoD1 are skeletal muscle-specific nuclear products which are expressed early in development, and may be critical in identifying poorly differentiated neoplasms in veterinary medicine, and have shown utility in differentiating embryonal and alveolar rhabdomyosarcomas in human cases.

The stalwart procedure of identifying cross-striations on a Masson's trichrome or

phosphotungstic acid hematoxylin (PTAH) stain or ultrastructural analysis has largely been supplanted by the use of immunostains and lack of disposable time and patience by today's veterinary pathologist.

The precise diagnosis in this case was also a subject of lively discussion. Some attendees favored a simple diagnosis of rhabdomyosarcoma, suggesting that the biphasic nature of this neoplasm may simply be a factor of sampling (as is often seen in osteosarcoma), or perhaps factors associated with metastatic environment. Ultimately, upon review of gross necropsy findings and current classification (as listed above), the group agrees with the contributor based on the distribution of metastases in multiple tissues as well as the alveolar pattern seem most prominently in the pulmonary metastatic focus.

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