Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2015-2016

Conference 3

23 September 2015

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CASE I: 8482-15 (JPC 4066661).

Signalment: Bovine neonate, male, Angus (*Bos taurus).*

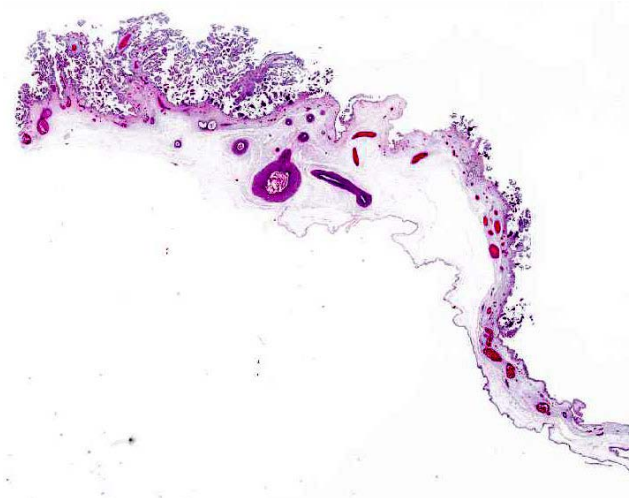
History: Fourteen-month-old heifer had been bred while still with her dam. Not knowing she was pregnant, she was vaccinated against brucellosis on January 15, 2015, using RB51 vaccine strain of *Brucella abortus*. The fetus was expelled April 9, 2015. The attending veterinarian and an assistant had been exposed to the fetus and fetal fluids.

Gross Pathology: None reported

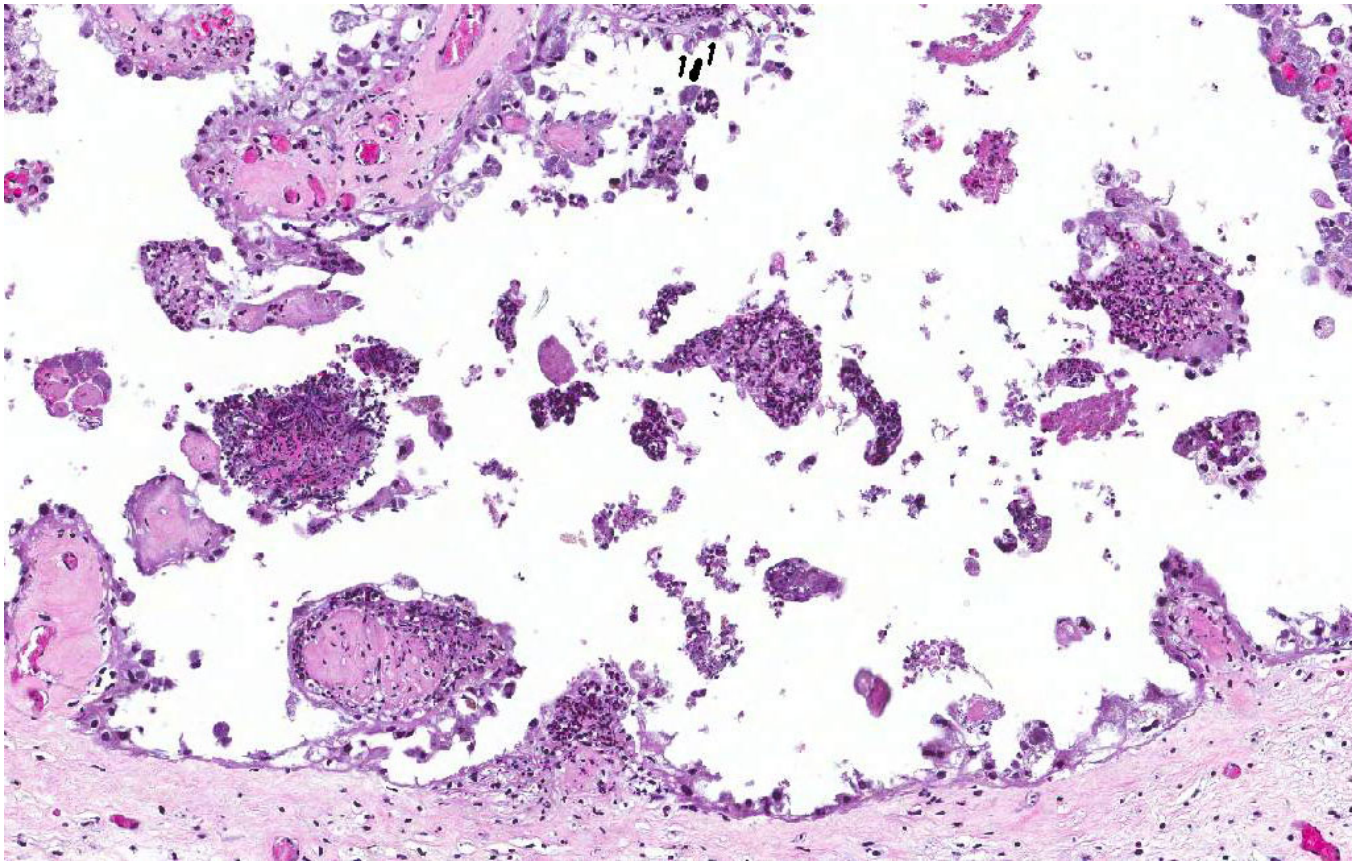
Laboratory Results: Bacterial cultures of placenta were positive for *Brucella abortus.* The isolate was confirmed as strain RB51with PCR by the USDA-APHIS National Veterinary Services Laboratory.

Histopathologic Description: Sections of cotyledon are characterized by multiple foci of necrosis of villi. Numerous degenerate inflammatory cells are present in the necrotic foci. Variable numbers of neutrophils can be seen scattered throughout the lamina propria. Margination of neutrophils as well as exocytosis is observed in blood vessels in the lamina propria. Occasional fibrin thrombi are present. Cytoplasm of many remaining trophoblasts is distended with coccobacilli.

Contributor's Morphologic Diagnosis:
1. Marked multifocal subacute necrotic and neutrophilic placentitis with intralesional coccobacilli.



The submitted section is composed of cotyledon (upper left), chorion, and allantois.



There is multifocal necrosis of chorionic vili infiltration of moderate numbers of neutrophils. (HE, 80X)

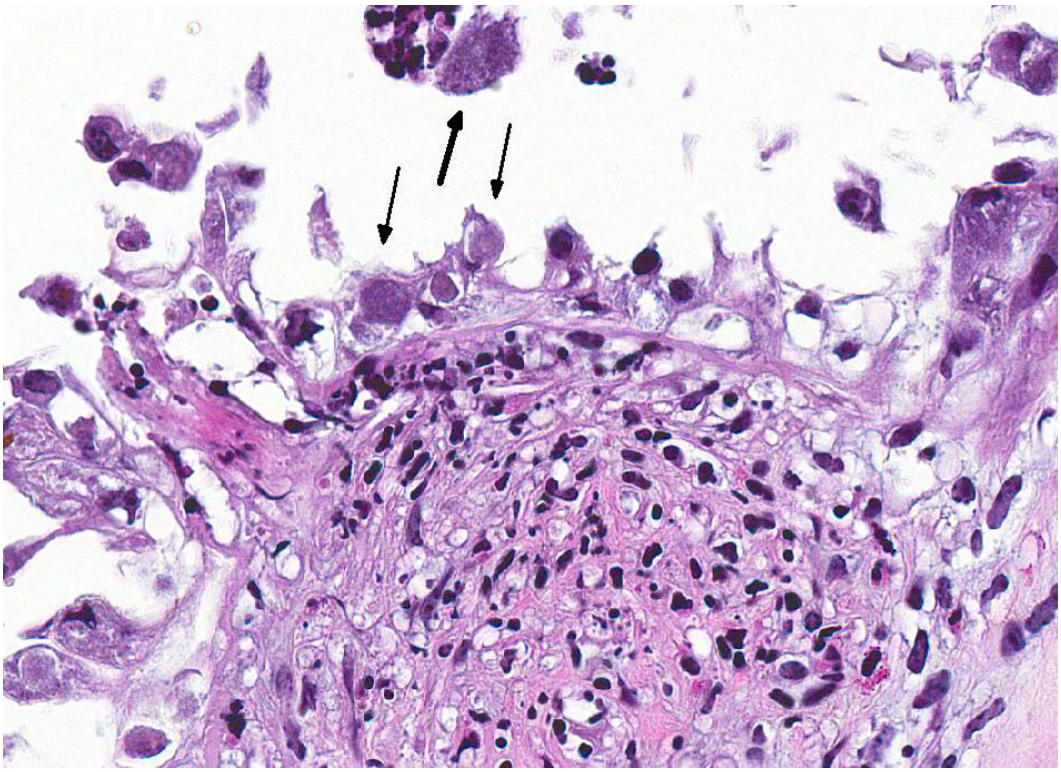
2. Moderate to marked multifocal subacute neutrophilic vasculitis.

Contributor's Comment: Bovine brucellosis is a disease for which the United States Department of Agriculture has implemented an eradication program due to its zoonotic potential. Vaccination plays a major role in the control strategy for brucellosis. The most recently developed vaccine utilizes strain RB51. RB51 is a spontaneous rough mutant of *B. abortus* 2308 that lacks a homopolymer of perosamine as the O-chain component of LPS.[8] The O-chain is an immunodominant antigen that can cause problems related to serologic diagnosis of vaccinated animals and is expressed in smooth colony types of *B. abortus*.[8] In addition to lacking the O-side chain of LPS, this isolate is less virulent compared to known virulent strains and is protective against infection with virulent *B. abortus*, making it a suitable vaccine candidate.[8] During infection, the majority of the organisms localize in cotyledons, placental membranes, and in allantoic fluid. Early infection of the placenta begins in intercotyledonary erythrophagocytic trophoblasts followed by replication in adjacent chorioallantoic trophoblasts.[1] After replication, there is necrosis of trophoblasts with release of massive numbers of organisms into the uterine lumen.[1] The usual source of infection for cattle is ingestion of organisms from an aborted fetus or placenta, or contaminated uterine discharge.[7]

This case is unusual in that abortion was the result of administration of the vaccine strain (RB51) of *B. abortus*. It has been shown that vaccinating pregnant cattle with RB51 can result in rare placentitis and preterm expulsion of the fetus.[5] Vaccination of pregnant cattle is ill-advised. Therefore, vaccination is limited to young, non-pregnant cattle. In spite of recommendations, instances of fetal wastage occur.[2] In the present case, the fact the pregnancy status was unknown resulted in fetal loss. It should be noted that animal workers are at risk of infection by *B. abortus* when assisting parturition or handling infected tissues from these animals.

JPC Diagnosis: Placenta, chorionic villi: Placentitis, necrotizing, multifocal to coalescing, mild with mild vasculitis and numerous intratrophoblastic bacilli.

Conference Comment: Conference participants were struck by the low degree of vascular and trophoblastic changes in the section of chorioallantois and speculated the mild nature of lesions was related to abortion being caused by vaccine strain (RB51) of *B. abortus*. The vasculitis was discussed as well as the importance of recognizing vasculitis in placental lesions and cases of abortion, which can help differentiate causes such as brucellosis and chlamydiosis from other etiologies. Participants described vessels as being infiltrated by low numbers of lymphocytes, plasma cells and neutrophils as well as the presence of fibrin and hemorrhage, accompanied by hypertrophic endothelium, but agreed the overall changes were mild. Vascular changes would also be expected in maternal tissues. Other common lesions seen with *B. abortus* infection include fetal pneumonia, which can vary in severity, as well as microscopic granulomas in the liver, spleen and lymph nodes.

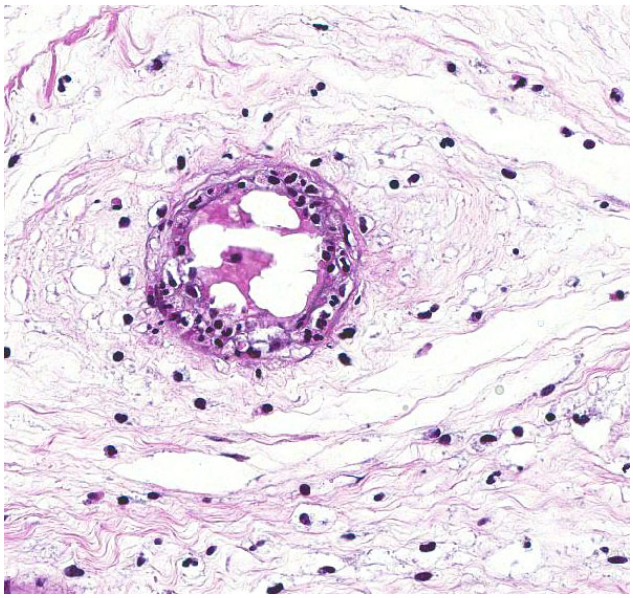


Trophoblasts lining necrotic villi contain numerous bacilli within their cytoplasm. (HE, 400X)

Necrotizing placentitis with intratrophoblastic bacteria is typical for, but not unique to, *B. abortus*, and would be expected to be much more severe in naturally occurring disease. Gross lesions commonly consist of a necrotizing placentitis with thick brown and/or bloody exudates. In general, gross and microscopic lesions can be very similar with the various agents of bacterial abortion. Differential diagnosis for bacte-rial placentitis with intratrophoblastic bacteria include other gram negative organisms such as *Coxiella burnetii*, where infected trophoblasts typically have a more foamy vacuolated appearance, and *Chlamydophila abortus*, where placental vasculitis would be very prominent. *C. burnetti* is more common in goats and is rare in the U.S., and *C. abortus* is more common in sheep. The gram-positive organism *Listeria monocytogenes* was also discussed, which, in addition to abortion, results in fetal septicemia and the presence of foci of necrosis in many organs. Other bacterial causes of abortion include leptospirosis, which would not result in necrotizing placentitis; and *Campylobacter* sp., which more commonly results in early embryonic death but can result in abortion with similar gross and histologic placental lesions as brucellosis, but lesions are generally less severe.[3]

There are both smooth and rough strains of *Brucella* spp., with rough strains lacking the expression of O side chain on the lipo-

polysaccharide (LPS), and include the RB51 vaccine strain. Smooth and rough strains enter phagocytic cells, preferably macrophages, differently and the rough strain is more rapidly targeted to the phagolysosomal compartment and is generally unable to replicate, whereas smooth strains are capable of intracellular replication within the phagosome. Virulent *Brucella* spp. employ multiple mechanisms to detoxify free radicals in order to survive in the phagosome, including expression of superoxide dismutases. *Brucella* spp. have also adapted mechanisms to avoid the innate immune system, such as decreased stimulatory activity of TLR4 receptors, being devoid of structures such as pili, fimbriae and capsules that would stimulate pattern recognition receptors (PRRs), as well as prevention of phagosome-lysosome fusion and inhibition of macrophage apoptosis.[4] Primary routes of infection are considered to be oral exposure to contaminated fetal membranes and aborted fetuses, and ingestion of contaminated milk. Once the infection is localized within lymph nodes, bacteremia results in extension of infection into multiple organs including the uterus, placenta and mammary glands. When the infection reaches the fetal membranes, bacteria replicate within trophoblast cells, and there is extensive necrosis and exudates as well as endometritis, and abortion is the eventual result.[4]



A mild vasculitis affects small vessels within the chorion. (HE, 300X)

Contributing Institution:

Veterinary Diagnostic Center, University of Nebraska-Lincoln, Lincoln, NE. vbms.unl.edu/nvdls

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CASE II: 2010-2 (JPC 3165093).

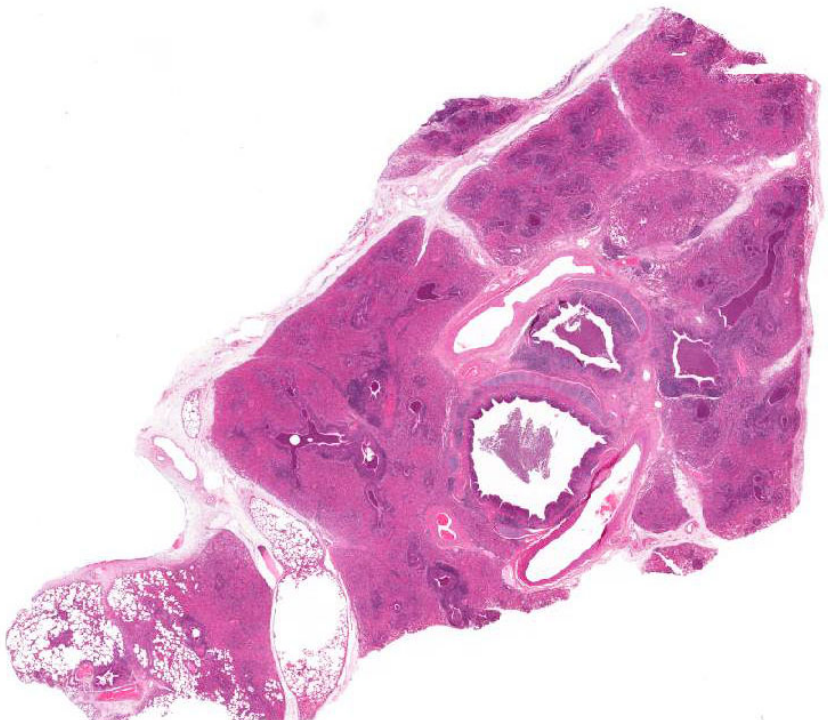
Signalment: Six yearling white-tailed deer (*Odocoileus virginianus*), male and female.

History: Over a period of 6-9 months 6 of 20 white-tailed deer confined to a small pasture developed weight loss, increased respiratory rate, intermittent nasal discharge, and labored breathing, with coughing upon forced exercise. The six affected deer were isolated in a sick pen and treated orally with antibiotics. In spite of antibiotic treatment and ad lib feed, minimal improvement was noted and all 6 deer were euthanized and examined.

Gross Pathology: Deer were thin, in poor body condition with minimal fat stores. Lesions were similar in all deer and varied only in degree of severity. Pulmonary lesions consisted of red to purple discoloration and atelectasis of cranial lung lobes with variable involvement of middle and caudal lung lobes. Discolored and atelectatic lung was sharply demarcated from less affected lung which generally remained pink and inflated. Affected lung was firm and on cut surface airways oozed purulent exudate. Affected lung did not float in formalin. Tracheal mucosa was diffusely reddened and contained intraluminal mucopurulent exudate. Tracheobronchial and mediastinal lymph nodes were moderately enlarged and edematous.

Laboratory Results: Bacteriologic culture of fresh lung from all deer yielded heavy, pure growth of *Pasteurella multocida*.

Histopathologic Description: Submitted sections of lung are from different animals and differ slightly in degree of involvement and severity. Multifocally, bronchi and bronchioles are variably filled with large numbers of degenerate and non-degenerate neutrophils, small amounts of fibrin, and detached epithelial cells. In the most severely affected airways, epithelium is characterized by multifocal areas of degeneration and necrosis with attenuation of remaining epithelial cells. Moderate numbers of neutrophils are seen within airway epithelium. Affected airways and vessels are surrounded by variable cuffs of lymphocytes, plasma cells and macrophages. The most pronounced lymphoid cuffs contain lymphoid follicles. In the remainder of affected lung alveolar interstitium is congested and alveoli contain neutrophils, erythrocytes and small amounts of fibrin with alveoli near bronchi/bronchioles being most severely affected. Interlobular regions are expanded by clear space due to edema and there are multifocal, mild infiltrates of neutrophils with the pleura.



Airways diffusely contain a cellular exudate and surround alveoli are consolidated and atelectatic. (HE, 6.0X)

Contributor's Morphologic Diagnosis: Lung, broncho-pneumonia, fibrinosuppurative, multifo-cal to focally extensive, acute to subacute, moderate, with multifocal airway epithelial necrosis and interlobular edema.

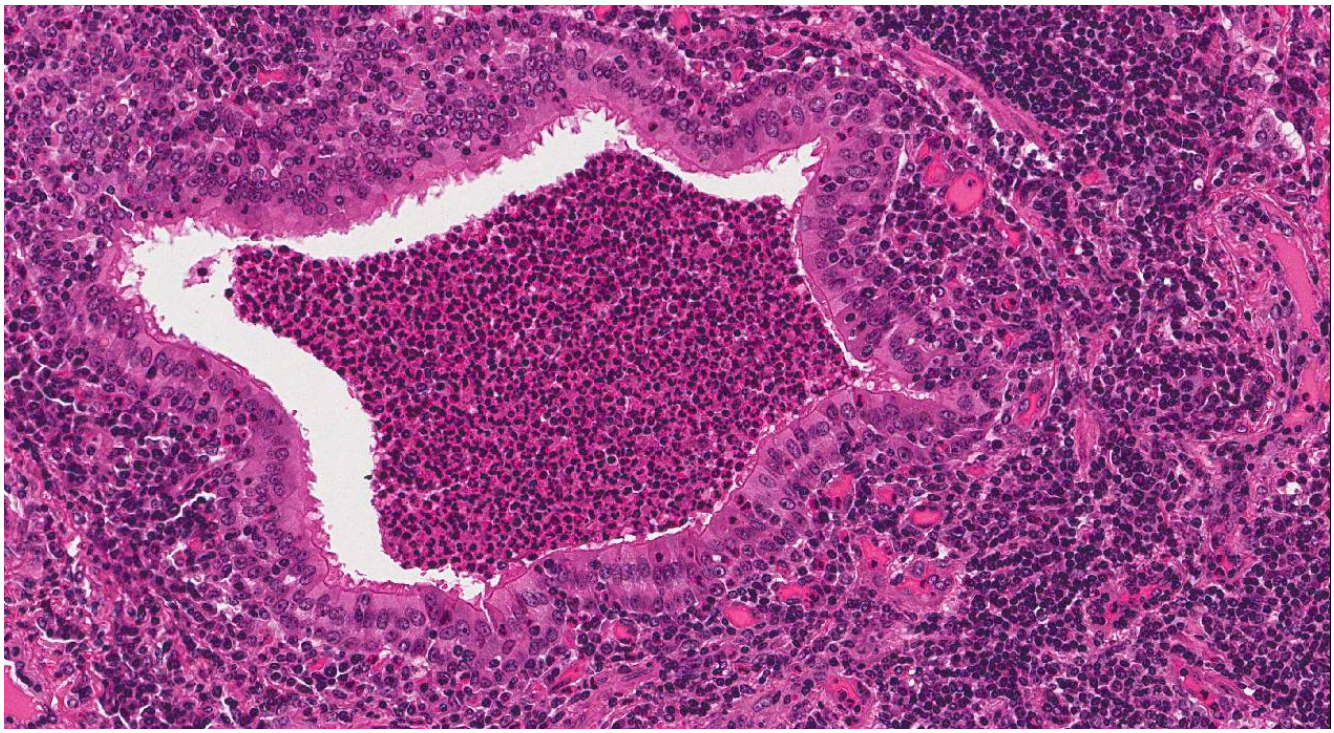
Contributor's Comment: Pasteurella organisms are

nonmotile, nonsporeforming, aerobic, fermentative, gram-negative coccobacilli. *Pasteurella* spp. are distributed worldwide and are a common etiology of diseases in cattle, bison, water buffalo, sheep, goats, domestic and wild birds, rabbits, laboratory animals and marine mammals.[2,4,5,7]

Pasteurella multocida is a common commensal organisms of the tonsil and nasopharynx in healthy ruminants.[2] Transition from infection to disease may be facilitated by stressors such as transport, intercurrent disease, climatic changes, social changes, or changes in feed or management.[2]

P. multocida has traditionally been classified into 5 capsular groups (A,B,D,E and F). Worldwide, serogroup A isolates are major causes of bovine respiratory disease. Serogroups A, and to a lesser extent D, cause fowl cholera in birds. Serogroups B and E are associated with hemorrhagic septicemia in cattle and water buffaloes in tropical regions of Africa and Asia. Serogroups B and E are rarely isolated from cattle in North America. In pigs, atrophic rhinitis and pneumonia are associated with toxigenic strains of serogroups D and A, respectively. Serogroup F strains are predominately isolated from diseased poultry.[4]

Pasteurellosis has been recorded in many species of deer including axis, black-tailed deer, mule deer, fallow, red, roe, sambar, reindeer, elk and white-tailed deer.[4,5,7] Disease generally presents as either hemorrhagic septicemia or pneumonia. Hemorrhagic septicemia has been responsible for outbreaks with high mortality in elk and fallow deer.[4,5] The pneumonic form, as seen in this case, is characterized by fibrinosuppurative bronchopneumonia, with or without necrosis and hemorrhage.



Characteristic of P. multocida infection, airways are filled with viable neutrophils without necrotic changes in the lining epithelium (HE, 140X)

While *Mannheimia haemolytica* (formerly *P. haemolytica*) can cause severe, acute fibrinonecrotic pneumonia and is an important component of the bovine respiratory disease complex, *P. multocida* is associated with less fulminating fibrinous to fibrinopurulent bronchopneumonia with limited lung necrosis.[2]

JPC Diagnosis: Lung: Bronchopneumonia, suppurative, chronic-active, diffuse, severe with intralobular edema and emphysema.

Conference Comment: This case nicely characterizes bronchopneumonia in a deer and allowed conference participants to discuss the neutrophil-rich, bronchial- and bronchiolar-centric nature of the disease process. Though all agreed that the extensive bronchopneumonia was most consistent with an infectious (most likely bacterial) etiology, most participants determined that infectious organisms were difficult, if not impossible, to visualize by routine histologic examination. Gram stains did not aid in identifying organisms. Additional histologic features discussed included type II pneumocyte hyperplasia, segmental atelectasis, and prominent bronchus- associated lymphoid tissue (BALT) hyperplasia, a finding that varied in severity between slides. Despite the slide variation, the presence of BALT hyperplasia along with the bronchial- and bronchiolar-centric inflammatory process lead conference participants to include *Mycoplasma bovis* as the main differential diagnosis. However, most agreed that the lack of well-differentiated nodules of necrosis made this diagnosis less likely. Another differential diagnosis discussed included *Mannheimia hemolytica*. However, "oat cells," fibrin rich exudate, and extensive necrosis, characteristic histologic features of *M. hemolytica*, were not present in this case. Additionally, bacterial aggregates are often easy to visualize in pneumonic mannheimiosis. The typical gross findings of *M. hemolytica* and *P. multocida* were compared as well. The classic gross postmortem findings of *M. hemolytica* including fibrin, hemorrhage, necrosis, and pleuritis with extensive "marbling" (due interlobular edema) particularly affecting the cranioventral lung regions were key features discussed in making the distinction between *M. hemolytica* and *P. multocida.* Other bacterial agents of respiratory disease in ruminants include *Histophilus. somni*, *Trueperella (*formerly *Arcanobacterium) pyogenes,* and *Bibersteinia. trehalosi*.

Compared to *M. hemolytica*, bacterial pathogenicity is reduced in *P. multocida* infection resulting in a slower onset of disease with absence of necrosis, vasculitis and exudation of abundant fibrin. In *P. multocida*, injury to cells of the respiratory system results from infiltration of inflammatory cells and release of their mediators and enzymes.[9] In contrast, *M. haemolytica* causes injury by release of bacterial toxins, such as leukotoxin, in addition to the effects of inflammatory mediators released from leukocytes, which results in a more necrotizing and fibrinous lesion. The leukotoxin, which is a member of the RTX group of toxins, is *M. hemolytica's* most important virulence factor, and can be directly toxic to cells as well as enhances the overall acute inflammatory response through activation of leukocytes and the complement system. Additionally, *M. haemolytica* has other virulence determinants such as polysaccharide in the capsule which can facilitate bacterial adherence and colonization, and inhibit their phagocytosis.[9] Environmental stressors and concurrent viral infection with agents such as bovine respiratory syncytial virus, bovine viral diarrhea, and infectious bovine rhinotracheitis virus result in increased susceptibility to infection by the bacterial agents.

P. multocida hemorrhagic septicemia is a reportable condition which occurs in cattle and water buffalo in Asia and Africa, and uncommonly in wild ruminants in the United States as previously mentioned by the contributor.[4,5] In this condition, the bacteria disseminate hematogenously to multiple organs resulting in congestion and hemorrhages in the respiratory, gastrointestinal and urinary tracts.[1] It is a high mortality condition with death being rapid,[6] and infection is thought to occur by inhalation or ingestion. Natural infections also occur in goats, but this species is considered more resistant to the infection.[8]

Contributing Institution:
National Centers for Animal Health; Ames, IA
http://ars.usda.gov/

References:

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buffalo and cattle following infection by *Pasteurella multocida* B:2. *Microb Pathog*. 2015;88:94-102.
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CASE III: 13-11676 (JPC 4032719).

Signalment: 1-year-old female Roosevelt elk **(***Cervis canadensis roosevelti)*

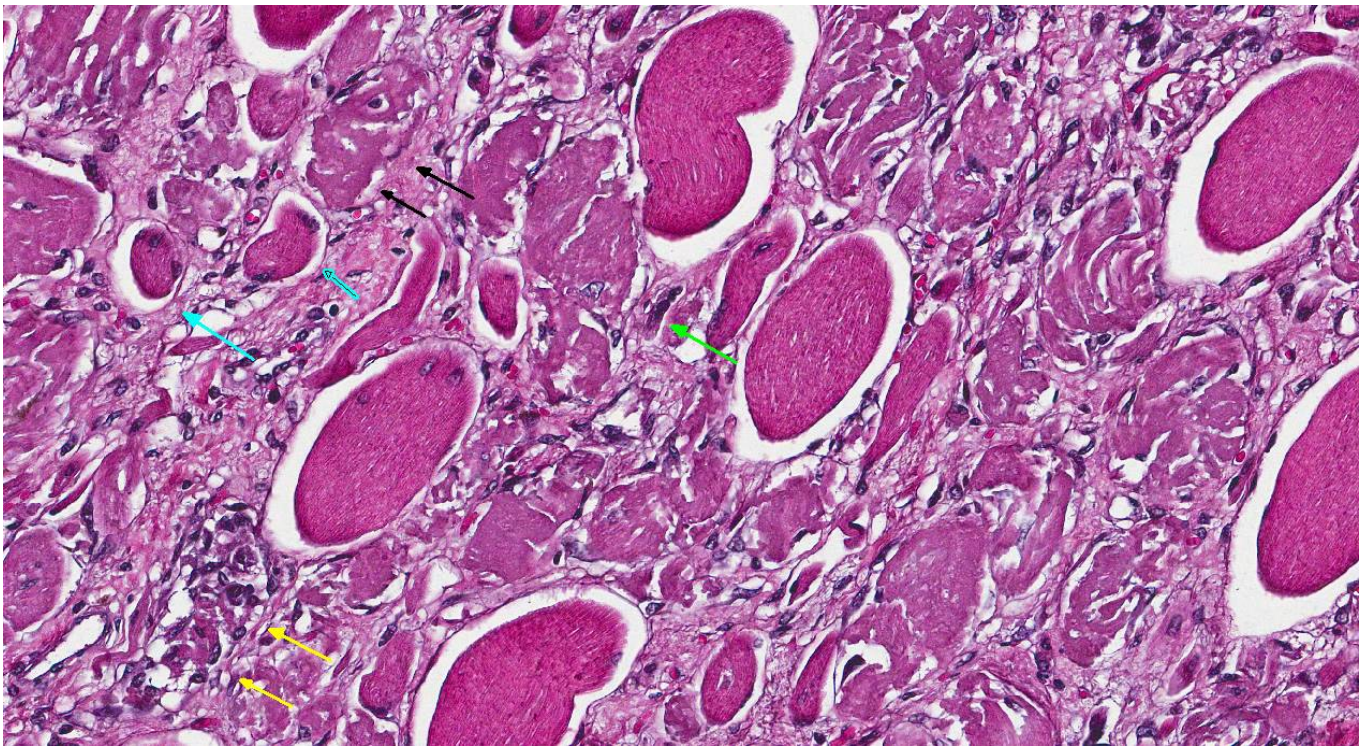
History: A one-year-old female Roosevelt elk (*Cervis canadensis roosevelti*) from an elk farm in Oregon received pour-on dewormer in a stanchion on April 20, 2013. Three days later, the animal appeared unstable on its front legs and subsequently became recumbent. Between April 23 and its euthanasia on May 7, the animal was seen by a veterinarian twice, once on April 26 and once on May 3 and received antibiotics and cortisol. The animal was not observed to stand during this entire period.

Gross Pathology: The animal was in good body condition with minimal post-mortem autolysis. Multifocal muscles within the caudal aspect of the left pelvic limb were firm and mottled pale white-tan to pink. The lesions frequently affected entire muscle bellies, but they appeared grossly to be restricted to the left pelvic limb. Similar lesions were not observed in the thoracic limbs, or anywhere else on the body.

Laboratory Results: None.

Histopathologic Description: Up to eighty percent of muscle fibers in examined sections are severely fragmented with a loss of cross striations and hypereosinophilia. These fibers are frequently difficult to distinguish from the extensive fibrous connective tissue in the perimysium. The remaining muscle fibers are markedly varied in size and there is extensive to complete loss of cross-striations. They also undergo one or more of the following changes: formation of contraction bands, hypereosinophilia, vacuolation or milder fragmentation of sarcoplasm, or centralization of nuclei. There is prominent proliferation of satellite cells at the periphery of myocytes. There are frequent centralized nuclei in large, mature muscle fibers.

There is extensive loss of myofibers with replacement by variably dense fibrous connective tissue that expands the epimysium and the perimysium. This fibrous connective tissue occasionally contains rare scattered macrophages and lymphocytes, but active inflammation is mild to absent overall. There are frequent, scattered hemosiderin-laden macrophages. Frequently surrounding or within areas of fibrosis there are multifocal elongated, narrow myocytes with lightly basophilic cytoplasm containing multiple centralized nuclei arranged in a row (strap cells).



This polyphasic skeletal muscle lesion shows many different forms of myocyte injury, including contraction band necrosis (black arrows), infiltration of macrophages (yellow arrows), atrophy (blue arrows), and myocyte regeneration with internalization of satellite nuclei (green arrow). (HE, 120X)

Multiple vessels, particularly small and medium caliber arterioles (but also some large veins) contain fibrin thrombi. Most of these thrombi are older and embedded in circumferential, proliferative spindle-shaped cells with foamy cytoplasm. This proliferation occurs in one to all layers of affected vessels and occludes the lumen, but there are small capillaries present with some red blood cells (recanalization). There is occasionally perivascular hemorrhage. Peripheral nerve bundles within the fascial planes tend to have the epineurium expanded by prominent myxomatous matrix.

Some slides contain myofibers expanded by well-demarcated, ovoid cysts filled with innumerable basophilic organisms (Sarcocystis species, suspected) that are not associated with inflammation.

Contributor's Morphologic Diagnosis:
Skeletal muscle: Locally extensive multiphasic myocyte degeneration, regeneration, necrosis, and loss with extensive fibrosis.

Small to medium caliber veins and arteries: Multifocal fibrin thrombi with transmural spindle cell proliferation and recanalization.

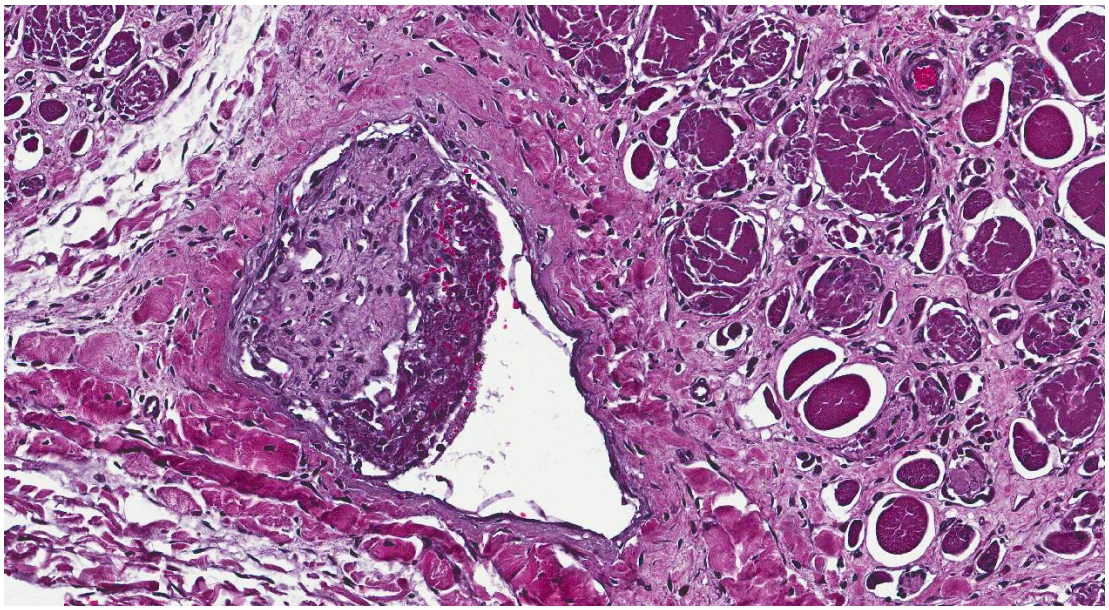
Contributor's Comment: Initially—particularly since Oregon has high rates of selenium deficiency in its farmed animals—the top differential diagnosis for this case was nutritional myopathy (white muscle disease). Liver samples were submitted to the Michigan State University Diagnostic Center for Population and Animal Health for selenium testing. The resulting liver selenium content of 3.6 is above the reference range of 0.7 to 2.5 micrograms per gram established for cattle. A previous study of 447 female farmed elk in Oregon found that liver selenium ranged from 0.002-3.15ppm. 42% of these animals had liver selenium levels considered low by cattle standards[1]. Although this animal's selenium

value does not necessarily rule out the presence of a vitamin E or selenium deficiency, the extremely localized nature of the lesion along with the history of being in a stanchion three days prior to the development of symptoms led us to consider the strong possibility of capture myopathy in this case.

Microscopically, the myocyte degeneration and regeneration in the lesion is multiphasic. This is not consistent with a traditional capture myopathy resulting from a single incident. Capture myopathy is thought to result from a combination of local hyperthermia and lactic acidosis coupled with altered blood flow due to massive catecholamine release when an animal (particularly a non-domesticated animal) struggles while being restrained. The resulting combination of hyperthermia, acidosis, and altered blood flow results in localized myonecrosis that can produce a number of distinct syndromes depending on the duration of stress, the extent of the lesion, and the nature of recuperation[2].

This lesion is problematic with regards to capture myopathy for two reasons: a case with a single reported capture event should produce a monophasic myonecrosis and vascular lesions are not considered a consistent aspect of capture myopathy. Upon extensive discussion among pathologists in the diagnostic laboratory, we felt that a possible explanation for the observed lesions was an initial incidence of capture myopathy or trauma (or any other possible reason for the initial bout of recumbency). Following the animal being recumbent, and perhaps due an initial exertional rhabdomyolysis, the pressure of the animal's weight on the limb likely produced a localized pressure ischemia.

Shifting of the animal's weight could have produced various incidences of ischemia and reperfusion over the course of disease, resulting in multiple incidences of muscle damage over time, which could explain the multiphasic nature of the myocyte degeneration and regeneration. The vascular lesions could indicate that the initiating injury could have been an infarct, but in the absence of a source of inflammation or thrombosis anywhere else in the body, it seems more likely that the proliferative and thrombotic vascular lesions are secondary to a combination of pressure ischemia and altered blood flow. Of course, the development of thrombi due to stagnation of blood in the vessels likely resulted in infarcts later on in the course of disease, adding to the ischemic lesions produced by pressure on the limb.



Multifocally, vessels contain organizing fibrin thrombi. (HE, 78X)

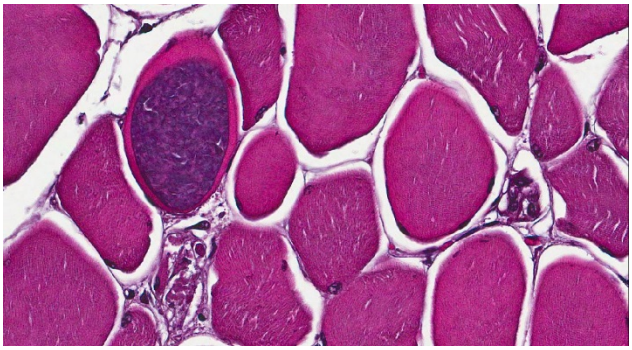
Another interesting aspect of this case was the absence of an active pigmentary nephrosis in this animal. Although there were a few chronic infarcts in the kidneys along with a few dilated tubules, there were no pigment casts present in the tubules. Despite the fact that the

lesions in the limb were likely ongoing, one can speculate that the myocyte degeneration in the limb was not severe enough to produce an active myoglobinurea.

JPC Diagnosis: 1. Skeletal muscle: Degeneration, necrosis and atrophy, diffuse, severe, with regeneration. 2. Skeletal muscle: Sarcocysts, multiple. 3. Skeletal muscle, vessels: Thrombosis, acute and chronic, multifocal, moderate.

Conference Comment: Conference participants agreed that there were confounding factors in this case and it is difficult to determine a specific etiology and chain of events due to the combination of vascular lesions, acute necrosis, atrophy and the presence of variable amounts of fibrous connective tissue. The combination of acute necrosis and atrophy is indicative of a polyphasic process, and participants agreed there is likely more than one event or process which contributed to this lesion. There was discussion regarding the amount of fibrous connective tissue within the section; some participants thought the fibrous connective tissue appeared prominent because of atrophy of surrounding muscle and not necessarily due to excessive fibrosis. Masson's trichrome stain demonstrated the amount of fibrous connective tissue is actually quite variable within the sections, with some areas being dominated by fibrous connective tissue and others having only small amounts, which is likely indicative of the polyphasic and dynamic nature of this lesion. Most agreed the precise cause of the vascular lesions, as well as their role in the pathogenesis of the muscular changes is unclear.

The size of the vessels and duration of obstruction play important roles in determining the size and nature of vascular-origin myofiber necrosis. Blockage of smaller capillaries results in less severe injury due to abundant vascular anastomoses, but can cause segmental myofiber necrosis along with regeneration and, if the cause is ongoing, the lesion would be polyphasic. This is in contrast to blockage of a large artery, where a large section of ischemic muscle would present as acutely necrotic and as a monophasic lesion, and which would eventually be replaced by fibrosis.[3] Causes of muscle ischemia and necrosis that may apply in this case include external muscle pressure and vascular obstruction (due to being recumbent), and swelling of a muscle confined within non-expandable fascia (i.e. compartment syndrome). Additionally, reperfusion injury can result when the animal is moved, which may also cause myofiber necrosis. Muscular injuries in "downer cows" can be either monophasic or polyphasic depending on duration of recumbency.[3]



Rare myocytes contain intracytoplasmic sarcocysts. (HE, 116X)

Following myocyte necrosis, remaining myofiber nuclei are unable to divide and rely on activation of adjacent satellite cells, which are more resistant to injury than myocytes. These cells divide and become activated myoblasts in order to regenerate the damaged segment of muscle. During the regenerative process, myoblast nuclei can be seen within the center of regenerating myofibers, which is a key finding indicative of regeneration. Success of regeneration depends on whether the basal lamina is intact and whether viable satellite cells are present. In the case of significant muscle trauma, there is often disruption of the basal lamina and most healing will, therefore, occur by fibrosis.[3]

Upon consultation, JPC physician neuromuscular pathologists did not believe there was a vascular or neurogenic contribution to the histologic changes.

There was slide variation with some sections having low numbers of sarcocysts which

contained numerous bradyzoites. Participants agreed that the presence of the sarcocysts did not contribute to the muscular lesion.

Contributing Institution:
Oregon State University Veterinary Diagnostic Laboratory
http://vetmed.oregonstate.edu/diagnostic

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CASE IV: 3309 (JPC 4066795).

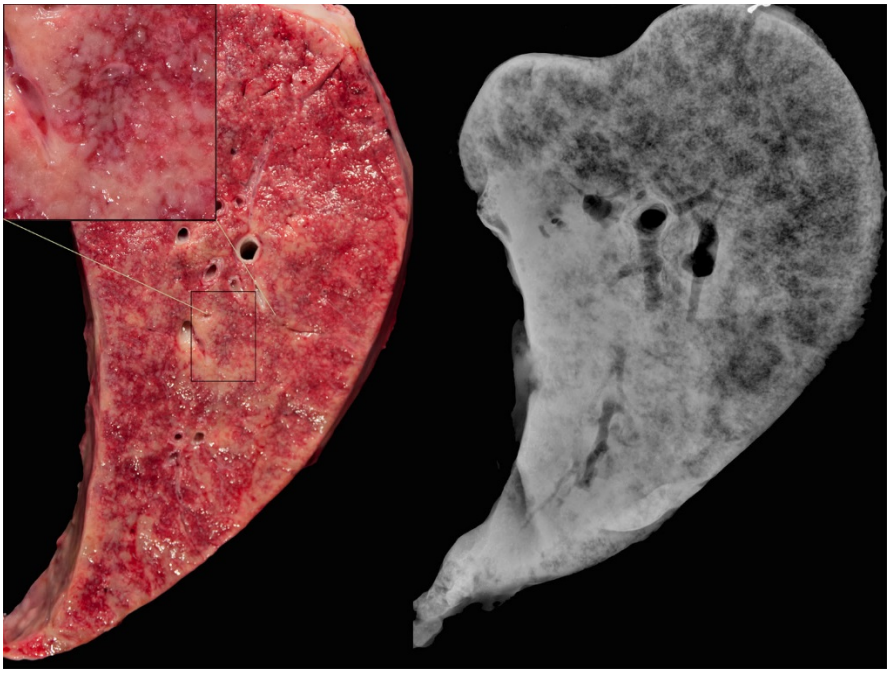
Signalment: 12-year-old, thoroughbred mare (*Equus caballus*).

History: This horse lived in Monterey County, California for at least 2 years. She had a six month history of progressive exercise intolerance, cough and tachypnea. On physical examination, the horse had frequent coughing within minimal exercise (e.g. walking) and markedly increased respiratory rate with abdominal effort and nasal flaring at rest. The horse also had moderate, generalized, decreased muscling. No nasal discharge or pyrexia was noted. Euthanasia was elected due to the severity and progression of the clinical signs.

Gross Pathology: Tan, pinpoint nodules, fibrous tracks and coalescing areas of firm consolidation replaced approximately 75% of pulmonary parenchyma. The nodules bulged on cut surface. Fibrous tracks and consolidated regions frequently extended into the pleural surface. Gritty particles were encountered upon full thickness, serial sectioning of the lung. The pelvis had thickening of the right ilial body and abundant dense fibrous tissue in the area of ischiatic spine (healing fracture callus).

Laboratory Results: Post-mortem radiographs of serial sections of the left lung revealed severe consolidation in the central and ventral lung fields. Small foci of radio-dense material were scattered throughout the lung. Complete blood count (CBC) analysis demonstrated mild fibrinogenemia 500 mg/dL (ref 100-400mg/dL); mild leukocytosis due to neutrophilia 12,155/µL (ref 2600-6800/µL) with slight toxicity; and mild monocytosis 541/µL (ref 0-500/µL). Severely compromised pulmonary parenchyma likely contributed to changes in the serum chemistry analysis that included elevated anion gap at 19mmol/L (ref 7-17mmol/L) with low normal bicarbonate at 23mmol/L (ref 23-32). A qPCR analysis for equine herpes virus 5 was negative on section of affected lung.

Histopathologic Description: There are multifocal to coalescing and nodular aggregates of inflammation and fibrosis that efface ~75% of the parenchyma; these aggregates consistently surround bronchioles and are also scattered within the alveolar parenchyma. Fibrosis diffusely thickens the visceral pleura. Nodules comprise aggregates of large foamy macrophages with fewer lymphocytes and plasma cells. Central areas of coagulation or liquefactive necrosis are occasionally present and mineralized. Multinucleated giant cells are



Side by side comparison of the gross (left) and radiographic (right) appearance of a transverse section through the left lung lobe. Approximately 75% of pulmonary parenchyma has multifocal to coalescing, and frequently nodular, regions of inflam mation and fibrosis that frequently extended to the pleural surface (gross image, left). The magnified insert of the affected region of the lung demonstrates the tan, inflammatory nodules bulging on cut surface. The radiograph reveals a diffuse, military interstitial pattern with severe ventral consolidation. Only few large bronchi contain air and the rest are obliterated by inflammation and fibrosis. (Image courtesy of: University of California Davis, Department of Pathology, Microbiology, Immunology
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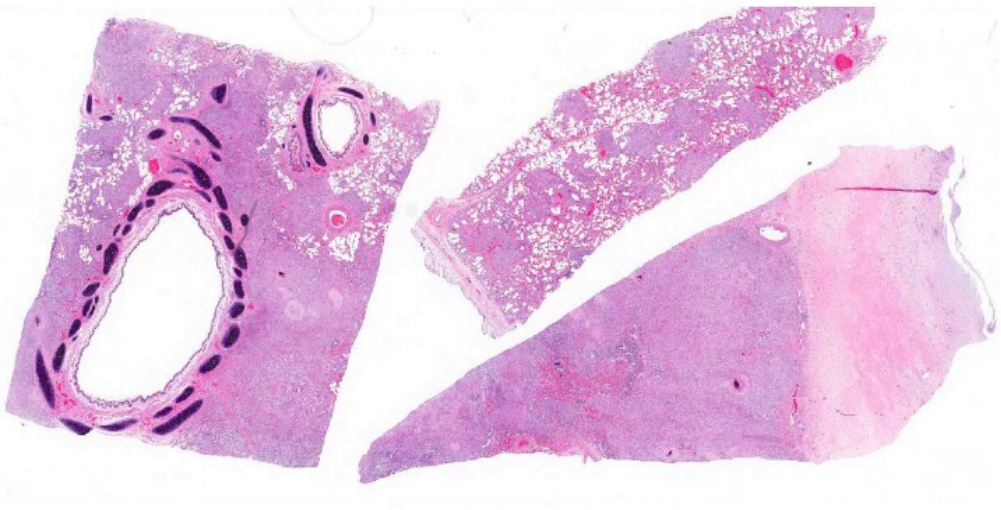
occasionally present. Macrophages frequently contain small numbers of refractile, birefringent, and angular crystals that are approximately one micron in diameter and smaller. Bronchiole lumina are occasionally collapsed and contain proteinaceous, granular debris. Small-caliber blood vessels are generally congested.

Contributor's Morphologic Diagnosis:
Lung: Severe, generalized, multi-nodular to confluent granulomatous pneumonia with intralesional crystals, parenchymal and pleural fibrosis, necrosis and mineralization

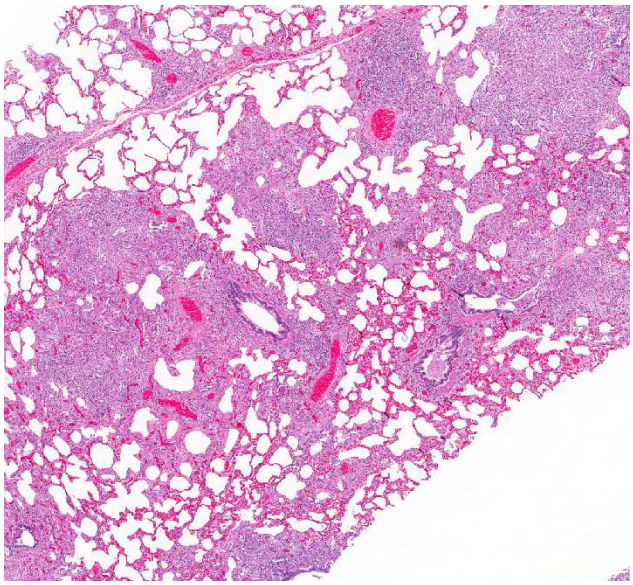
Tracheobronchial, cervical, mediastinal, thoracic aortic lymph nodes (not submitted): Necrotizing and fibrosing granulomatous lymphadenopathy with mineralization and intralesional crystals

Contributor's Comment: Approximately 75% of lung parenchyma was effaced by generalized granulomatous and fibrosing pneumonia, which is consistent with the history of respiratory compromise, tachypnea, cough and exercise intolerance. The morphology of these lesions with intralesional crystals, combined with the exposure to cristobalite in Monterey County, CA, is consistent with pulmonary silicosis (PS). PS is defined as silicate pneumoconiosis and accompanying pulmonary fibrosis.[1] Lesions are caused by inhalation of cytotoxic silica dioxide (SiO_2) crystal polymorphs including quartz, cristobalite, and tridymite.[1] PS is seen with increased frequency in horses from the Monterey, Carmel, Napa and Sonoma regions of California where soils have high levels of



There is multifocal to coalescing areas of inflammation which are primarily centered on small airways, as evidenced by the middle section. (HE, 6X)

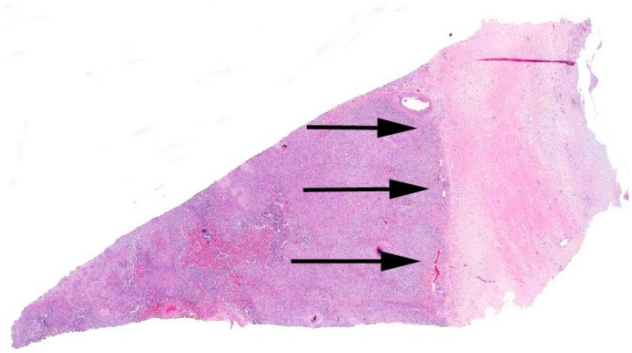
cristobalite.[6] Although PS occurs commonly in the aforementioned areas, a few cases have been reported outside of this region.[2]



Higher magnification demonstrates the bronchiolocentricity of the inflammation in this section of lung. (HE, 12X)

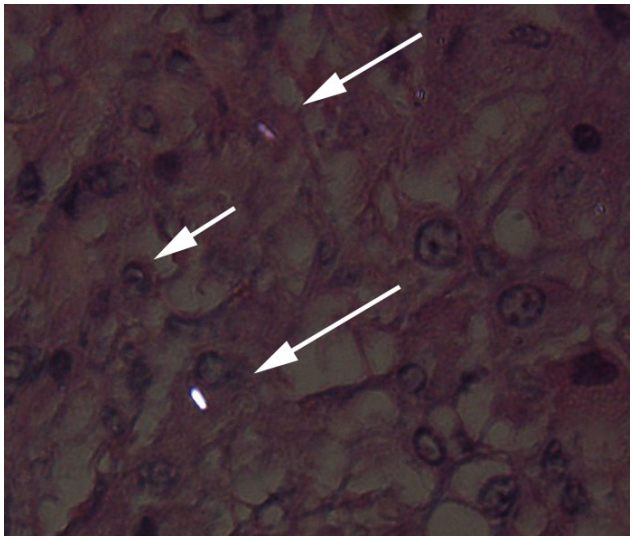
Common presenting signs in horses with silicosis include weight loss, exercise intolerance, respiratory distress, or increased respiratory effort.[2] Typical pulmonary radiographic findings in cases of PS have been described in detail, and are characterized by a diffuse, interstitial pattern ranging in distribution from miliary to micronodular to linear; alveolar consolidation is commonly seen.[2] Gross lesions in uncomplicated cases of PS are usually limited to the pulmonary system. Histologically, lesions are similar to the case presented here with lesions being limited to the pulmonary system and draining lymph nodes. PS is sometimes observed concurrently with bone fragility syndrome. Lesions of the latter have been statistically correlated to pulmonary silicosis; in these cases, the term silica-associated osteoporosis has been proposed (SAO).[3] Gross lesions of SAO reflect systemic osteoporosis and are most apparent in the axial skeleton, particularly the scapulae, ribs, spine and pelvis. For example, bowing of the scapulae and irregular thickening of the scapular spine is one of the prominent features of advanced SAO. Multiple pathological fractures in the ribs and regional expansion of the cortex with porous bone in the axial skeleton are common post mortem findings. Pathological fractures of the scapulae, pelvis and vertebrae are common as well.[1] Osteolytic foci and loss of cortical bone definition are variably observed radiographically in the bones of the axial skeleton and proximal portion of the appendicular skeleton.[1] Increased, heterogeneous radiopharmaceutical uptake associated with increased bone turnover are commonly found in cervical vertebrae, scapulae, ribs, sternebrae and pelvis (Optional JD. Anderson et al 2008). The histological feature of SAO are numerous, large osteoclasts with supernumerary nuclei and foamy cytoplasm that excessively resorb trabecular and cortical bone within osteolytic lesions.[1] Large cavities and thin disordered trabeculae commonly replace parent cortical and trabecular bone. Additionally, disordered osteonal formation results in a mosaic pattern of lamellar bone packets.[1] The etiopathogenesis of SAO is unknown.[1]

In this case, definitive silicosis-associated skeletal lesions were not identified. Although the mare had a healing fracture callus in the pelvis, which is commonly seen in cases of SAO, typical osteoporotic lesions were not observed grossly or radiographically. This case represents an uncomplicated case of PS without confirmed SAO.



One section of lung shows a thick band of pleural fibrosis. (HE 6X)

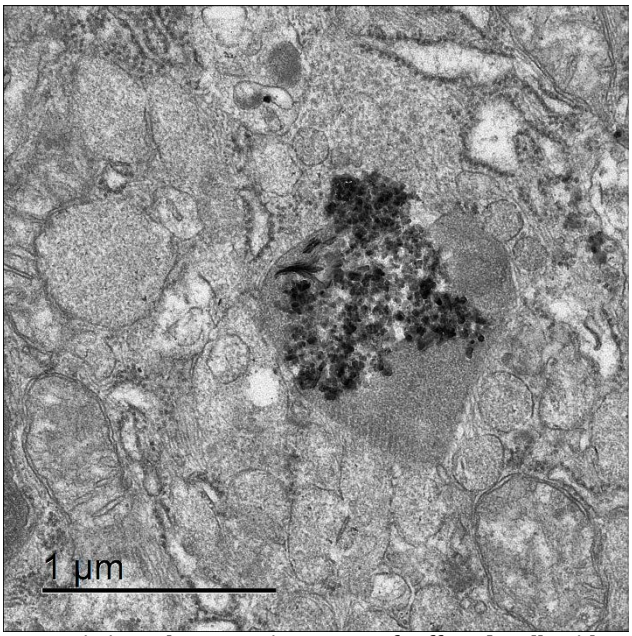
JPC Diagnosis: Lung: Pneumonia, nodular and granulomatous, multifocal to coalescing, severe, with pleural fibrosis and edema, and scattered intrahistiocytic birefringent crystals.



Histiocytes scattered throughout the section occasionally contain small birefringent crystals. (HE, 400X)

Conference Comment: This very interesting case generated lively discussion among participants following review of the history, signalment, and contributor's findings and proposed etiology. Like the contributor, most conference participants agreed the pulmonary changes are centered on small airways and dominated by granulomatous inflammation and fibrosis. Other histologic features discussed included the secondary pleural changes of fibrosis, subpleural edema and mesothelial hypertrophy, as well as the few areas of dystrophic mineralization. That said, participants also commented on the severity of the pathologic changes seen in the sections available for evaluation, which seem excessive in comparison to the relatively low number of birefringent crystals present in most areas of the lesion.

This case was additionally studied in consultation with the Department of Pulmonary and Mediastinal Pathology, whose pathologists have more familiarity and experience with the pathology of pulmonary silicosis, a common occupational exposure in humans. Similar to the contributor and conference participants, the pathologists described the lesions in this case as being centered on small airways (bronchiolocentric), an observation more apparent in the less affected areas of the lung. Specific changes they described include airway constriction, distortion, obliteration and metaplasia secondary to granulomatous inflammation; the bronchiolocentric nature of the lesions indicates the airway as the route of injury. That said, they interpreted the pattern of the granulomatous inflammation as more commonly associated with an infectious etiology versus pulmonary silicosis, such as that from a fungal or mycobacterial agent; in humans, the inflammatory pattern can also occur with inhaled antigens. In their evaluation, the histologic changes in this horse would be atypical for pulmonary silicosis, as the condition in humans typically consists of well-circumscribed nodules with a central area of lamellated collagen bundles surrounded by a thick rim of histiocytes. Furthermore, the nodules seen in human cases of pulmonary silicosis most often contain many more birefringent crystals compared with the extremely low number seen in this individual. Various tissue histochemical stains of submitted unstained serial sections did not demonstrate microbial organisms in this horse.



Transmission electron microscopy of affected cell with a ruptured organelle containing crystals. (Image courtesy of: University of California Davis, Department of Pathology, Microbiology, Immunology, 1 Garrod Dr, UC Davis, Davis CA 95616)

In addition to pulmonary silicosis, conference participants also included equine herpes virus 5 (equine multinodular pulmonary fibrosis) and mycobacterial infection in the differential

diagnosis. Equine multinodular pulmonary fibrosis is histologically characterized by marked interstitial fibrosis with alveolar like architecture lined by cuboidal epithelial cells. Macrophages and neutrophils are present in airways in variable numbers, and intranuclear inclusion bodies are occasionally seen in macrophages.[7] Mycobacterial agents known to cause granulomatous pneumonia in horses include *Mycobacterium avium complex, Mycobacterium tuberculosis* and *Mycobacterium bovis.* Unlike ruminants, the granulomas in horses typically do not have prominent casseous necrosis or calcification in the center of lesions, and extensive fibrosis is seen with chronicity, giving the lesions a sarcomatous appearance.[4]

We thank the contributor for providing excellent supporting materials with the case submission, which immensely increased the teaching and learning value of this very interesting case, including the superb gross and electron microscopy images, radiographs and clinical pathology data.

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