Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2019-2020

C o n f e r e n c e 25

13 May 2020

CASE I: 15/692 (JPC 4137583).Tissue from a horse (*Equus caballus*).

Signalment: 11 years, mare, American Quarter Horse, *Equus caballus*, horse.

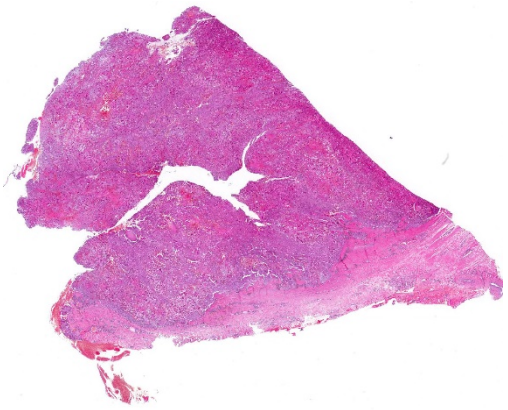
History: The horse had a firm, well-defined tumor on the left side of the neck. The tumor was excised for pathological examination.

Gross Pathology: A skin biopsy measuring 4x2x2 cm was received. There was a tumor in the dermis, not adherent to the epidermis, that consisted of a brown, homogeneous but somewhat lobulated, medium firm tissue. The tumor was surrounded by a thin sheath of connective tissue in the deeper areas and on the sides.

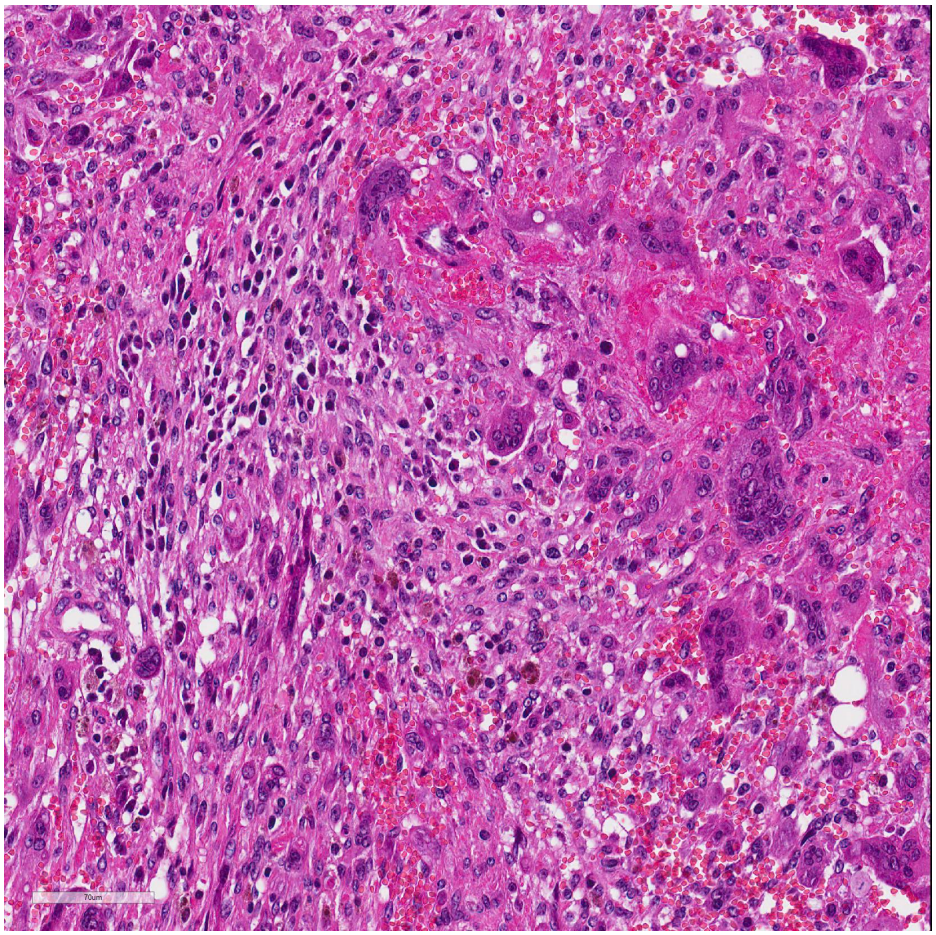
Laboratory results: None.

Microscopic Description: In the deep dermis of the skin there is a non-encapsulated, infiltrative, medium cell rich, moderately demarcated tumor that consists of spindle shaped cells and numerous multinucleated giant cells (MGCs), in sparse amounts of stroma. There are two growth patterns in the tumor, one with tightly packed spindle shaped cells growing in bundles with few MGCs and another with numerous MGCs and fewer loosely arranged spindle cells.

The spindle shaped tumor cells are medium sized, with small to moderate amounts of eosinophilic cytoplasm and an oval to cigar



Subcutis, horse: Dermal elements are effaced by a multilobular neoplasm which extends to three cut borders. (HE, 5X)



Subcutis, horse: The neoplasm is composed of two distinct cell types with a spindle cell component (left), and multinucleated giant cells (right). The stroma contains low to moderate numbers of lymphocytes and plasma cells, hemorrhage, and hemosiderin-laden macrophages. (HE, 300X)

shaped nucleus with finely stippled chromatin and 1-3 small nucleoli. The spindle shaped cells have mild to moderate anisocytosis and anisokaryosis. The MGCs are elongated, oval or round, they have abundant eosinophilic cytoplasm and oval or round nuclei with finely stippled chromatin and 1-3 small nucleoli. The MGCs are highly variable in shape, size, and nucleus number (up to 37). Mitotic figures are 2 per 10 HPF.

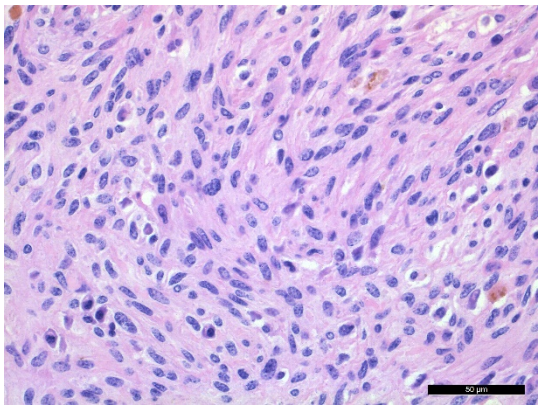
There are some multifocal necrotic areas in the tumor tissue. The areas with numerous MGCs often have hemorrhage, and there are multifocal inflammatory cell infiltrates with lymphocytes, plasma cells and macrophages with yellow to brown pigment (hemosiderin). Small amounts of pigment is also present in the cytoplasm of some MGCs. No acid-fast mycobacteria was detected in a Ziehl-Neelsen stained section.

Immunohistochemistry staining with the muscle marker desmin showed positive staining in the spindle shaped cells. Immunohistochemistry staining with antibody for α smooth muscle actin was variable. Areas with compact growth of spindle shaped cells had a combination of strongly positive cells and negative cells. In areas with numerous MGCs, the spindle cells were weakly positive. All MGCs were negative, in both desmin and α smooth muscle actin stained slides.

Contributor's Morphologic Diagnosis:

Skin: Undifferentiated pleomorphic sarcoma with numerous MGCs

Contributor's Comment: Undifferentiated pleomorphic sarcoma is a rare tumor that also has been termed malignant fibrous histiocytoma, giant cell tumor of soft parts, extraskeletal giant cell tumor and anaplastic



Subcutis, horse: Higher magnification of the spindle cell component of the neoplasm. (Photo courtesy of: Norwegian University of Life Sciences, Faculty of Veterinary Medicine www.nmbu.no)

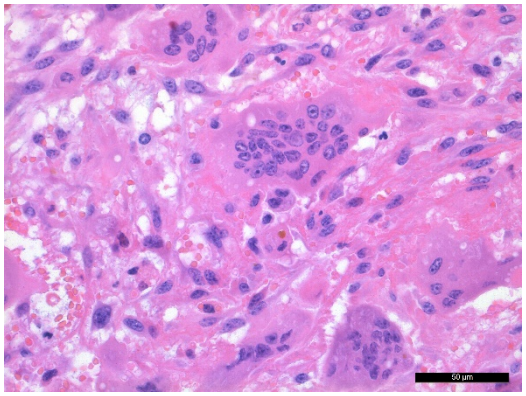
sarcoma with giant cells. The cellular origin of the tumor cells are controversial. Sarcomas with combined spindle shaped cells, vacuolated histiocyte-like cells, and variable amounts of pleomorphic MGCs and a collagenous stroma have in the past often been diagnosed as malignant fibrous histiocytoma. But the term undifferentiated pleomorphic sarcoma is now preferred as these tumors most likely represent a diverse group neoplasms.[5,6]

A meta-analysis of 43 case reports and case series, totaling 82 human patients, showed that giant cell rich malignancies may be of highly variable origin, and may arise from both skeletal and many extraskeletal tissue types.[4]

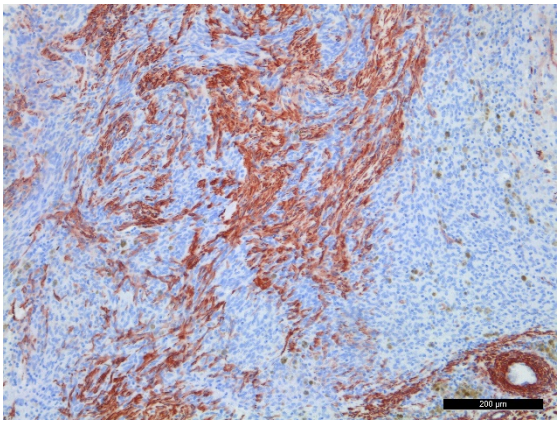
The tumor in the present case is dominated by spindle shaped cells and numerous MGCs, with few mononuclear histiocyte-like cells. Also in areas with numerous MGCs, most other cells were spindle shaped. The spindle shaped cells were immunohistochemically positive for desmin and variably positive for α smooth muscle actin.

In a study of 21 cases of equine giant cell tumor of soft parts,[2] tumors with a morphology comparable to the present case were described. The tumor cells were immunohistochemically positive for vimentin and negative for cytokeratin, smooth-muscle actin, CD3, CD79a, CD31 and desmin. CD18 expression was detected only in the MGCs in the tumors, and it was suggested that the MGCs represent a secondary non-neoplastic cellular population.

It is suggested that within the undifferentiated pleomorphic sarcoma group there is a tumor of primitive myofibroblasts origin in dogs and cats that is analogous to the entity malignant fibrous histiocytoma (MFH) in humans.[5] The fibroblastic/myofibroblastic cells in MFH stain positive for vimentin and variably for actin and desmin, and the MGCs should show the same positivity as the fibroblastic cells.[5] This was however not the case in the present tumor, where desmin and actin negative MGCs were present between desmin and smooth muscle actin positive spindle shaped cells.



Subcutis, horse: Higher magnification of the multinucleated component of the neoplasm. (Photo courtesy of: Norwegian University of Life Sciences, Faculty of Veterinary Medicine www.nmbu.no)



Subcutis, horse: Spindle cells demonstrate multifocal smooth muscle actin positivity. (Photo courtesy of: Norwegian University of Life Sciences, Faculty of Veterinary Medicine www.nmbu.no) (anti-SMA, 200X)

Specific antibodies for further differentiation between myofibroblasts and smooth muscle cell differentiation or origin were not available. Thus a distinction between a myofibroblastic fibrosarcoma with MGCs or a leiomyosarcoma with MGCs could not be made. In human leiomyosarcomas, a giant cell variant is recognized, and in these tumors the MGCs are proposed to be osteoclast-like cells.[3]

Contributing Institution:

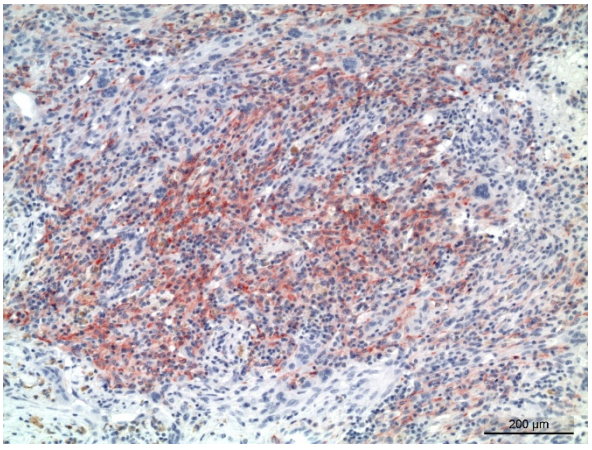
Norwegian University of Life Sciences, Faculty of Veterinary Medicine
www.nmbu.no

JPC Diagnosis: Subcutis (presumptive): Pleomorphic (anaplastic) sarcoma with giant cells.

JPC Comment: The contributor has provided a concise review of this tumor, which has been shrouded in controversy for many years. It has appeared twice in the WSC in the subcutis of the horse (WSC 1994-1995, Conf 20 Case 3 and WSC 1991-1992 Conf 3, Case 4). Two submissions of malignant fibrous histiocytoma in the skin of the rat have also been submitted (WSC 1976-1977, Conf 29 Case 1 and WSC 1987-1988, Conf 21, Case 4.)

Giant cell tumors of soft parts have been described in a number of species, including baboons, Syrian hamsters, cats, and in a mule, as well as humans.[2] A recent publication describes them in rabbits.[1] In the rabbit, they most often arise in the subcutaneous tissues as well. In humans, they may be classified as superficial (affecting the skin and subcutis) or deep (affecting tendons, fascia, and skeletal muscles of the thighs). Deep tumors often recapitulate giant cell tumors of bone, and are positive for CD68 and tartrate-resistant acid phosphatase (TRAP) and occasionally positive for smooth muscle actin (as seen in this case).[2]

One of the major differentials for this neoplasm, both from a morphologic and a immunohistochemical standpoint, is



Subcutis, horse: Spindle cells demonstrate multifocal desmin positivity. (Photo courtesy of: Norwegian University of Life Sciences, Faculty of Veterinary Medicine www.nmbu.no) (anti-desmin, 100X)

histiocytic sarcoma. In the study of 21 horses with this neoplasm, 19/21 horses demonstrated CD18 positivity within the multinucleated giant cell population, but not in the spindle cell population.[2] For this reason, several authors have posited that the multinucleated cell population may be a reactive population of histiocytic cells in a spindle cell neoplasm[2,5], but exactly why they arise in a neoplasm with a fibroblastic/myofibroblastic phenotype is yet unclear.

A recent report of a tumor of this type in a Warmblood horse discusses another variant of this tumor, which has years ago been included under the umbrella term "giant cell tumor of soft parts" but more recently termed "benign giant cell tumor of tendon sheath."[7] This particular variant has been reported in dog, cats and horses. Other authors believe this neoplasm actually represents a form of pigmented villonodular synovitis, as it often arises in or near joints and shares a common feature of numerous hemosiderin-laden macrophages.[7] A case of pigmented villonodular synovitis in a reticulated giraffe appeared in the WSC 2015-2016 as Conference 9, Case 1, and the case discussion reflects a similar controversy of nature and origin.

The immunohistochemistry reported by the contributor in this case is also somewhat problematic, as the 21 cases in the retrospective by Bush *et al.*[2] were negative for smooth muscle actin and desmin, but this particular neoplasm demonstrated patchy immunopositivity. This is in agreement with the current thinking that this neoplasm, which has gone under many names over the years, is not a single neoplasm with different variants, but a collection of several neoplasms which are histologically and immunohistochemically diverse.[2,5] Consultation with a veterinary pathologist with extensive experience in similar tumors suggested an alternative theory on the origin of these lesions, in that they may be reparative in nature rather than neoplastic (personal communication, L. Craig, University of Tenn.) A similar morphology is seen in peripheral giant cell granulomas in the oral cavity of the dog and cat – a biphasic population of spindle and multinucleate cells within a background of granulation tissue. (See WSC 2017-2018 Conf 11, Case 4). While the origin of these tumors is not yet elucidated in veterinary species, human pathologists have considered peripheral giant cell granulomas to represent a reparative rather than neoplastic response.

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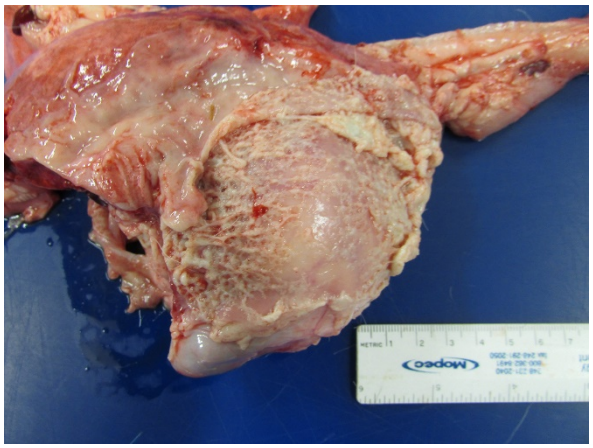
CASE II: MU11478 (JPC 4115990).
Tissue from a horse (*Equus caballus*).

Signalment: A 20-day-old female brown and white goat (*Capra aegagrus hircus)*.

History: This goat had a weeklong history of lameness in the left front leg. It improved slightly after a dose of banamine and florfenicol and then lameness progressed. At the time of euthanasia, there was swelling and clinical lameness in both carpal joints.



Heart, goat. The pericardial sac contains abundant fibrin. (Photo courtesy of: Veterinary Medical Diagnostic Lab, University of Missouri, www.vmdl.missouri.edu)

The goat was nursing poorly, and the owner requested euthanasia on humane grounds.

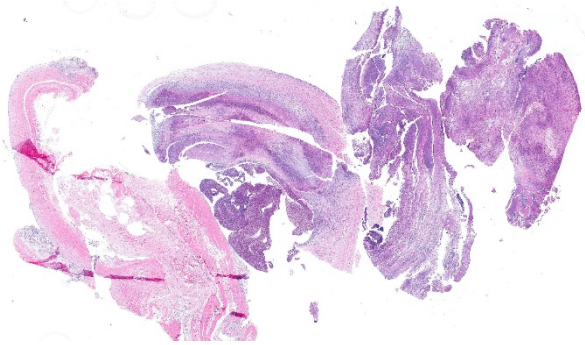
Gross Pathology: The goat kid weighed 5.7 kg at necropsy. Lesions were limited to the joints. In addition, there was marked, tan to white, caseous to fibrinous exudate, with increased synovial fluid volume, expanding multiple joints and multifocally infiltrating into the surrounding extracapsular tissue. The left and right shoulders, carpi, coxofemoral, right stifle, and right tarsal joints were affected.

The thoracic cavity contained marked, multifocal, yellow-tan fibrinous exudate that was multifocally adhered to the pleura, body wall, and pericardium, with numerous easily broken adhesions. The lungs are multifocally tan and red, mildly firm, and float in formalin.

Laboratory results: Small colonies of bacteria were isolated on sheep blood agar from swabs of the right carpus, right shoulder and left fetlock. Polymerase chain reaction identified them as *Mycoplasma mycoides* subsp. *capri.*

Microscopic Description: In all joints examined, there was marked edema and inflammation of the synovial membrane. The least affected specimen still contains recognizable synovial villi (Fig 2). Neutrophilic inflammation occurred in the joint lumen and infiltrating villi (Fig 3), admixed with other leukocytes. The synovial lining was ulcerated in other joints, with more fibrin in the joint lumen. The synovium and joint capsules were expanded by edema, moderate fibrosis and granulation tissue. Multifocally within the synovium,

capsule, joint space, ligament, and adjacent fat and muscle, there were aggregates of



Joint, goat. The joint capsule and synovium is at left, several large lamellated clots of suppurative inflammation are present to the right. (HE, 7X)

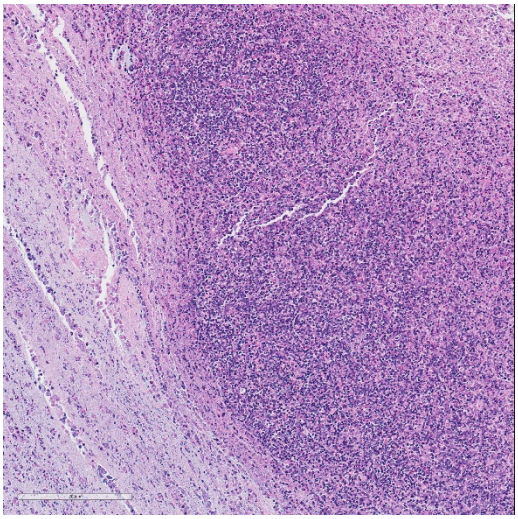
necrotic neutrophils with fewer macrophages, similar to those comprising the separated inflammation in the specimens submitted. Unfortunately, in these particular specimens, it is only possible to identify the sheets of necrotic neutrophils, not their location. The extent of inflammation is illustrated in Fig 4, taken from another affected joint.

Contributor's Morphologic Diagnosis:

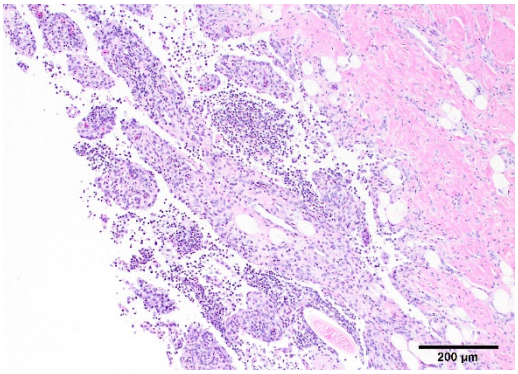
Subacute fibrinosuppurative and necrotizing polyarthritis

Contributor's Comment: The synovial lesions found in this goat are characteristic of *Mycoplasma mycoides* subsp. *capri* infection, with regard to the extent and severity of inflammation. Clumps of degenerate neutrophils and extension of inflammatory cells across the joint capsule and occasionally into muscle are dissimilar to arthritis of neonatal bacteremia, or early lentiviral infection. Several species of mycoplasma, including *M. capricolum* subsp. *capricolum* (the etiology of contagious caprine pleuropneumonia), *M. putrifaciens* and *M agalactia* can produce similar arthritis.[9,11] This particular goat had fibrinous pleuritis as well, and pleuropneumonia is often described as the most common pathologic finding in herd outbreaks, which can produce explosive disease and high morbidity and mortality. In contrast, arthritis associated with neonatal bacteremia yields a more localized suppurative intra-articular exudate with less extensive synovitis. Lentiviral infection leads to synovial proliferation and a lymphohistiocytic exudate.

Mycoplasma mycoides subsp. *capri* has been described in herd outbreaks involving hundreds of goats,[3,5,12] and, although this outbreak involved only kids less than a month of age, adults can also be affected. Adults can carry the organism in the ear canal, and isolation or PCR are the main methods of detecting carriers to eliminate them from the herd.[1] Kids acquire the infection by ingesting the agent through milk or colostrum containing mycoplasma.[5] Experimental infection after systemic



Joint, goat. Higher magnification of the lamellated suppurative exudate within the joint. (HE, 182X)



Joint, goat. Remnant synovial villi are surrounded and infiltrated by large numbers of neutrophils. (Photo courtesy of: Veterinary Medical Diagnostic Lab, University of Missouri, www.vmdl.missouri.edu) (HE, 200X)

innoculation[8] recapitulates the disease in multiple organ systems.

It is important to recognize that organisms of the mycoides group of mycoplasmas is somewhat distantly related from most other species[6,4] and that PCR tests that can speciate this group of organisms must be used or the organism may escape detection.[2,7,10]

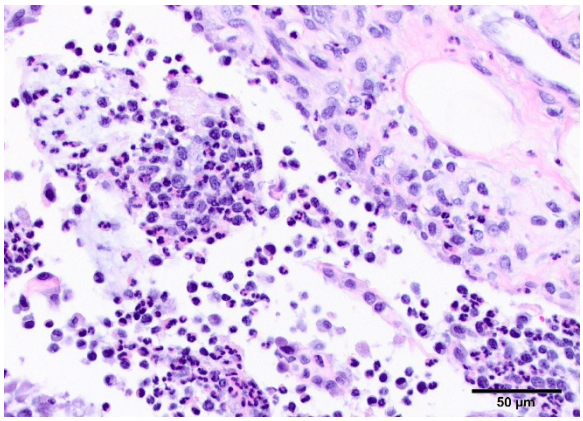
Contributing Institution:

Norwegian University of Life Sciences, Faculty of Veterinary Medicine
www.nmbu.no

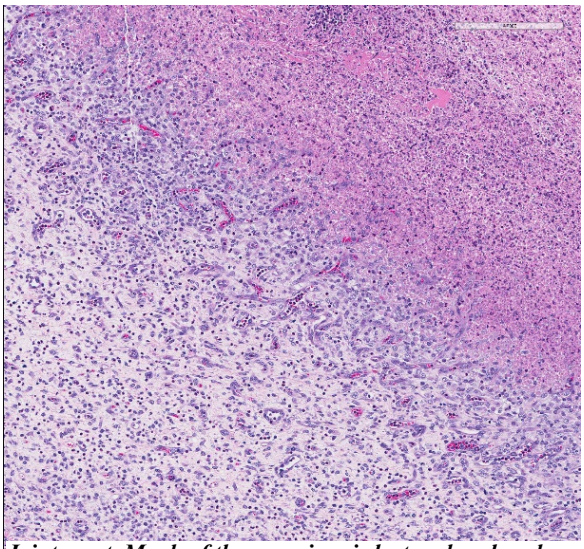
JPC Diagnosis: Synovium and periarticular soft tissue: Synovitis, fibrinosuppurative, chronic, diffuse, severe, with synovial ulceration and granulation tissue formation.

JPC Comment: Outbreaks of disease in young goats due to *Mycoplasma mycoides* subsp. *capri* have been reported to cause serious disease around the world for many years. In addition to polyarthritis, the agent may also result in septicemia as well as respiratory disease in kids. The disease is introduced into a herd by healthy carriers, and as mentioned by the contributor, transmitted to young kids via infected milk (up to 50% of does shedding mycoplasma in the milk do not show clinical signs).[1] The feeding of pooled milk by bottle appears to be the most common route of infection. Disease outbreaks often occur following stress, such as the onset of cold weather or overcrowding. Infected animals may be successfully treated with tylosin, and closer attention to husbandry practices; a long period allowed for kids to nurse from their dams appears to be useful in preventing recurrence of the disease in out years.

Mycoplasma agalactiae, the causative agent of contagious agalactiae in goat, may also cause arthritis in neonatal goats.[5] An outbreak in Greece demonstrated polyarthritis in 1-3 day old kids the season after the doe herd had contracted contagious agalactia, and the dams of the affected kids had been treated during that time for mastitis. (Affected dairy goats usually shed mycoplasma in the milk for a year, although prolonged shedding for periods up to 8 years



Joint, goat. Higher magnification of the remnant synovium. (Photo courtesy of: Veterinary Medical Diagnostic Lab, University of Missouri, www.vmdl.missouri.edu)



Joint, goat. Much of the synovium is lost and replaced with densely cellular granulation tissue. (HE, 100X)

have been reported.)[5] *Mycoplasma mycoides* subsp. *mycoides* has also been reported to cause polyarthritis in goats as well.[5]

Since the submission of this case, the contributors have published a case study in the Journal of Veterinary Diagnostic Information. Over a three-year period, 8 goat kids, averaging 2 weeks of age, were submitted for autopsy. 5 of the 8 goats had concurrent respiratory disease (pleuropneumonia, interstitial pneumonia, or pleuritis), 2 had meningitis, and 1 (presumptive the individual in this case) had pericarditis as well. PCR testing of colonies grown on sheep blood trypticase soy agar from affected animals using universal 16S rRNA forward and reverse primers identified the isolates as *M. mycoides* subsp. *capri.*

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CASE III: 17-137-W4 (JPC 4117671)**.**

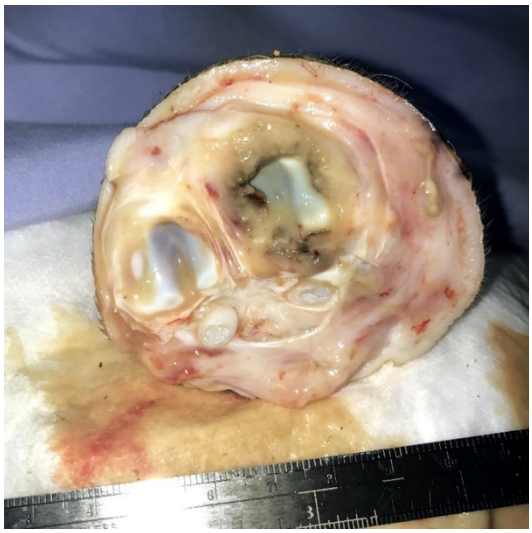
Signalment: ~6 month old, female, Yorkshire cross, *Sus scrofa*

History: Animal arrived at the university on 10/3/17. Observed limping on right front limb on 10/6/17, three days after arrival. The lameness progressed despite NSAID and antibiotic administration. The animal was also observed coughing occasionally with mucous production. Radiographs indicated soft tissue swelling and evidence of pneumonia. The decision was made to euthanize by the investigator on 10/12/17 due to lack of response to treatments and

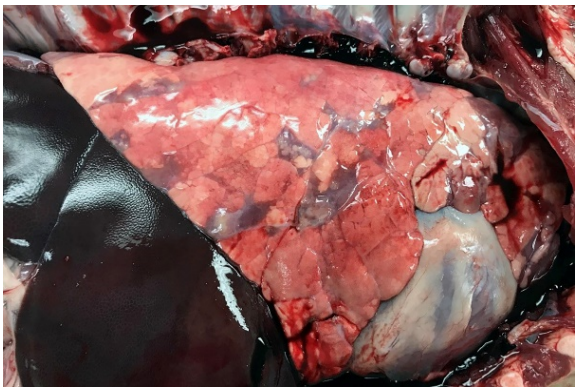
Right front foot, pig. There is marked swelling of the right front foot with several abrasions on the cranial surface. (Photo courtesy of: University of Pittsburgh, Division of Laboratory Animal Resources, http://www.dlar.pitt.edu/)

inability to use in the following week's study. A complete necropsy was performed by the veterinary pathologist on 10/13/17.

Gross Pathology: The right front foot swelling was associated with a large joint abscess between P2 and P3. Approximately 20mL of tan foul smelling fluid was expressed when the joint capsule was cut.



Right front foot, pig. The lateral joint space of P2 and P3 contains suppurative exudate and the synovium is proliferative and grey-red. (Photo courtesy of: University of Pittsburgh, Division of Laboratory Animal Resources,



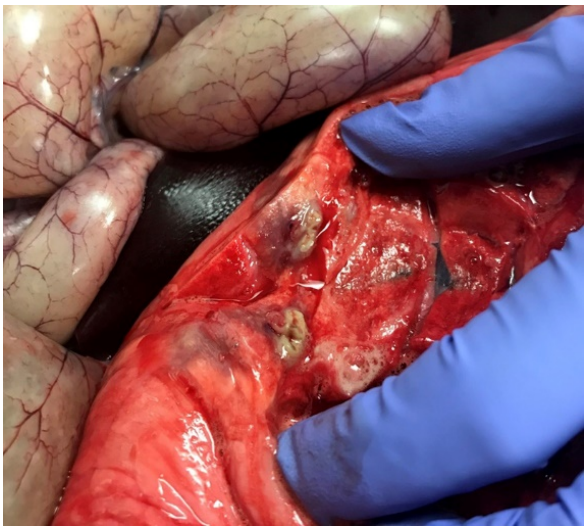
Lung, pig. Multiple lobules are depressed and dark red with extensive greyish foci. (Photo courtesy of: University of Pittsburgh, Division of Laboratory Animal Resources, http://www.dlar.pitt.edu/)

The synovium was proliferative and tan-grey to dark red between the lateral P2 - P3 joint. There was also circumferential severe swelling of the surrounding soft tissues. The lungs contained multifocal lobular dark red firm areas. On cut section, lobules were dark red to grey with white-grey foci that seemed to be associated with the airways. Cultures of the lung were submitted.

Laboratory results: Cultures from both the lungs and joint were positive for the bacterial organism *Trueperella pyogenes.*

Microscopic Description: Lungs: Affecting 75% of the section, there are multifocal to coalescing areas of necrosis composed of numerous viable and degenerate neutrophils with fewer macrophages admixed with abundant karyorrhectic and eosinophilic cellular debris and cloud-like bacterial colonies. Necrotic foci are surrounded by many epithelioid macrophages, reactive fibroblasts, and fewer lymphocytes with an outside rim of organizing granulation tissue and collagen. In less affected areas, the perivascular interstitium and alveolar septa are infiltrated and expanded by variable numbers of macrophages, neutrophils, and lymphocytes. Alveoli are often filled with proteinaceous fluid (edema), fibrin, foamy macrophages, and neutrophils. Most airways contain various amounts of neutrophils, fibrin, edema fluid, and sometimes bacterial organisms. Numerous blood vessels in the lung sections contain fibrin thrombi.

Joint, P2-P3: The synovial lining is diffusely ulcerated and covered in a layer of hyalinized fibrin and necrosuppurative debris. The joint capsule contains microabscesses composed of a central necrotic core surrounded by a rim of epithelioid macrophages, more peripheral reactive fibroblasts and lymphocytes, with an outside layer of organizing granulation tissue and collagen. The synovial membrane is thickened up to 5 times normal and forms villous projections in areas. Villi are expanded by edema, fibrin, congested blood vessels, fibroblasts, organizing granulation tissue and fibrous connective tissue.



Lung, pig. On cut section, the greyish foci that appear to be associate with the airways.(Photo courtesy of: University of Pittsburgh, Division of Laboratory Animal Resources, http://www.dlar.pitt.edu/)

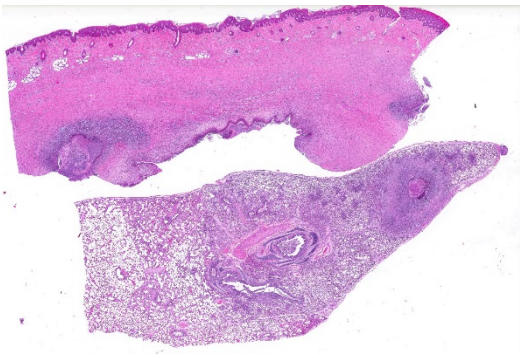
Contributor's Morphologic Diagnosis:

Pneumonia, necrosuppurative, multifocal to coalescing, severe, chronic, with bacteria, etiology consistent with *Trueperella pyogenes.*

P2-P3 synovitis, necroulcerative, diffuse, chronic, with microabscesses and bacteria, etiology consistent with *Trueperella pyogenes.*

Contributor's Comment: The lesions in this animal indicated chronic infection in both the joint space (P2-P3) and the lung was due to *Trueperella* which was cultured from both locations. Due to the severity and chronicity of the lesions, it is not possible to determine whether they occurred as a result of direct infection (i.e.: inhalation, focal wound) or embolic spread. The large amount of airway inflammation in the lungs suggests possible infection by inhalation, but embolic spread was likely also a factor.

T. pyogenes is a commensal and an opportunistic pathogen that causes abscessation and pneumonia in several large animal species including pigs and ruminants. In addition to pneumonia and abscesses, other potential lesions include metritis, udder lesions, pneumonia, arthritis, endocarditis, lymphadenitis and osteomyelitis. It is a gram-positive, non-motile, non-spore-forming, short, rod-shaped, coryneform bacterium that is ubiquitous in the environment and can be found on the mucous membranes of domestic animals leading to subsequent spread to any other organ system in the body.[2,3,4,7]



Haired skin, lung: Sections of lung and haired skin are submitted for examination. Both have one or more abscesses and the deep margin of the skin section appears to be a draining tract. (HE, 5X)

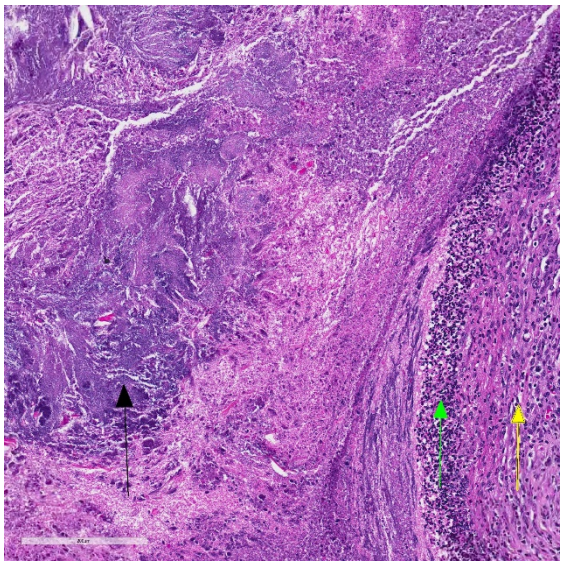
Although *T. pyogenes* is can infect companion animals and humans, it is an uncommon clinical pathogen.[1] In ruminants and pigs, however, it can cause significant disease and lead to condemnation at slaughter. Although treatment with antibiotics can clear the infections, this pathogen can also develop resistance to commonly used antibiotics such as tetracycline and doxycycline.[2,4,7]

Contributing Institution:

University of Pittsburgh, Division of Laboratory Animal Resources

http://www.dlar.pitt.edu/

JPC Diagnosis: 1. Haired skin, deep dermis: Abscess, with draining tract.



Haired skin: High magnification of the dermal abscess with numerous bacterial colonies (black arrow), peripheral layer of degenerating neutrophils and eosinophils (green arrow), abscess wall (yellow arrow). HE, 186X)

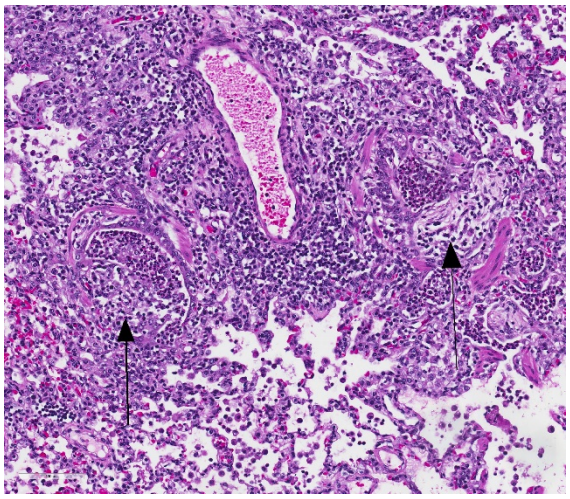
2. Lung: Pneumonia, embolic, chronic-active, focally extensive, severe.

JPC Comment: Well-known for its opportunistic infections in ruminants, *Trueperella pyogenes* is a common cause of suppurative lesions in swine as well. *T. pyogenes* is a common inhabitant of the mucosal flora in healthy animals, and can be cultured from feces of healthy swine. Infected swine can shed it in urine, feces and discharges from the upper respiratory tract, teats, and vulva.[6]

As an opportunist, it colonizes areas of inflammation or infection caused by other agents, and is a common isolate in longstanding lesions such as abscesses when the inciting agent has long disappeared. The bacterium possess a number of virulence factors which help in colonization of a number of surfaces, including neuraminidases, fimbria and collagen-, fibrin-, and fibrinogen binding proteins. Other virulence factors assist in tissue damage, including pyolysin, a cholesterol-binding exotoxin which results in cytotoxicity in a variety of cells, including neutrophils, macrophages, epithelial cells, erythrocytes, and fibroblasts.[5] The cytolytic activity of pyolysin is similar to other exotoxins secreted by gram-positive bacteria, by binding to the cell membrane and forming transmembrane pores.[5]

T. pyogenes is one of, if not the most common, bacterium isolated with suppurative processes in swine, and it may be responsible not only for focal lesions, but (as a result of hematogenous dissemination (as demonstrated in this case)) multisystemic infections as well. It is a common agent of pneumonia, pleuritic, osteoarthritis, polyarthritis, mastitis, endocarditis, valvulitis, and reproductive infections.[5,6] It may result in pyelonephritis or mastitis in non-pregnant sows, and pyometra with fetal maceration in pregnant sows. It may also be part of a mixed infection, and infections are often associated with immunosuppression from common swine viruses, such as porcine arterivirus (PRRSV).[5]

Abscesses in a variety of organs are characterized by a thick wall and yellow-green pus. Intramuscular or subcutaneous abscesses may show few clinical signs except a loss of body condition in pigs, but will often result in carcass condemnation at slaughter, especially in cases with multiple abscesses or visceral involvement.[5]



Lung, pig. Plugs of fibrous connective tissue are growing into bronchioles following effacement of the wall y suppurative and eosinophilic inflammation (bronchiolitis obliterans). (HE, 218X)

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CASE IV: 25681 (JPC 4115969).

Signalment: 2 year old neutered male American Quarter Horse (*Equus ferus caballus)*

History: The horse had a history of muscle wasting and pyrexia that did not respond to corticosteroids or NSAIDS. There was ultrasonographic evidence of mineralization of multiple internal organs that led to euthanasia.

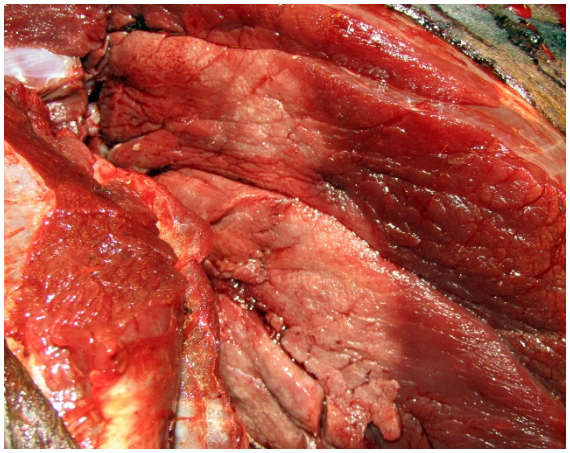
Gross Pathology: The horse weighed 406 kg and was fair body condition. Skeletal muscle of the gluteal and biceps groups of both rear limbs contained patchy pale areas. Tongue and cardiac muscle are similarly

affected. The lungs failed to collapse, even after incision, and were firm and somewhat gritty. Patches of endocardium had a wrinkled appearance, were hard and had a pale gray color. Both kidneys had multiple wedge-shaped, pale, gritty cortical foci. Petechial hemorrhages and erosions were found in the glandular stomach.

Laboratory results:

Test	**December 7**	**December 16**	**Reference**
WBC	15.44 X 10^3 /ul ↑	21.76 X 10^3 /ul ↑	5.40-14.3 X 10^3 /ul
Segs	13.74 X 10^3 /ul ↑	17.41 X 10^3 /ul ↑	2.26-8.85 X 10^3 /ul
Fibrinogen	0.4 gm/dL	0.6 gm/dL ↑	0.1-0.4 gm/dL
Urea Nitrogen	21 mg/dL	39 mg/dL ↑	11-24 mg/dL
Creatinine	0.9 mg/dL	2.1 mg/dL ↑	0.9-1.7 mg/dL
Cl-	96 meq/L	94 meq/L	95-105 meq/L
Calcium	11.5 mg/dL	11.1 mg/dL	11.0-12.9 mg/dL
Phosphorus	4.1 mg/dL ↑	8.7 mg/dL ↑	1.8-2.1 mg/dL
Triglyceride	58 mg/dL ↑	84 mg/dL ↑	14-62 mg/dL
AST	>10,000 U/L ↑	5324 U/L ↑	203-415 U/L
CK	>80,000 U/L ↑	6136 U/L ↑	112-496 U/L
Urine 2/07/18	Brown, opaque, pH 9.0		
Heme	3+		
Urine specific gravity	1.063		
Urine Sediment Cytology	Marked amorphous material		
Strep equi SEM ELISA	Moderately positive		

Microscopic Description: Two sections of muscle are submitted, one minimally and one extensively affected (gluteal). There is multifocal, degeneration, necrosis and mineralization involving single or clustered myofibers. Associated with degenerating fibers are infiltrating macrophages, multi-nucleate giant cells, lymphocytes and neutrophils. Foci of degenerate fibers are associated with mild to moderate interstitial fibrosis. Multinucleate regenerating muscle fibers have lightly basophilic nuclei, and central nuclei are present. Small to medium-



Skeletal muscle, horse. There are patches of pallor within the gluteal muscles. (Photo courtesy of : Veterinary Medical Diagnostic Lab, University of Missouri, www.vmdl.missouri.edu)

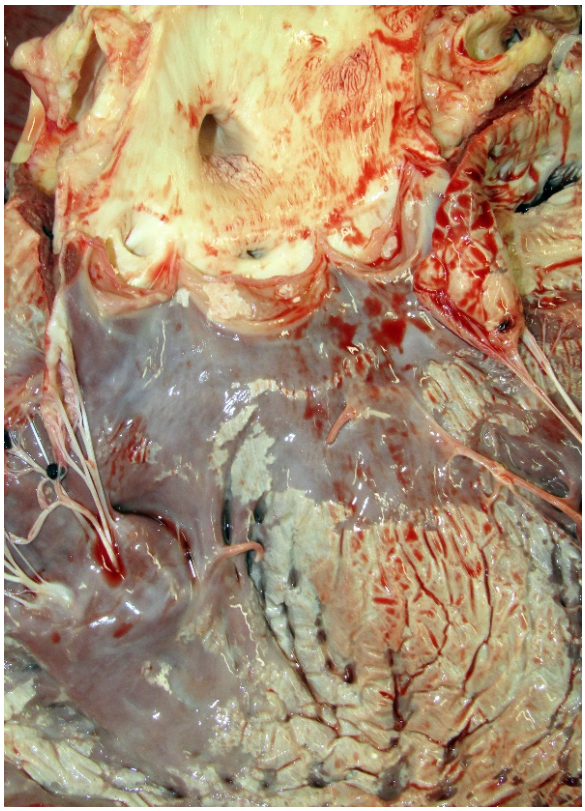
sized arterioles in affected areas are characterized by coagulative necrosis and mineralization affecting the tunica intima and tunica media.

Other microscopic diagnoses (not found on the tissues submitted) include mineralization of alveolar walls and arteries of the lung, mineralization of the epicardium, endocardium and muscle of the heart and aorta, and multifocal mineralization of the renal cortex, gastric mucosa and tongue muscle.

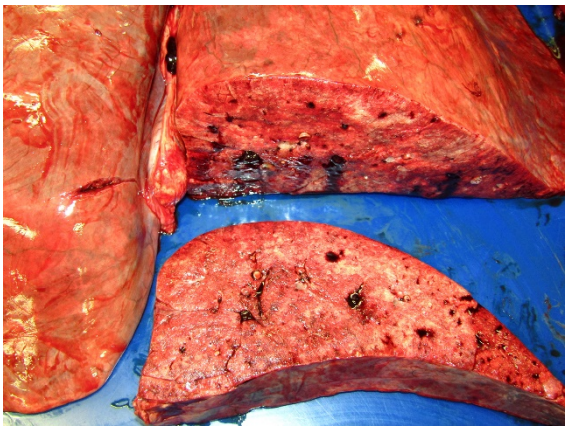
Contributor's Morphologic Diagnosis:

Chronic-active necrotizing myodegeneration with myofiber mineralization and attempted muscle regeneration

Contributor's Comment: This animal has characteristic lesions in muscle and other organs consistent with systemic calcinosis.[3,5,8] Skeletal muscles affected by this condition undergo gross atrophy. Large muscles, especially the gluteal, have more severe and extensive lesions, and the more severely affected specimen submitted is gluteal muscle. Other muscles can be much less affected. Histologic lesions are severe muscle necrosis. Necrosis in this case is associated with acute contraction bands, sarcoplasmic vacuolation, loss of striations and dystrophic calcification. Foci involving multiple fibers within a single muscle appear to be random, and segmental. Involvement of multiple fibers in one spot, in association with vascular mineralization suggests that vascular damage may influence lesion distribution. Macrophage infiltrate and regenerative changes are often concurrently present in foci where multiple fibers are involved. Acute and chronic damage exists



Heart, horse. There is significant mineralization of the left ventricular endocardium, aorta, and aortic valves. (Photo courtesy of : Veterinary Medical Diagnostic Lab, University of Missouri, www.vmdl.missouri.edu)



Lung, horse. The lung lobes fail to collapse and contain multiple calcified foci. (Photo courtesy of : Veterinary Medical Diagnostic Lab, University of Missouri, www.vmdl.missouri.edu)

side-by-side, although the regeneration does not appear to be conspicuously successful.

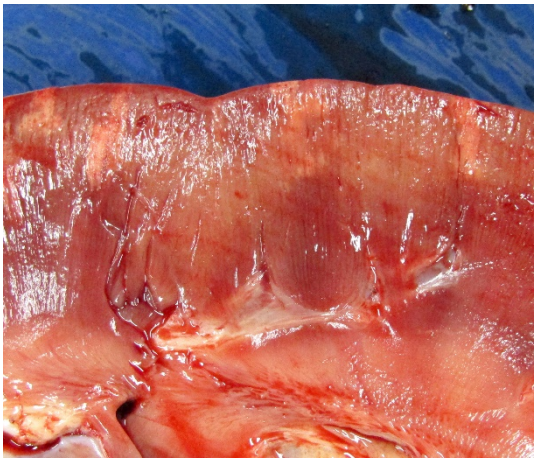
Systemic calcinosis is an invariably fatal multisystemic disease of horses that is thought to be a manifestation of calciphylaxis. Caliciphylaxis, or Systemic Uremic Arteriopathy in humans most often occurs in advanced uremia, prolonged dialysis, and renal transplantation. Muscle manifestations can be present, may be the presenting complaint,[4,6] and are manifest as a subacute proximal myopathy. Cutaneous calcification is the most common site of calcium deposition in people, along with calcification of multiple internal organs. Patients also have elevated serum phosphate and normal serum calcium.

The equine disease affects younger horses, primarily Quarter horses and Paint horses. Tissue mineralization is multisystemic, and clinical signs relate to the severity of mineral deposition in various organs. This animal presented with muscle weakness and ataxia, and the muscular system is usually involved. In this horse, mineralization of the lung and heart was also severe, even though the horse presented with signs of lameness. Numerous organs can be affected, including the liver and intestine in the analogous human disease. Only a handful of equine cases have been reported.

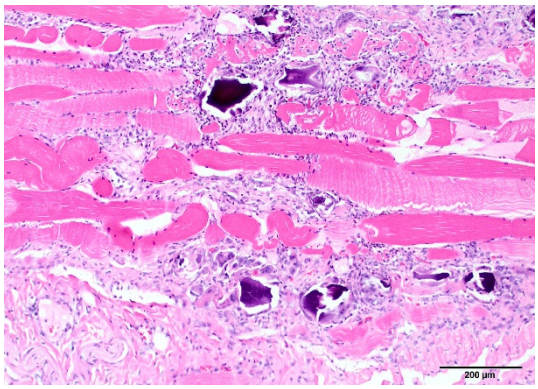
The equine disease may be immune-mediated, with some similarities to dermatomyositis, but there is not yet immunologic support for this hypothesis. In some humans, the presence of suspected lupus was found in one patient,[6] and vascular complement deposition has been found in another patient, providing potential support for this idea.[1]

Mineralization of soft tissue may be dystrophic (deposition of mineral on dead tissue) or metastatic (deposition of mineral on otherwise normal tissue).

Muscle calcification can occur without vascular damage. Direct sarcolemmal damage causes contraction bands in muscle fibers. Calcium entry into muscle fibers eventually results in mitochondrial overload.



Kidney, horse. Gritty pale foci in the renal cortex have an infarct-like distribution (HE, 275X). (Photo courtesy of : Veterinary Medical Diagnostic Lab, University of Missouri, www.vmdl.missouri.edu)



Skeletal muscle, horse: A central, degenerating blood vessel (arrow) co-exists with degenerating and mineralized fibers. (HE, 400X)

Mineralization is a hyperacute event that is virtually concurrent muscle degradation by calpains.[2]

However, dystrophic mechanisms are more likely in calciphylaxis due to the occurrence of vascular mineralization and thrombosis that is associated with calcification of myofibers. This multifocal distribution of muscle lesions that contain more to less acute damage also suggests that arteriolar damage is primary. Calcification can develop on otherwise normal tissue (metastatic) when the Ca X P product exceeds 65. In this patient, the Ca X P product was 47 on a first chemistry and 97 on a second. Calcification may be a result of RANK-l and TNF activation of osteoclasts, and hyperphosphatemia. The mechanism is thought to be through parathyroid hormone release. Vitamin D can also produce hyperphosphatemia, which usually results in hypercalcemia; serum calcium was within normal limits in this horse and the amplified Ca X P product was primarily due to hyperphosphatemia. Attempts to measure parathyroid hormone in the patient were unsuccessful due to prolonged sample storage.

In people, administration of parathyroid hormone and corticosteroid exacerbates the calciphylaxis and leads to death. It is argued that arteriolar smooth muscle is not normal in uremic patients[7] and uremic patients without calciphylaxis have vascular mineralization anyway. In any event, since corticosteroids are a mainstay of therapy for myositis in veterinary medicine, misdiagnosis is unlikely to improve clinical outcome.

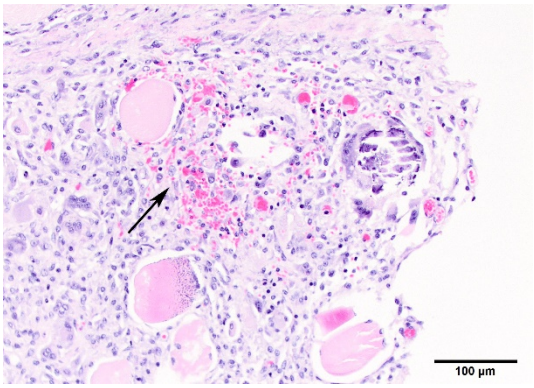
Contributing Institution:

Veterinary Medical Diagnostic Lab
University of Missouri
www.vmdl.missouri.edu

JPC Diagnosis: Skeletal muscle: Myositis, necrotizing and granulomatous, polyphasic, diffuse, severe, with mineralization.

JPC Comment The contributor has provided an excellent review of systemic calcinosis in the horse, as well as cellular mechanisms of tissue calcification. In this particular specimen, the wide range of changes in affected myofibers (ranging from acute swelling and hyalinization, to fragmentation and mineralization) indicates a polyphasic lesion, one that occurs over time. In monophasic lesions, such as may be seen with acute myopathic toxic injury, such as ionophore toxicosis, skeletal muscle lesions are approximately at the identical stage or degeneration or necrosis.[3]

A wide range of conditions can result in



Skeletal muscle, horse. Acute degeneration coexists with mineralization, interstitial fibrosis, and regenerative multinucleate cells in one focus of damage. (HE, 200X)

local or systemic muscular calcification in horses, both dystrophic and metastatic. Traumatic damage to myofibers, such as may be seen with injections, will result in monophasic dystrophic calcification. A range of plant toxicoses may result in either monophasic lesions (in acute overwhelming intoxications) or polyphasic lesions (which are more common, in prolonged grazing). Toxicosis with sublethal doses of ionophores may result in monophasic lesions with mineralization of effected myofibers. Some species of *Cassia* result in monophasic skeletal muscle lesions in horses and ruminants; recumbent animals often do not recover. A number of plants including *Cestrum diurnum*, *Trisetum flavescens*, and plants of the genus *Solanum* accumulate analogs of activated Vitamin D, which result in excessive absorption of calcium from the intestine and subsequent metastatic calcification of skeletal muscle and many other organs. These lesions are polyphasic and progressive, and skeletal muscle mineralization is considered to be one of the earliest affected sites. Vitamin E-selenium imbalance may result in significant polyphasic damage to myocardial and skeletal muscle (particularly the muscles of deglutition and the neck and shoulder muscles) in foals, and less severe lesions within the skeletal muscles in adult horses. Recurrent non-lethal cases of exertional myopathies may present as polyphasic lesions.[3]

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