WEDNESDAY SLIDE CONFERENCE 2013-2014

Conference 24

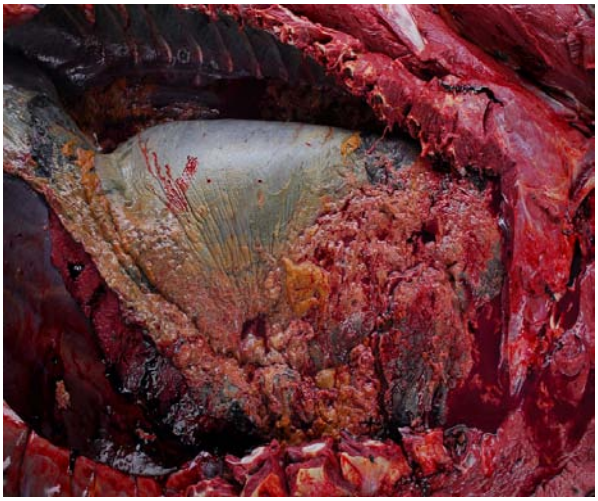
30 April 2014

CASE I: R08-185 (JPC 3165077).

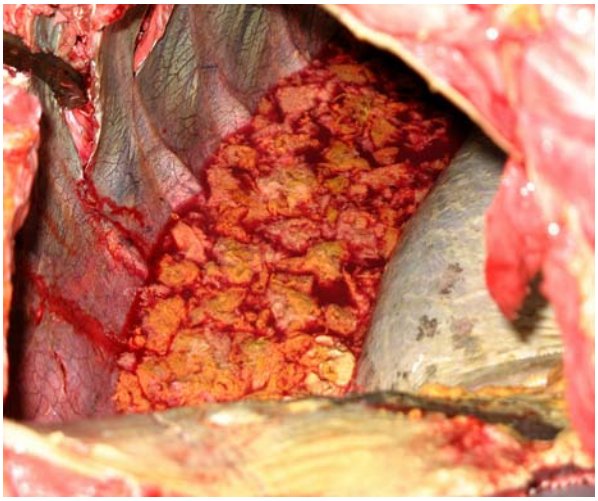
Signalment: Adult castrated male horse, (*Equus caballus*).

History: During quarantine, five horses imported from New Zealand to Taiwan exhibited depression, anorexia and dyspnea with elevated body temperature (40.2-40.5°C) on day one after arrival. They were treated with antibiotics and supportive therapy; however, one was found dead three days later.

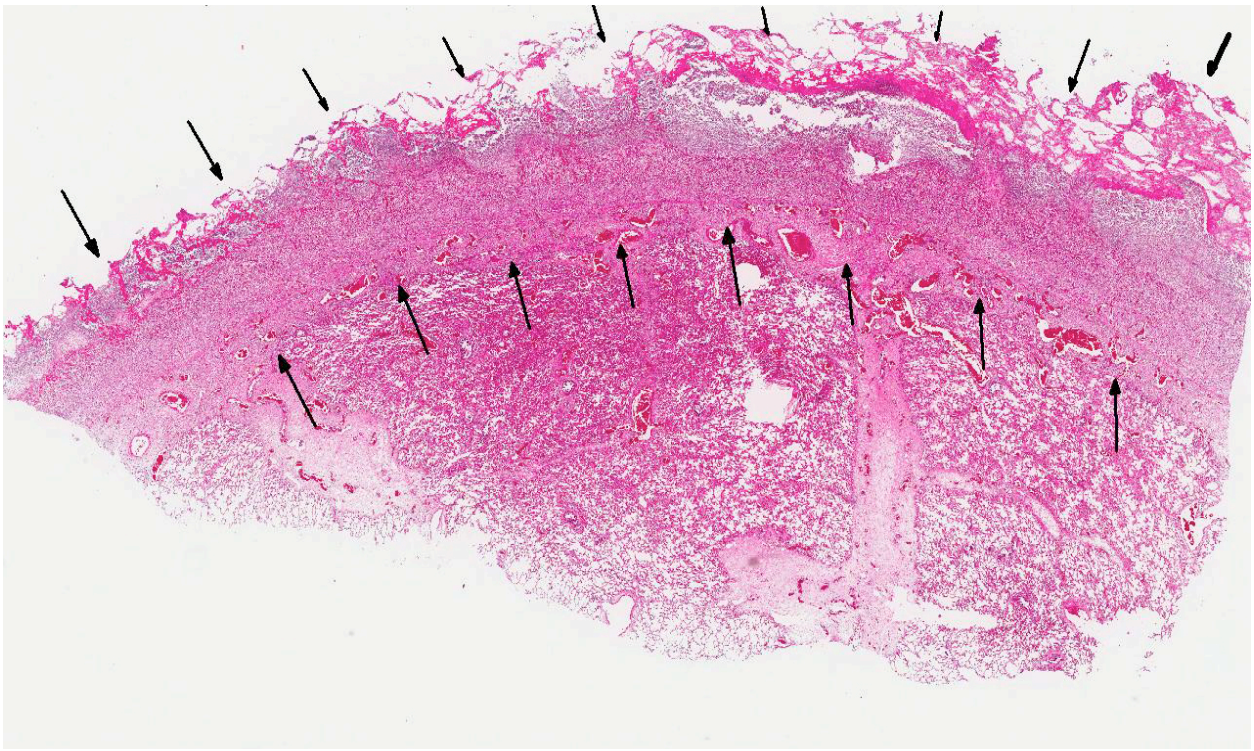
Gross Pathology: The horse shows poor body condition with moderate dehydration. The thoracic cavity is filled with turbid, serosanguineous and fibrinous pleural effusion, with marked thickened attachments between the



1-1. Lung, horse: Grossly, the pleural surface was covered by dense mats of fibrin and suppurative inflammation with numerous adhesions between the visceral and parietal pleura. (Photo courtesy of: Division of Animal Medicine, Animal Technology Institute Taiwan, http://www.atit.org.tw/ATIT/)



1-2. Lung, horse: The thoracic cavity is full of purulent exudate with flocculent fibrin clumps. (Photo courtesy of: Division of Animal Medicine, Animal Technology Institute Taiwan, http://www.atit.org.tw/ATIT/)



1-3. Lung, horse: The pleura is markedly expanded (arrows). (HE 0.63X)

parietal and visceral pleura. Many yellowish nodules, 0.3 to 10 cm in diameter, are scattered around the parenchyma of the lung. The tracheobronchial lymph nodes and spleen are moderately enlarged.

Laboratory Results: *Streptococcus equi* subsp. *zooepidemicus* was identified from thoracic fluid, lung and spleen.

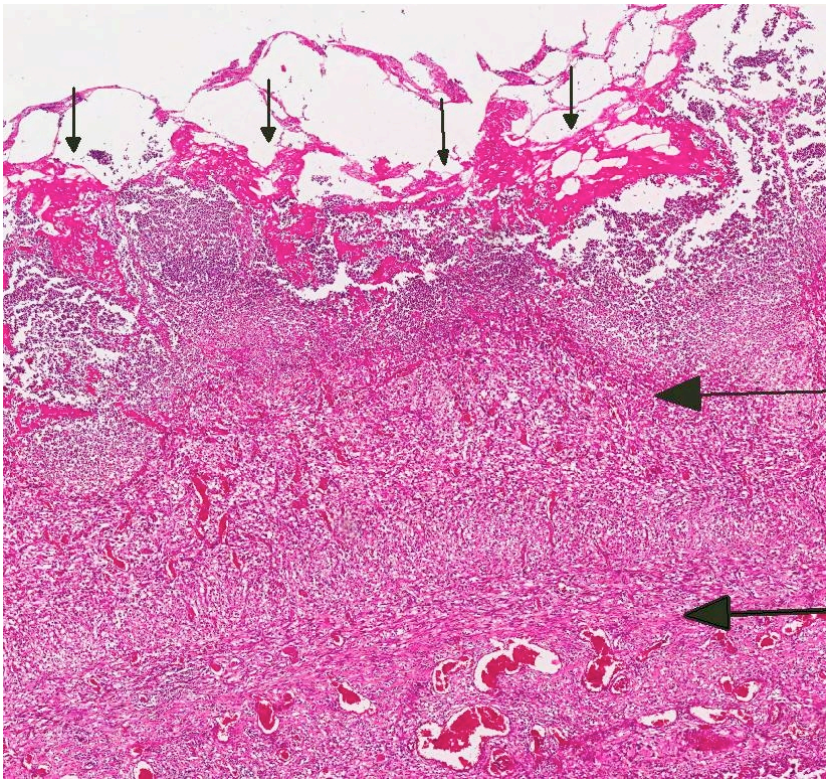
Histopathologic Description: A dense band of degenerate neutrophils admixed with bacterial colonies and fibrinous exudates covers the visceral pleura. The subpleura contains many well-vascularized, congested vessels and organized loose fibrous connective tissue underlain by inflammatory cells. The subpleural areas are filled with viable and degenerate plasma cells, epithelioid macrophages, fewer multinuclear giant cells and lymphocytes. There is diffuse intra-alveolar and interlobular edema and congestion. Alveolar septa are congested. Some sections of lung contain large areas of abscessation (not provided). These foci contain sheets of degenerate neutrophils admixed with fewer macrophages, necrotic debris, and colonies of cocci.

Contributor's Morphologic Diagnosis:

1. Lung: Pleuritis, fibrinopurulent, diffuse, severe, subacute to chronic, with intralesional cocci colonies.
2. Lung: Abscesses, multifocal, moderate, subacute, with intralesional cocci.

Contributor's Comment: *Streptococcus equi* subsp. *zooepidemicus* is a beta-hemolytic gram-positive cocci that belongs to the Lancefield group C of streptococci and causes disease in a variety of mammals.[1,2,6] It consists of 3 subspecies, *S. equi* subsp. *equi*, *S. equi* subsp. *zooepidemicus*, and *S. equi* subsp. *ruminatorum*.

Transmission is mainly via aerosol, wound contamination, oral or contagious route.[2] Aerosol transmission is the most likely route in this case. *S. equi* subsp. *zooepidemicus* is considered to be an opportunistic pathogen in horses and alpaca; it is commonly found in the nasopharynx of clinically normal horses.[1,6] There are reports of sporadic or outbreaks in many species, including humans. *S. equi* subsp. *zooepidemicus* has been associated with mastitis,[12] abscesses, meningitis, endocarditis, reproductive system disease, orchitis, arthritis, septicemia and respiratory and uterine infections.[1,3,7] Human infections caused



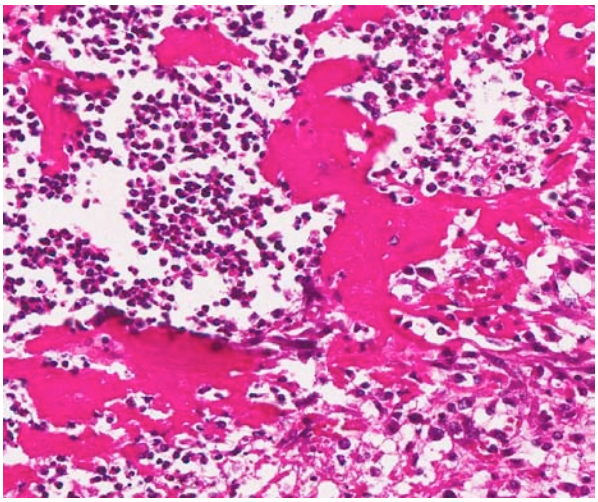
1-4. Lung, horse: Higher magnification demonstrates a thick superficial mat of fibrin and suppurative exudate (small arrows), covering a thick layer of granulation tissue (large arrows). (HE 24X)

by *S. equi* subsp. *zooepidemicus* include outbreaks of foodborne illness, meningitis, septicemia, arthritis, pneumonia, glomerulonephritis, and streptococcal toxic shock syndrome, in both immunocompromised and immunocompetent patients.[3] Severe *S. equi* subsp. *zooepidemicus* induced pleuropneumonia has been observed in horses. In Peru, infection can cause a mortality rate of 50-100% of affected alpacas (known as alpaca fever).[4,6] In China, *S. equi* subsp. *zooepidemicus* is the most commonly isolated secondary pathogen in swine since 1975. Humans may be infected via contact with sick animals, unpasteurized milk or other dairy products;[12] therefore, this bacterium poses a zoonotic health risk to animals and humans.

Since 2004, this pathogen has been isolated from three batches of horses imported (from the United States and New Zealand) to Taiwan. The source of infection in the present cases is presumably associated with transport stress. There have been no reports of *S. equi* subsp. *zooepidemicus* infection in livestock, zoo/wild animals or pets in Taiwan prior to this report.

JPC Diagnosis: Lung: Pleuritis, fibrinosuppurative, chronic, diffuse, severe, with granulation tissue, mild fibrinous interstitial pneumonia and rare colonies of cocci.

Conference Comment: Conference participants are urged to review WSC 2013-2014, conference 5, case 1 for a detailed review of various *Streptococcus* species important in veterinary medicine. *S. equi* subsp. *equi* is the causative agent of strangles, a contagious infection of the equine upper respiratory tract and local lymph nodes with occasional hematogenous dissemination to internal organs (bastard strangles). Bronchopneumonia (due to aspiration of nasopharyngeal exudate), guttural pouch empyema, laryngeal hemiplegia ("roaring" due to recurrent laryngeal nerve compression from retropharyngeal lymphadenopathy) and facial paralysis/Horner syndrome (secondary to compression of adjacent sympathetic nerves) are common sequelae to strangles. *S. equi* has also been linked with immune-mediated vasculitis and purpura hemorrhagica in horses. *S. equi*, unlike *S. equi*



1-5. Lung, horse: Higher magnification of the most superficial layers of pleura, with dense polymerized fibrin and numerous viable and degenerate neutrophils. (HE 128X)

subs. *zooepidemicus,* is not part of the normal nasal flora.[8,9,13] *S. zooepidemicus* is associated with reproductive disease and bursitis (or fistulous withers) in horses,[12] as well as cervical lymphadenitis (or "lumps") in guinea pigs.[10] It was also implicated in an outbreak of acute hemorrhagic pneumonia in more than 1,000 shelter dogs in California[11] and has been associated with increased severity of clinical signs in dogs affected by the canine infectious respiratory disease complex, which likely involves challenge with both bacterial (*Bordetella bronchiseptica, Pasturella* spp., *Mycoplasma* spp., *S. zooepidemicus*) and viral (canine parainfluenzavirus and canine adenovirus) agents.[3] *S. equi* subsp. *ruminatorum* has been associated with mastitis in domestic sheep and goats, as well as abscesses (similar to strangles) with subsequent pneumonia in spotted hyenas and zebras.[5]

Contributing Institution: Division of Animal Medicine
Animal Technology Institute Taiwan
http://www.atit.org.tw/ATIT/

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CASE II: H13-3451 (JPC 4037901).

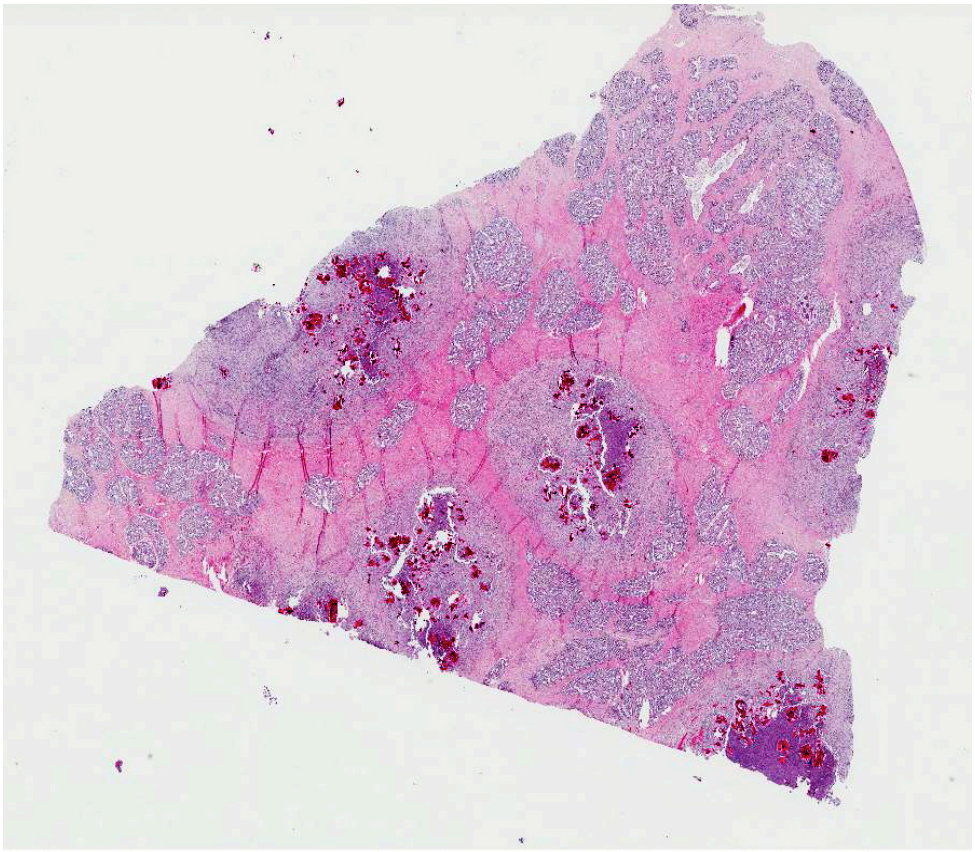
Signalment: 7-year-old female breed unspecified ox, (*Bos taurus*).

History: This 7-year-old cow calved 4-5 months previously, and had been at pasture since. Found dead without premonitory signs.

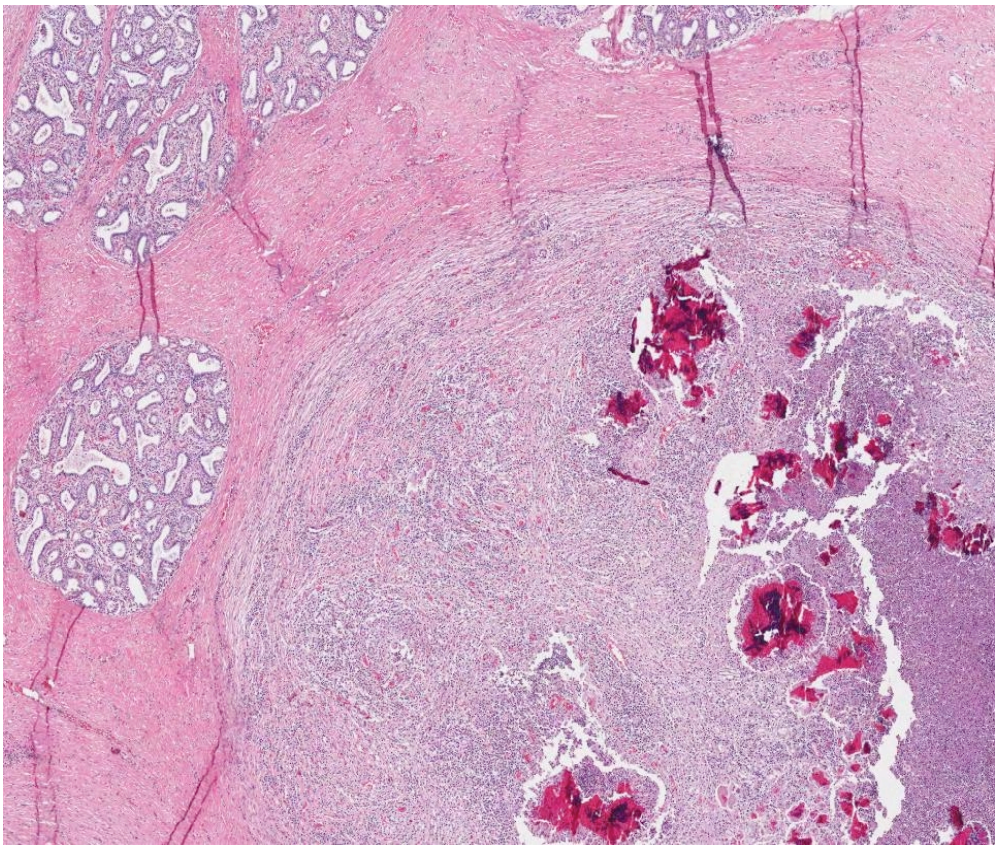
Gross Pathology: Carcass preservation is poor, but the animal is in good body condition. Gross lesions are confined to urinary tract, mammary gland and supramammary lymph nodes: bilateral, chronic, fibrinopurulent and severe pyelonephritis; chronic, necrosuppurative, severe cystitis; urolithiasis due to multiple sand-like calculi; and induration of the left-fore mammary gland quarter. No milk could be expressed. On cut surface, the gland parenchyma was markedly fibrosed with multifocal 2-3 mm areas of suppuration. The left supramammary lymph node was enlarged with a 1 cm diameter focus of suppuration.

Laboratory Results: Beta-haemolytic, coagulase-positive *Staphylococcus aureus* was isolated from the supramammary lymph node. *Corynebacterium renale* was isolated from the kidney.

Histopathologic Description: Mammary gland. Separating lobules of atrophic acini are extensive bands of mature fibrous tissue which contain multifocal to coalescing pyogranulomas up to 10 mm in diameter. Pyogranulomas are centered on colonies of gram-positive cocci (gram-stained section not submitted) within up to 100 µm long radiating columns of hyaline eosinophilic material (Splendore-Hoeppli material). These in turn are surrounded by variably sized zones of viable and degenerate neutrophils, occasionally within a large area of necrosis, bounded by large numbers of macrophages and varying numbers of multinucleated giant cells mixed with lymphocytes, plasma cells and small numbers of neutrophils, bounded by concentric bands of fibrous tissue. Mammary acini are devoid of secretory product and are lined by cuboidal to low columnar epithelium which is multifocally vacuolated or necrotic and sloughing. Multifocally there is exocytosis of low numbers of neutrophils and lymphocytes into acinar epithelium and multifocally acini contain small amounts of eosinophilic fibrillar material (fibrin) and small numbers of neutrophils and/or macrophages. Within the interstitium of the secretory tissue there is vascular congestion and a diffuse mild infiltration of lymphocytes, plasma cells and fewer macrophages and a diffuse mild fibroplasia.



2-1. Mammary gland, ox: Glandular tissue is expanded by multiple non-confluent abscesses. (HE 0.63X)



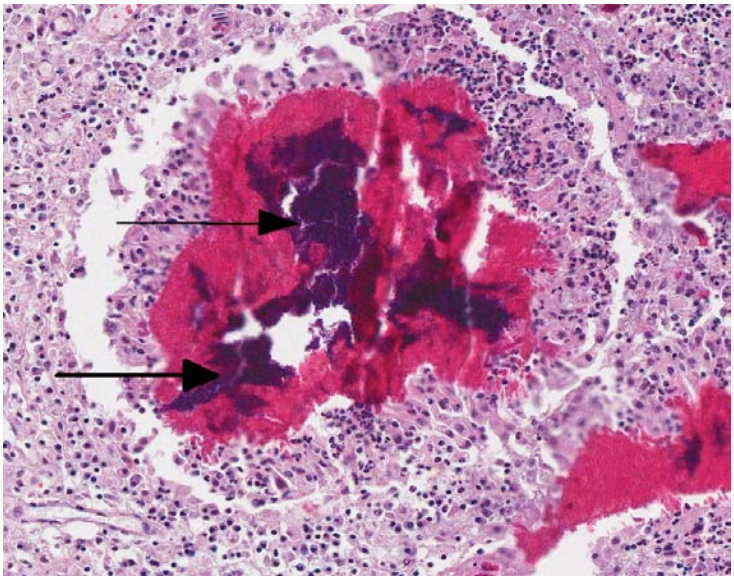
2-2. Mammary gland, ox: Higher magnification of one of the abscesses, which is centered on numerous bacterial colonies encased in brightly eosinophilic Splendore-Hoeppli material, and surrounded by a dense fibrous capsule. (HE 25X)

Contributor's Morphologic Diagnosis: Mammary gland: pyogranulomatous mastitis, chronic with intra-lesional bacteria.

Contributor's Comment: The cause of death in this case was renal failure due to pyelonephritis. The chronic mastitis was judged to be incidental with respect to the presentation of sudden, unexpected death. *Staphylococcus aureus* was isolated from the supramammary lymph node. Although culture was not performed on the mammary gland, gram positive nature of the intralesional cocci would be consistent with *Staphylococcus* spp.

S. aureus is one of the most common causes of bovine mastitis. Clinically, staphylococcal mastitis may be peracute and fulminating or milder and more chronic. The acute forms of disease generally occur shortly after parturition and tend to produce gangrene of the affected quarters with high mortality.[9] The chronic or subclinical forms are more common and thus associated with the most important economic losses. The clinical presentation may be related to the strain of *S. aureus*; strains differ in their ability to spread within herds, and to cause somatic cell count elevation, clinical mastitis, or persistent infections or loss in milk production. *In vitro*, strains differ in their ability to withstand killing by neutrophils or invade mammary epithelial cells.[1]

The main reservoirs of infection are infected quarters and lesions on the skin of the udder and teat. Once *S. aureus* contaminates the teat orifice, it can persist and multiply before entering the teat canal and sinus and disseminating within the mammary gland. Colonization of the distal part of the mammary gland may be achieved by adhesion to specific receptors on the surface of epithelial cells. The adhesion varies from very low to extremely high numbers of bacteria per cell. *In vitro* adhesion depends on multiple factors including strain and origin of mammary epithelial cells.[5] The host response to the penetration includes degeneration and necrosis of epithelial cells and exudation of neutrophils into the interlobular tissue and secretory acini. If the exudation is massive and the organisms highly toxigenic, the acute and gangrenous forms of the disease occur. *S. aureus* can also invade more deeply into the inter-acinar tissue and establish persistent foci of infection that provoke botryomycotic granulomatous reactions associated with marked fibroplasia.[9] Acinar atrophy may be due to pressure from this fibrosis and also from occlusion of small ducts by



2-3. Mammary gland, ox: Dense aggregates of Splendore-Hoeppli material surround large colonies of staphylococci (arrows). (HE 225X)

exudate or granulation tissue causing obstruction of milk flow from unaffected lobules.[9]

JPC Diagnosis: Mammary gland: Mastitis, pyogranulomatous, multifocal, severe, with numerous cocci and Splendore-Hoeppli material.

Conference Comment: The tissue sections examined in this case are composed primarily of lobules of mammary ducts; mammary glands/ acini are largely atrophied or lost, likely due to pressure necrosis secondary to abundant inflammation. Mastitis is the single most common disease syndrome of adult dairy cows. Routes of infection vary from ascending infection of the teat canal (most common) to hematogenous or percutaneous. The most commonly bacterial isolates are *Streptococcus* spp., *Staphylococcus* spp., and gram-negative coliforms, especially *Escherichia coli* (also *Enterobacter aerogenes, Klebsiella pneumoniae, Citrobacter* spp., *Pasteurella multocida, Pseudomonas aeruginosa*, *Serratia* spp., and *Proteus* spp.). The mammary gland is the principal site of persistence or reservoir for certain bacterial species, including *Streptococcus agalactiae, Staphylococcus aureus* and *Mycoplasma bovis*, while infection with coliforms is typically acquired via teat contamination from the external environment (e.g., fecal contaminated bedding, soil or water). *Streptococcus uberis* and *S. dysagalactiae* can persist in either location. Other pathogens associated with bovine mastitis include *Trueperella pyogenes, Prototheca zopfii, Nocardia asteroides*, *Mycobacterium* spp., and less commonly, *Brucella abortus*, *Mannheimia haemolytica*, *Salmonella* spp., *Cryptococcus neoformans*, and *Candida* spp.[4,7,9]

Staphylococcus aureus is the most commonly reported etiology of mastitis. *S. aureus* isolates range from nonpathogenic to highly pathogenic; catalase and hemolysin production are the best indicators of bacterial pathogenicity.[4,9] Factors in normal milk which inhibit bacterial growth are listed in table one.[7,9] *S. aureus* has developed multiple virulence factors to overcome these defense mechanisms, which are listed in table two.[2-5,7]

Although most problematic in cattle, mastitis also affects many other domestic animal species. The major agents recovered from sheep and goats with necrotizing or gangrenous mastitis are *S. aureus* and *Mannheimia haemolytica*. For mycoplasmal mastitis, the typical causative agents are *Mycoplasma agalactiae* or *M. mycoides*. Additionally, goats and sheep infected with the small ruminant lentiviruses, caprine arthritis and encephalitis virus and maedi-visna virus, respectively, develop "hard udders" with agalactia. Equine mastitis is sporadic, and *Streptococcus zooepidemicus* is the typical cause.[4,7,9] In swine, mastitis usually occurs in lactating or recently weaned sows. Gram-negative coliforms are the most commonly isolated etiologic agents; gram-positive bacteria such as *Streptcoccus, Staphylococcus* and *Aerococcus* spp. are reported less frequently.[6] In dogs and cats mastitis is uncommon; dogs are more likely to present with mammary neoplasia, while fibroadenomatous hyperplasia (mammary hyptertrophy) is the most prevalent mammary lesion in cats. When present in dogs or cats, mastitis tends to occur in early lactation, due to *Staphylococcus* spp., *Streptococcus* spp. or *E. coli* entering lactiferous ducts via fissures in nipples.[4] *E. coli, Klebsiella pneumoniae* and *Streptococcus zooepidemicus* are commonly encountered in guinea pig mastitis, while *S. aureus* (type C) and *Pasturella multocida* tend to affect rabbits. Mastitis is also reported in rats (typically due to *Pasteurella pneumotropica, S. aureus, Corynebacterium* spp., or

Pseudomonas spp.), and hamsters (beta-hemolytic *Streptococcus* spp., *P. pneumotropica, E. coli*).[8]

Table 1. Antibacterial factors in milk.[7,9]

Antibacterial Factor	
Phagocytic cells	Phagocytosis is less efficient in milk than in serum
Lactoferrin	Iron-binding protein that inhibits bacterial multiplication
Lysozyme	Lyses bacterial cell wall peptidoglycan
Lactoperoxidase	May inhibit *S. aureus* and streptococci
Hydrogen peroxide	A weak oxidizing agent that is a byproduct of bacterial fermentation of milk carbohydrates
Immunoglobulins	Primarily IgG, which promotes opsonization; less IgA, which may reduce bacterial adherence at epithelial surfaces

Table 2. Select virulence factors of *S. aureus*.[2-5,7]

Virulence Factor	
Leucocidin	Cytolytic to bovine leukocytes
Alpha-toxin	Binds cell membranes forming hexameric pores; not produced by all *S. aureus* isolates
Beta-toxin	A phospholipase C or sphingomyelinase
Protein A	Antiphagocytic factor that binds to the F_c fragment of IgG
Extracellular enzymes	Coagulase, hyaluronidase, phosphatase, nuclease, lipase, catalase, staphylokinase (fibrinolysin), superantigens and proteases
Bacterial capsule	Interferes with opsonization, phagocytosis, and complement activity; not present in all strains of *S. aureus*
Penicillinase	Splits beta-lactam ring of penicillin

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CASE III: 12-503 (JPC 4017811).

Signalment: 15-year-old male castrated Maine Coon cat, (*Felis catus*).

History: 1-week history of lethargy and anorexia. The cat presented recumbent and minimally responsive; it went into respiratory arrest the next morning and was euthanized.

Gross Pathology: The liver and spleen contain numerous 0.5 mm white nodules. Most lymph nodes are enlarged and mottled red and white.

Laboratory Results:

Hematocrit: decreased (21)
ALT: increased
Albumin: decreased

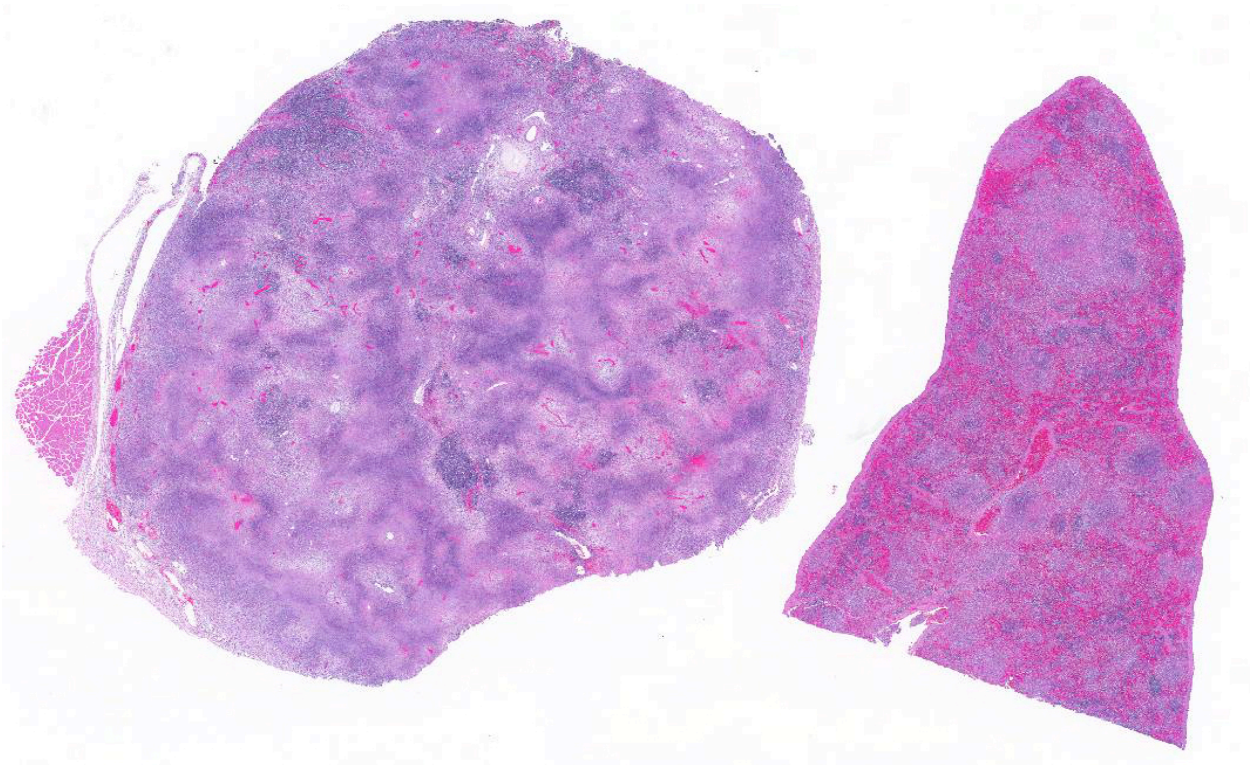
Histopathologic Description: Lymph nodes and spleen have multifocal coalescing areas of necrosis containing macrophages and a few neutrophils. The spleen also has numerous aggregates of mast cells.

Contributor's Morphologic Diagnosis: Lymphadenitis and splenitis, necrotizing, granulomatous, multifocal, severe.

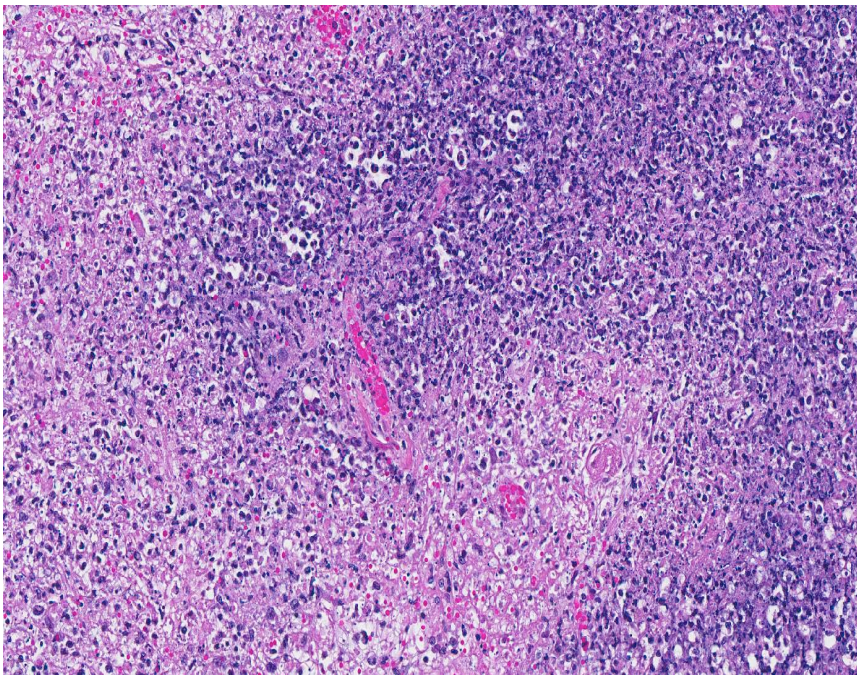
Contributor's Comment: This is a case of tularemia caused by *Francisella tularensis*. The organism was not cultured from the tissues but its presence was confirmed by PCR. *Francisella tularensis* is a potential agent of bioterrorism and is a reportable disease.

Francisella tularensis is a gram-negative bacterial rod and a facultative intracellular pathogen of macrophages. The organism is difficult to grow in artificial media and the diagnosis is best confirmed by PCR.

Francisella tularensis is the cause of the disease tularemia. Tularemia has a worldwide distribution and affects a variety of mammals, birds, amphibians and fish. The organism is maintained in the environment by various terrestrial and aquatic mammals, primarily rabbits and rodents, and is transmitted by a variety of arthropods including ticks, mites, blackflies, fleas, mosquitoes and lice.[2] The organism may also be transmitted by contact with infected vertebrates,



3-1. Lymph node and spleen, cat: The lymph node, and to a lesser extent, splenic architecture is effaced by confluent areas of lytic necrosis. (HE 0.63X)



3-2. Lymph node, cat: The lymph node show almost total effacement of architecture by lytic necrosis and almost total lymphorrhexis. (HE 208X)

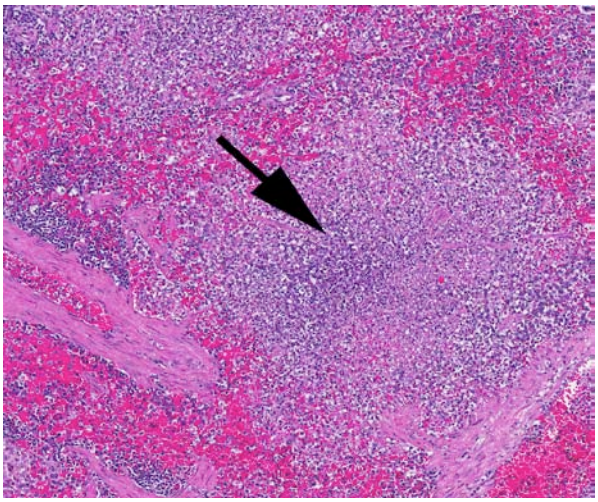
by inhalation of feces-contaminated dust, or ingestion of insufficiently cooked infected carcasses. Human infections usually result from skinning or dressing rabbits, and rabbits are the source of infection in 90% of human cases.[4]

Gross lesions of tularemia are multifocal white spots in liver, spleen and lymph node varying in size from pinpoint to several millimeters. Microscopic lesions are multifocal to confluent areas of necrosis with a mild influx of macrophages and neutrophils.

JPC Diagnosis: 1. Spleen: Splenitis, necrotizing, multifocal to coalescing, severe.
2. Lymph nodes: Lymphadenitis, necrotizing, multifocal to coalescing, severe.

Conference Comment: Tularemia, a zoonotic disease also known as rabbit fever or deer-fly fever, has a worldwide distribution, is currently listed as a category A select bioterrorism agent and is thus a reportable disease in the U.S.[5] Two main biovars have been described. The more virulent biotype A (*F. tularensis* subsp. *tularensis*) ferments glycerol and spreads (predominantly in North America) via ticks and hemophagous insects (mosquitoes and biting flies). The primary tick vectors described in the U.S. include the wood tick (*Dermacentor andersoni*), the American dog tick (*Dermacentor variabilis*), the Pacific coast tick (*Dermacentor occidentalis*) and the lone star tick (*Amblyomma americanum*). The less virulent biotype B (*F. tularensis* subsp. *holarctica*) does not ferment glycerol, exhibits a complex aquatic epidemiology and is more common in Eurasia, where infection usually occurs due to ingestion of infected prey or water contaminated by rodents. Type A strains are typically responsible for clinical disease in rabbits, cats and humans.[1,3] *F. tularensis* has an extensive host range, and infections in more than 200 animal species (primarily mammals, but also birds, fish, amphibians, and reptiles) have been reported. Infection rates appear to demonstrate seasonal variability; cases are reported most frequently from May to August, likely due to the activity of arthropod vectors.[5]

F. tularensis can pass transovarially within tick vectors, which are infected for life.[3] Additionally, the bacterium can survive for weeks to months in water, soil, and decaying animal carcasses.[5] Bacteria are typically inoculated into the host



3-3. Spleen, cat: Multifocal areas of lytic necrosis are present throughout the section of spleen, primarily affecting white pulp. (HE 125X)

during tick feeding/defecation or following ingestion of infected rabbits or rodents. Dogs appear to be fairly resistant while cats and rabbits are susceptible. The infectious dose can be quite low; inhalation or parenteral inoculation of 10 to 50 colony-forming units can induce clinical disease, while 10^8 colony-forming units are required for oral infection. *F. tularensis* persists within macrophages where it inhibits phagosome-lysosome fusion; the bacterial capsule is thought to be important in intracellular survival.[1,3] Necrosis generally centers upon lymphoid tissue within the spleen and lymph nodes, although lesions within the liver and lungs (especially if bacteria are inhaled) are also commonly described.[3,5] The gross lesions associated with *F. tularensis* (foci of splenic, lymph node and hepatic necrosis and/or pyogranulomatous inflammation) are indistinguishable from lesions associated with yersiniosis. Histologically, *Yersinia* spp. often form large colonies, while *F. tularensis* coccobacilli are typically found within macrophages,[6] although individual bacterial were not readily apparent in this case.

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CASE IV: O20/09 (JPC 3167509).

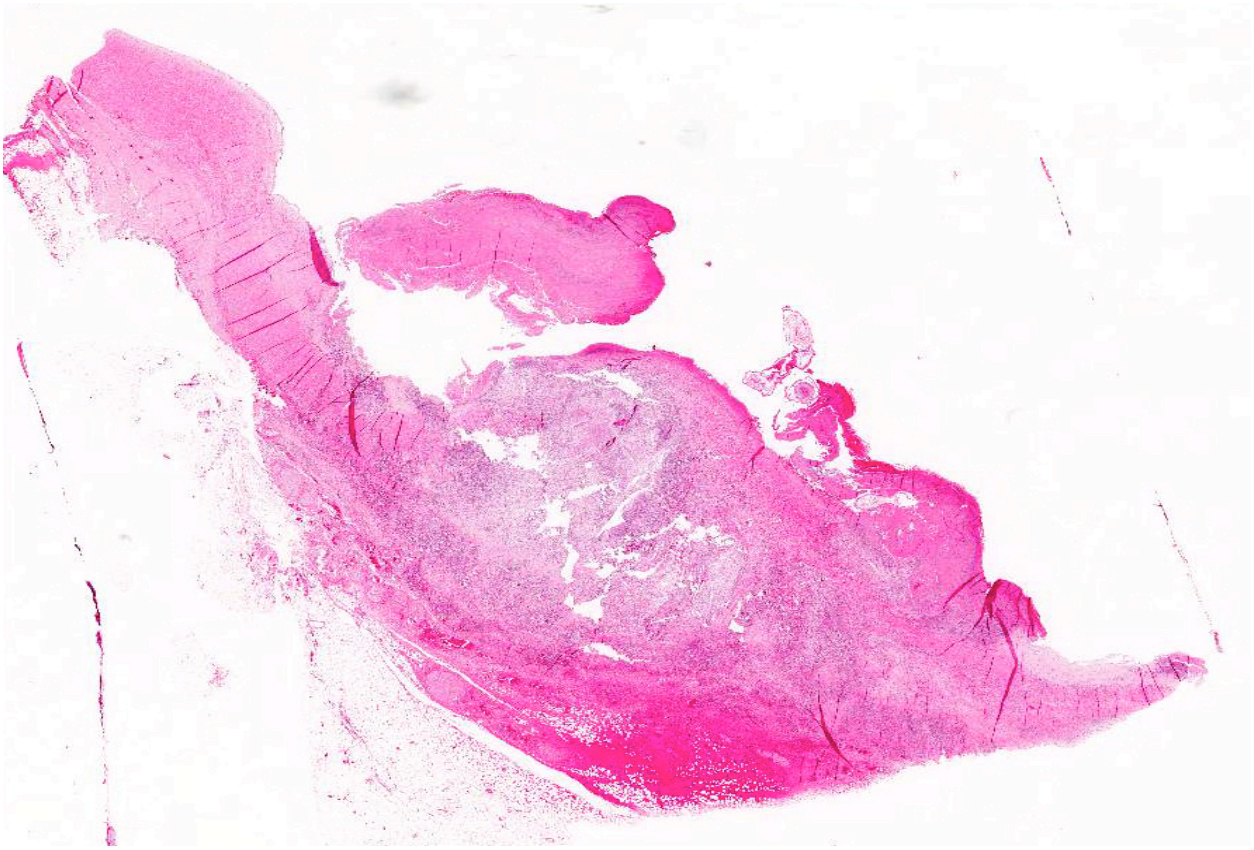
Signalment: 5-year-old female Swedish riding pony, (*Equus caballus*).

History: The horse was euthanized, and presented for necropsy after traumatic injury to the left hock, with suspected septic bursitis of the left calcaneal bursa and possible bone involvement.

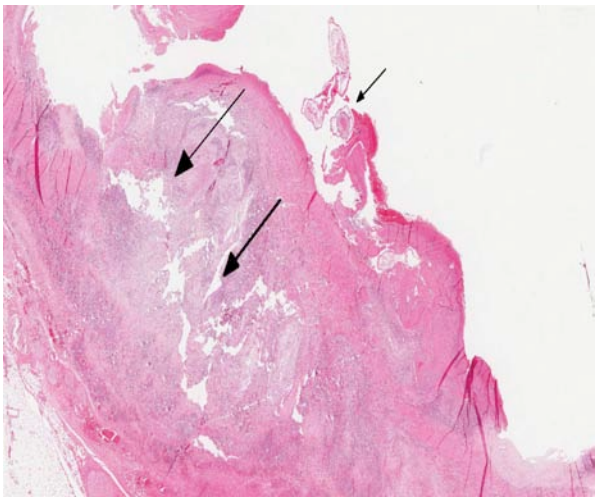
Gross Pathology: There is diffuse thickening of the dermis and focal ulceration (approximately 2x2 cm) on the dorsal aspect of the left hock, exposing dry granulation tissue. The calcaneal bursa contains fibrinopurulent and hemorrhagic material and there is hypertrophy and ecchymotic hemorrhages of the synovial membrane.

Within the cranial mesenteric artery, there is a focal area with thickening of the arterial wall and vegetative thrombotic masses attached to the vascular intima with presence of several slender nematode parasites, less than 20 mm in length.

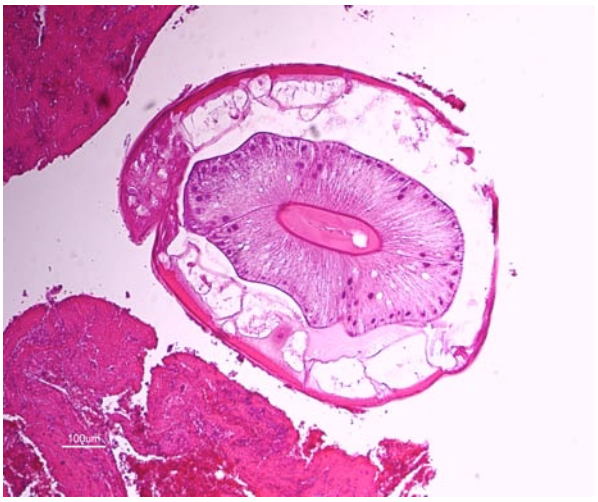
Histopathologic Description: Large muscular artery and surrounding soft tissues: There is a marked inflammatory reaction involving tunica intima, media, and adventitia with multifocal to coalescing infiltrates of a mixed population of inflammatory cells, which in all arterial layers consist of large numbers of plasma cells and lymphocytes, and moderate to large numbers of eosinophils and histiocytes. There is extensive multifocal to coalescing necrosis, predominantly involving the tunica media and intima. Continuous with and disrupting/destructing the endothelial lining of the tunica intima, are extensive depositions of deeply eosinophilic homogenous to finely granular fibrinous masses (thrombi) containing moderate amounts of cellular debris, multifocal accumulations of erythrocytes, neutrophils, and multifocal sheets of large numbers of lymphocytes, plasma cells, and moderate numbers of eosinophils (with focal areas of mineralization present in some sections). The tunica intima also shows thickening, fibrosis and multifocal small hemorrhages.



4-1. Mesenteric artery, horse: The lumen of this opened section of artery is compromised by the presence of a large adherent thrombus. (HE 0.63X)



4-2. Mesenteric artery, horse: Cross sections of adult nematodes (small arrows) are present at the luminal surface of the fragmented and inflamed thrombus (large arrows). (HE 90X)



4-3. Mesenteric artery, horse: A cross-section through the adult strongyle demonstrates a smooth eosinophilic cuticle, prominent coelomyarian-polymyarian musculature, one visible lateral chord and an intestine lined by multinucleated cells with a prominent brush border. (HE 150X) (Photo courtesy of: Department of BVF, Division of Pathology, Pharmacology & Toxicology, SLU (Swedish University of Agricultural Sciences), Box 7028, SE-750 07 Uppsala, Sweden http://www.bvf.slu.se/)

On the luminal side of the intima and partially embedded within the thrombotic masses, are several metazoan parasites (measuring approximately between 0.6 and 1.2 mm), covered by a smooth cuticle, and showing a thin hypodermis, platymyarian musculature, and a large intestine with few multinucleated cells and a prominent brush border (strongyle larvae).

In the tunica media, the heavy infiltration of inflammatory cells and areas of necrosis disrupt myocyte alignment and the borders between the tunica media and adventitia and intima. In the tunica adventitia and surrounding soft tissue, the inflammatory infiltrates are predominantly perivascular and lymphoplasmacytic, and there is hyperemia of venules. Moderate to large numbers of slender rod-shaped bacteria are also seen (post-mortem bacterial growth).

Contributor's Morphologic Diagnosis: Cranial mesenteric artery: Arteritis, lymphoplasmacytic and eosinophilic, chronic, transmural, severe, with multifocal necrosis, thrombosis and intra-luminal/-lesional strongyle larvae.

Contributor's Comment: The location and nematode morphology suggest that the presented case represents verminous arteritis caused by *Strongylus vulgaris*. No parasites were observed in the large intestine, which may have related to the necropsy being performed during winter when intestinal numbers of *S. vulgaris* are low.[2]

Strongyle nematodes are important gastrointestinal parasites of the horse. Strongyles have a worldwide distribution, and in a recent Italian study, large strongyles (*Strongylinea*) were the most abundant and most prevalent (34%) equine large intestinal parasites.[10] The three common genera of migratory large strongyles that affect horses (*Strongylus edentatus, S. equinus,* and *S. vulgaris)* live as adults in the large intestine of the horse, but have differing migratory larval routes.[11] The adults are found in the colon and cecum, and produce eggs that are passed via the feces and that develop to infective third-stage larvae (L3) outside the host.[11] Ingested L3 of *S. vulgaris* exsheath in the small intestine, penetrate the intestinal mucosa of the small intestine, colon and cecum, and moult in the submucosa to L4.[7,11] Fourth-stage *S. vulgaris* larvae then enter small intestinal arteries and migrate to the cranial mesenteric artery and its main branches, which are predilection sites for lesions caused by the larval stages.[7,11] A marked seasonality in proportions of affected arteries and arterial worm burdens have been reported, with the highest numbers encountered during winter.[9] After further development of L4 in the mesenteric circulation, L5 larvae return via the vasculature to the intestine, where nodule formation arise around

larvae trapped in the smaller vessels of the intestinal wall, before young adult nematodes are released into the intestinal lumen.[11] The prepatent period for *S. vulgaris* is 6-7 months.[11]

Histopathologic arterial wall changes from necropsies most often reveal chronic (fibrosis of intima and/or media, with or without mild accumulation of mononuclear inflammatory cells) or chronic active (neutrophils, eosinophils and necrotic foci are also present) arteritis.[8] Severity of inflammatory changes have been shown to be directly related to presence of larvae, which may be entrapped in intimal thrombi, the intima itself, and less commonly in the media or adventitia.[8] Verminous arteritis can be found in necropsies of horses with no history of colic,[7,12] such as in the presented case. However, thromboembolism in the branches of the cranial mesenteric and the ileocaecocolic arteries and ischemia or infarction, interference of gut innervation related to pressure on abdominal autonomic plexuses, and release of toxic products from dying larvae have all been discussed as causes of clinical disease.[7,12]

Pathogenic effects in the large intestine relate to adult worms feeding on mucosal material and incidental damage to blood vessel, but also to disruption of the mucosa associated with the emergence of young adults.[11] Infestation may lead to poor condition and performance, anemia, temporary lameness, intestinal stasis, colic, and rarely intestinal rupture and death.[11]

JPC Diagnosis: Mesenteric artery: Arteritis, proliferative, eosinophilic and granulomatous, transmural, diffuse, severe, with thrombosis and multiple strongyle larvae.

Conference Comment: Nematodes of the subfamily *Strongylinae* (family *Strongylidae*) are "plug feeders" or "blood suckers" commonly found in the cecum and colon of horses and tend to undergo extensive extraintestinal migration.[1] As noted by the contributor, the three major genera of the *Strongylinae* subfamily are *S. vulgaris, S. edentatus* and *S. equinus.* Conference participants briefly discussed the microscopic characteristics associated with the larvae of large strongyles (also known as true strongyles), including platymyarian-meromyarian musculature, prominent lateral cords, a pseudocoelom, and a large, central intestine lined by few multinucleated cells with a prominent brush border.[4] *S. vulgaris* larvae preferentially migrate up the cranial mesenteric artery, leading to arteritis, thrombosis and, occasionally, segmental colonic necrosis. Aberrant migration into the aorta, renal and coronary arteries is also described.[1,7] Upon ingestion, infective third stage larvae of *S. edentatus* penetrate the intestinal wall, enter the liver via the portal vein, molt to L4, and migrate through the hepatic parenchyma, causing eosinophilic, neutrophilic and mononuclear inflammation and hemorrhagic/necrotic tracts. Resultant tags of fibrous scar tissue on the hepatic capsule can often be detected as incidental findings on gross necropsy. After leaving the liver via the hepatorenal ligament, larvae travel to the retroperitoneal tissue of the flank. Here they molt to L5 prior to returning to the cecum/colon, where they form nodules and hemorrhagic plaques within the gut wall (one possible cause of hemomelasma ilei) and eventually penetrate the lumen and lay eggs.[1] Aberrant migration of *S. edentatus* is also occasionally reported as a cause of orchitis in young stallions.[3] *S. equinus* is less prevalent then either *S. vulgaris* or *S. edentatus* and infection is generally clinically insignificant. Exsheathed third stage larvae penetrate into the deep layers of the ileum, cecum and colon, where they produce subserosal nodules. Fourth stage larvae migrate throughout the liver, pancreas and peritoneum, molt to L5, and ultimately return to the cecum/colon, causing mild eosinophilic inflammation.[1]

In contrast to true strongyles, members of the subfamily *Cyathostominae* feed on intestinal contents and are essentially non-pathogenic as adults, although emergence of histotropic larval stages from the intestinal wall may cause disease. Cyathostomes, also known as small strongyles, can number in the hundreds of thousands in the equine large intestine. Larvae burrow into the submucosa to develop, where they may undergo hypobiosis; emergence of large numbers can cause rupture of the muscularis mucosae with severe inflammation and edema. Clinically, affected horses present with diarrhea, ill-thrift and hypoalbuminemia, while grossly the colonic mucosa may exhibit numerous umbilicated red-black nodules containing encysted larval nematodes.[1] Much like *S. vulgaris*, the clinical syndrome (larval cyathostomiasis) associated with these infections is typically seasonal in onset, occurring most commonly in younger animals in

late winter or early spring.[2] The moderator also pointed out that these small strongyles often exhibit variable degrees of ivermectin and moxidectin resistance, which can confound attempts at control/treatment.[5,6]

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