WEDNESDAY SLIDE CONFERENCE 2015-2016

C o n f e r e n c e 24

11 May 2016

CASE I: JPC WSC 1 (JPC 4069824).

Signalment: 2 year-old Gyr cow (*Bos indicus*)

History: This 2-year-old, unvaccinated, pregnant cow was down for less than 24 hours. On physical examination, she had increased patellar reflexes in both hind limbs and the left hind leg was swollen and had demonstrable crepitus.

Gross Pathology: A 2x1 cm raised, roughened, hairless area with minimal crusting is on ventral midline over the xiphoid (presumed stephanofilariasis). Sub-cutaneous crepitus is palpable over the hind limbs, most prominent over the caudal thighs, with a lesser amount palpable on the forelimbs (emphysema). The skeletal muscles of both hind limbs contain large, multifocal to coalescing, dark red to black, dry areas with an odor of rancid butter and occasional clusters of gas bubbles (emphysematous and hemorrhagic myositis), that is worse over the caudal thigh muscles. Similar, less severely affected areas are multifocally found in the skeletal muscle of
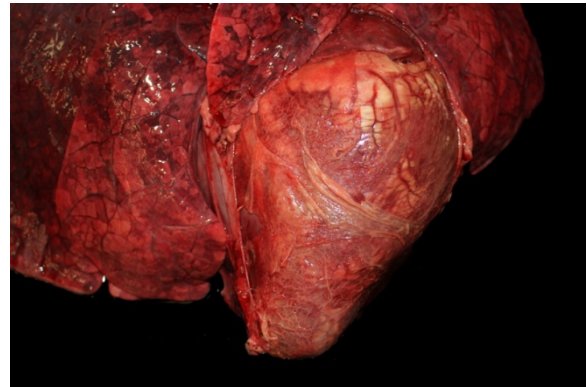
Heart, ox. Abundant fibrin covers the myocardium and interior epicardial surface. (Photo courtesy of: Dept Vet Pathobiology, College Vet Med Biomed Sciences, Texas A&M University, http://vetmed.tamu.edu/vtpb)

the forelimbs. A moderate amount of subcutaneous, clear, dark red, gelatinous tissue surrounds the gastrocnemius muscles (edema). The dorsal pleural surface contains multifocal to coalescing dark red foci (ecchymoses). Multifocal to coalescing, 1-100 mm, black foci are on the pericardial sac and jugular vein (petechiae). Covering the epicardium and loosely adhered to the pericardial sac is yellow, friable, fibrillar material (fibrin, fibrinous epicarditis). A 5x3 cm, dark red, irregularly marginated, flat area on the epicardium extends multifocally into the myocardium (myocardial necrosis).

Heart, ox. A 5-3cm irregular dark red area of necrosis is present within the myocardium (at left). (Photo courtesy of: Dept. Vet Pathobiology, College Vet Med Biomed Sciences, Texas A&M University, http://vetmed.tamu.edu/vtpb)

Laboratory Results:

Toxic WBCs present on CBC.

Chemistry abnormalities:

[Ca] 6.5 (7.4-11.5 mg/dl); total protein 5.8 (6.5-9.3 g/dl); albumin 2.6 (3-4 g/dl); aspartase aminotransferase 1420 (53-173 U/L); creatinine kinase >16,000 (55-392 U/L); anion gap 21.9 (10-18 mmol/L); fibrinogen 1000 (300-700 mg/dl); leucopenia [WBC] 3800 (4000-12,000).

Cytology:

Smear from a left thigh aspirate: Low cellularity with few, intact, nucleated cells and numerous erythrocytes in a light pink hazy background with many, thick, box-like, bacterial rods often seen in end-to-end pairs. The nucleated cell population is composed of non-degenerate neutrophils, lymphocytes, and monocytes in numbers and proportions consistent with peripheral blood. No overtly neoplastic cells are observed.

Skeletal muscle FA: *Clostridium chauvoei*-positive

Histopathologic Description: A section of myocardium with epicardial surface is examined. Moving from the epicardium centrally into this inflammatory lesion, the epicardium has an or-ganized fibrin coat with embedded, degenerate neutrophils. The ep-icardium, epicardial fat and ex-ternal myocardium have variable amounts of free erythrocytes and degenerating and necrotic neutrophils that dissect and infiltrate tissues. Moving further inside the necrosis, is intense in the cardiomyocytes and neutrophils with more pyknotic nuclei, hyalinized homogeneous cardiomyocytes (necrosis), lysed ey-rthrocytes and fibrin between fibers. The inflammation abruptly stops, and in a central zone (surrounded by the previously described wall of inflammation) the histoarchitecture remains with retention of homogenous fibers separated by fibrillar, pink material (fibrin) and a pale interstitium (edema and lysed erythrocytes) and few, scattered pyknotic nuclei. Clusters and individual bacterial rods with polar spores are seen. At least 10, round to oval, basophilic, approximately 130X250 u, or-ganisms with a <5u wall and numerous, 25u-longX8u-wide, basophilic structures (sarcocyst with bradyzoites) randomly expand cardiomyocytes without tissue response. They are intricately septate.

Contributor's Morphologic Diagnosis:

1. Severe, diffuse, acute, fibrinosuppurative epicarditis; multifocal to coalescing nec-rohemorrhagic myocarditis with centrally extensive coagulative necrosis, emphysema and abundant, intralesional, gram-positive, spore-forming bacilli (*Clostridium chauvoei*).

2. Multifocal *Sarcocystis cruzi* schizonts/sarcocyts presumed.
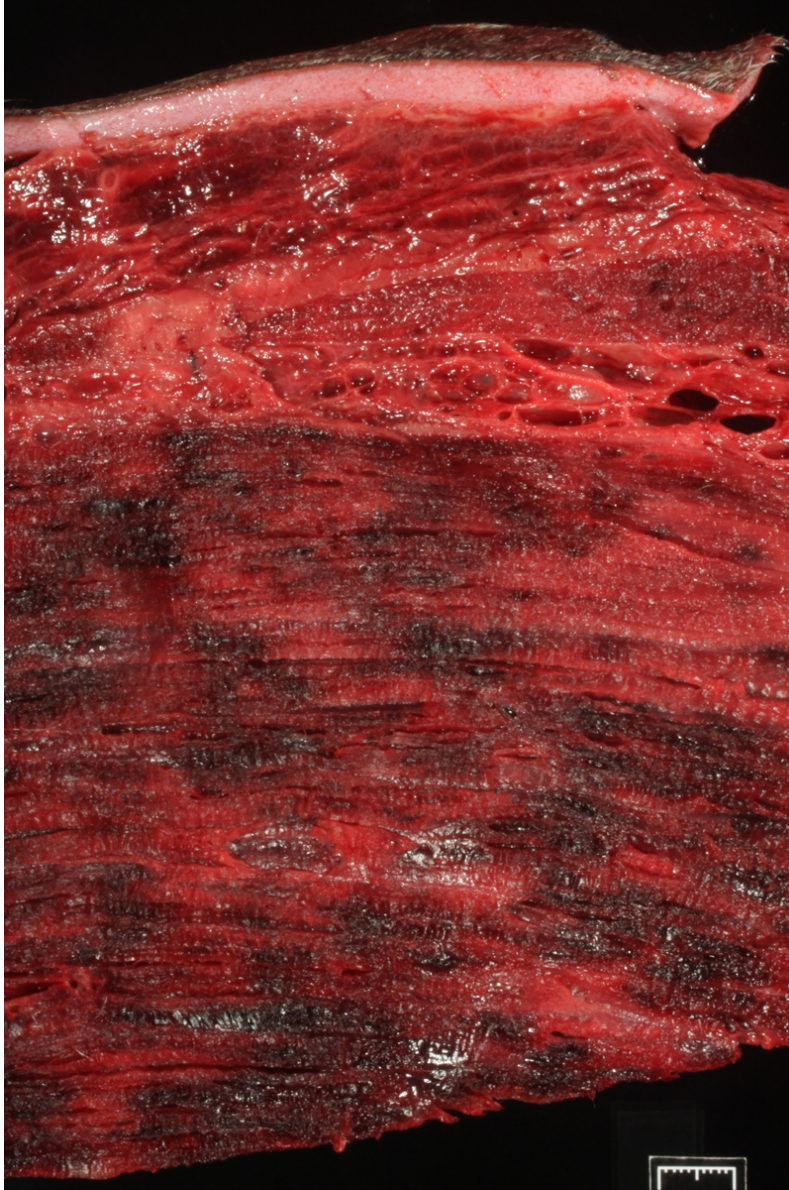
Skeletal muscle, ox. Hindlimb skeletal muscles contain multifocal areas of necrosis, hemorrhage, and gas formation. ((Photo courtesy of: Dept. Vet Pathobiology, College Vet Med Biomed Sciences, Texas A&M University, http://vetmed.tamu.edu/vtpb)

Contributor's Comment: Blackleg is a disease of pastured young animals, especially cattle and sheep, and is often associated with moist pasture in the summer. This is a case of blackleg in a two-year-old cow seen during this year's warm and rainy, late spring. The pathogenesis involves activation of latent *C. chauvoei* in muscle.[7]

Ingested spores replicate in the gut and presumably remain in macrophages with muscle for long periods. Spores are activated by a low oxygen tension in their environment, and the organisms proliferate producing potent, necrotizing toxins and gas. Rapid death with hemorrhagic, crepitant lesions deep in muscles of the pelvic and pectoral girdles are seen. The pathogenesis is recapitulated in the histology with a central zone of coagulative necrosis with individual and clusters of gram-positive bacteria with polar spores and no inflammation that peripherally blends into viable tissue with a zone of primarily degenerate neutrophils and nonlysed erythrocytes. The inciting factor/lesion is not known. When sectioned, the skeletal muscle lesions are seen as dry (coagulative necrosis) areas surrounded by gas, hemorrhage and edema. The various vaccines (polyvalent, 2 to 8-species vaccines) control the disease in endemic areas if timed and applied properly. Although there are published cases of *C. chauvoei* myositis without ex-traskeletal/visceral lesions,[6] clostridial my-ositis can be seen in the myocardium, tongue and diaphragm.[2,4,7]

This cow's heart had a fibrinosuppurative epicarditisand when the heart was sectioned, the necrohemorrhagic myocarditis was noted to penetrate the myocardium. Visceral lesions may be small and are missed in cursory necropsies. It is important to note that the visceral sites are heavily used

muscle sites, predisposed to having a lower pO2. Diagnosis in this case was straight-forward because the carcass was fresh, and only *C. chauvoei* was seen with the FA test; however, *C. chauvoei* does not proliferate post-mortem,[7] and in autolized carcasses, other clostridial species may grow. In making a clostridial diagnosis, four basic aspects/criteria should be considered:[1] a) clinical history, b) post-mortem findings, c) relative numbers of pathogenic organisms seen in the microscopic fields, and d) the postmortem interval. If animals are id-entified early and treated with antibiotics, they will live several hours longer and will

Sarcocystis (*Sarcocystis cruzi* or *S. hirsuta*) are ubiquitous in cattle muscles, and as seen in this case, the sarcocyst full of bradyzoites does not incite a host response except in cases of eosinophilic myositis.[5]

JPC Diagnosis:

1. Heart: Pancarditis, necrosuppurative and fibrinous, acute, diffuse, severe with necrotizing vasculitis, fibrin thrombi and moderate numbers of bacilli.

2. Heart, myocardium: Sarcocysts, few.
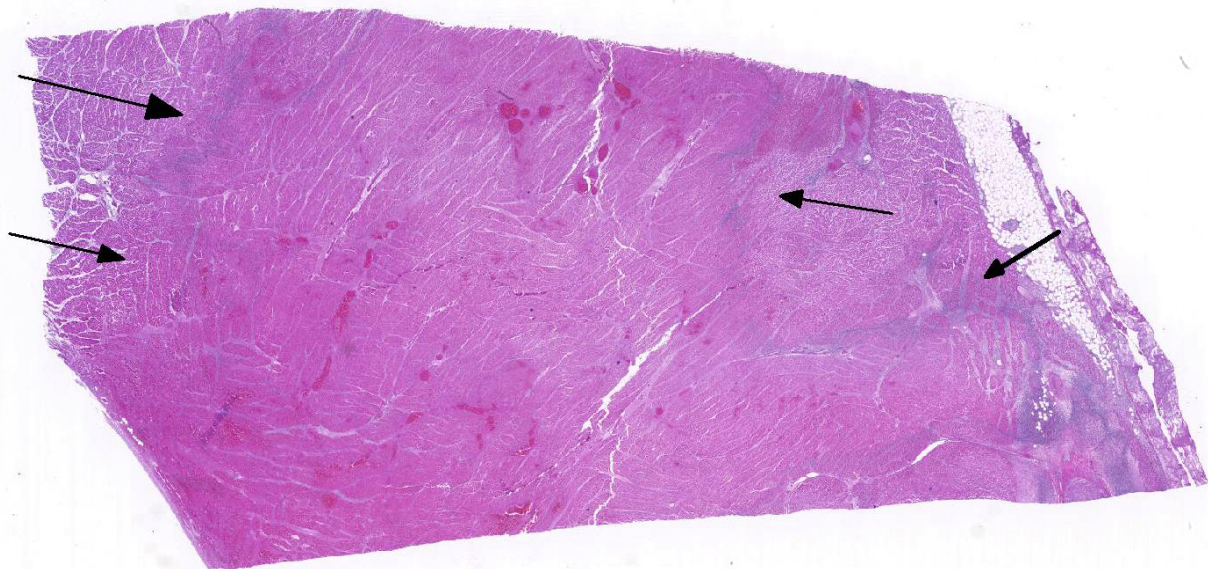
Heart, ox. Subgross image of the area of necrosis within the myocardium (epicardium at left). A line of cellular debris (arrows) delineates the margin. (HE, 5X).

have fibrinous and hemorrhagic transudates covering the pleura, pericardium and pe-ritoneal surfaces (contributor's observation). Such a pleuritis without pneumonia is common with blackleg.

Outbreaks of clostridial myocarditis caused by *C. chauvoei* are reported in calves and sheep.[3,8] In ruminants, other causes of nec-rosuppurative (non-traumatic) myocarditis are *Histophilus somni* and listeriosis.[5]

Conference Comment: Once clostridial spores are ingested in the environment, they may remain dormant in the small intestine or germinate and inhabit the small intestine. Spores eventually gain access to ma-crophages where the spread to muscle and germinate once muscle damage occurs, which creates a low oxygen tension en-vironment. Tissue injury in cases of bl-

ackleg occurs due to necrosis of muscle and supportive tissues due to α and β toxins released from the clostridial organisms. Toxins include oxygen-stable hemolysin, neuraminidase and hyaluronidase, among others. The toxins are released at the site of bacterial replication damaging adjacent muscle and spreading outward through the muscle, supporting tissues and associated vessels.[11] Neuraminidase is particularly important as it helps facilitate disease spread.[9]

Other clostridial agents cause disease in a fashion similar to *Clostridium chauvoei*,

sordelli and *C. novyi*. Malignant edema also has a similar pathogenesis to big head in sheep where wounds of the head sustained during fighting allow germination of *Clostridium novyi* spores followed by release of toxins similar to what is described for blackleg and malignant edema. This results in significant edema in the head and neck region and may extend into the thorax. In black disease, ingested *C. novyi* spores are present in the liver and fluke migration establishes the anaerobic environment needed for spore germination resulting in extensive hepatic necrosis.[11]
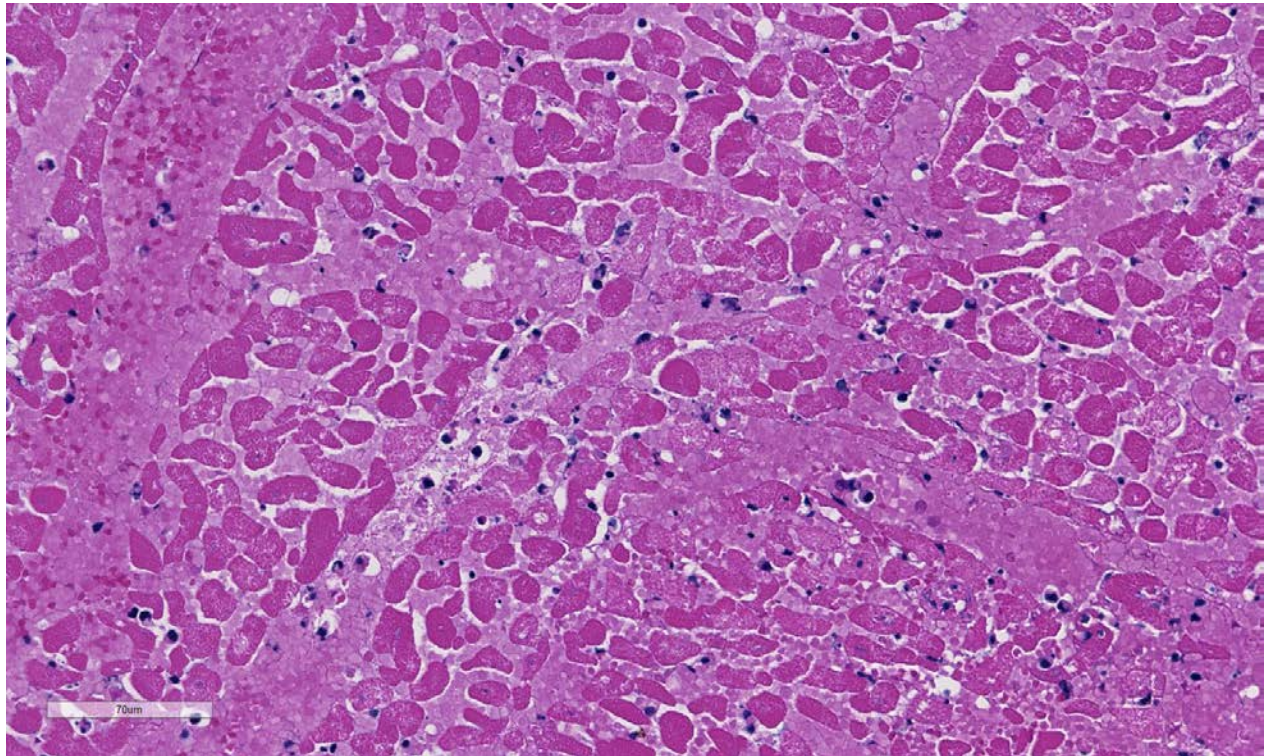
Heart, ox. Large areas of myocardium demonstrate coagulative necrosis. (HE, 320X)

including *C. septicum*, the agent of pseudoblackleg and a potential etiology of malignant edema. As opposed to ingestion, spores of *C. septicum* are often encountered via wound infection. Spores germinate in the anaerobic environment created in the wound, releasing toxins which cause muscle damage similar to what is seen in blackleg.[11] Other agents of malignant edema include *C.*

Conference participants commented that although emphysema is commonly seen in this entity, and was present in the excellent gross image provided by the contributor, it is not a prominent histologic finding in the slides provided. Conference participants also noted necrotizing vasculitis as a feature in this case, although it is typically not associated with this entity,[10] as well as the
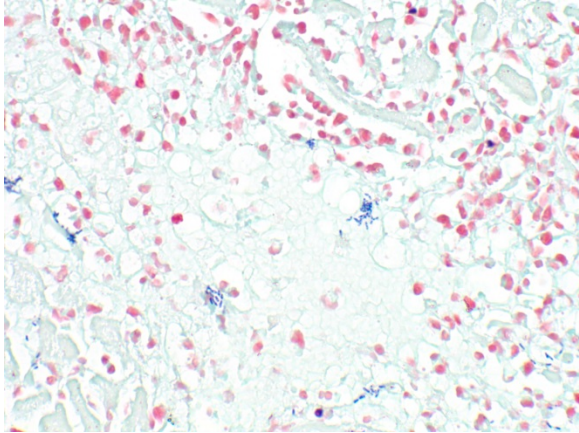
Heart, ox. Aggregates of robust gram-positive bacilli are scattered throughout the necrotic areas of the section. (Gram, 600X)

presence of fibrin thrombi variably filling vessel lumina and adhering to vessel walls. A tissue Gram stain highlighted bacilli in this slide.

The decreased calcium level in this case is the result of entry of calcium into damaged muscle cells. Albumin is a negative acute phase protein and is decreased in cases of acute inflammation. The increased anion gap is likely due to a combination of lactic acidosis and elevation of renal acids secondary to dehydration. Elevations in as-
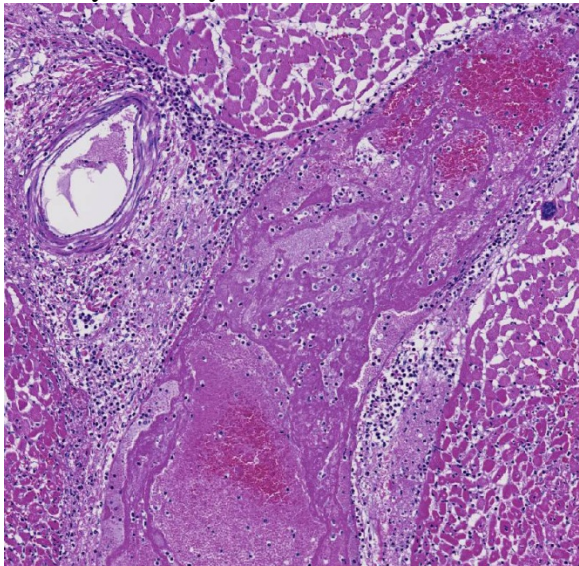
Heart, ox. Within the affected areas, occasional veins and lymphatics are occluded by fibrinocellular thrombi. (HE, 80X).

partase aminotransferase and creatinine kinase are secondary to muscle damage. Other agents which may cause necrotizing myositis / myocarditis and were discussed during the conference include *Trueperella pyogenes* which typically results in abscesses and *Bacillus anthracis* which results in the presence of abundant dark, unclotted blood exuding from multiple orifices. Other differential diagnoses discussed include ionophore toxicity, vitamin E /selenium deficiency and eosinophilic myositis.

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CASE II: NF-11-604 (JPC 4017089).

**Signalment:** 2 year old, intact female, Dorper Sheep, (*Ovis aries).*

History: This 2-year old intact female Dorper sheep (*Ovis aries)* had a history of weakness, decreased pupillary light reflexes, abnormal mentation, bloody nasal discharge, dyspnea, and thin body condition (BCS 2/5). The ewe was from a flock of 186 adult animals; 10 of which were found dead.

Gross Pathology: An intact ewe, weighing 33.90-kg was necropsied. The animal was in thin body condition, appeared mildly dehydrated, and was moderately autolyzed. On external examination, there is a small amount of yellow mucoid discharge present on the external nares bilaterally. The conjunctiva and mucous membranes are diffusely pale pink to white. Upon internal examination, the abdominal cavity lacked significant adipose tissue. Pericardial and epicardial fat is also absent. Approximately 50-ml of yellow serous fluid was present within the thoracic and peritoneal cavities. A thick mat of white, friable material (fibrin) iss loosely adhered to the pleural suface to the left lung. Fibrin was also present covering the pericardium adjacent to the left lung. The cranioventral left lung is diffusely firm and dark red. On cut section, a moderate amount of white, purulent material oozes from the airways. Diffusely, the caudodorsal aspect of both lungs is mottled red and pink, is rubbery, and fails to collapse. The lumen of the distal 1/3 of the trachea, contained a moderate amount of white, frothy fluid. The tracheobronchial lymph nodes are diffusely enlarged to approximately 2-3 times their normal size. Within the peritoneal cavity, a small focus of hemorrhage is noted surrounding the capsule of the right kidney. Within the abomasum, a single 5-10-cm long, threadlike nematode, consistent with *Haemonchus contortus*, is present. The contents of the colon were scant and soft, green.

Laboratory Results:

Bacterial culture and sensitivity (lung):
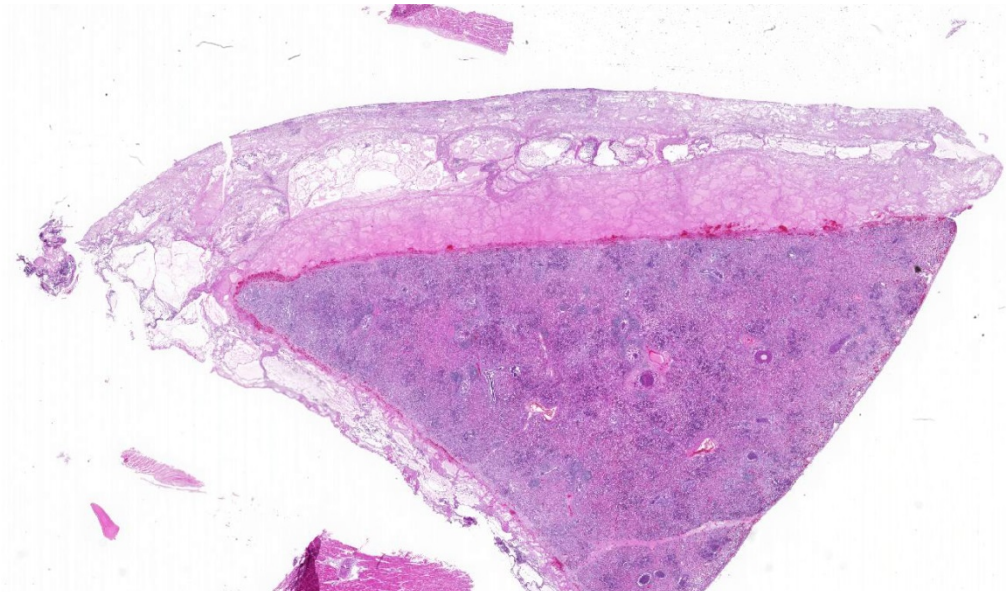
Lung, sheep. A wedge shaped section of lung exhibits diffuse parenchymal consolidation and a marked fibrinous pleuritis (top.) (HE, 5X)

Moderate *Mannheimia haemolytica;* Moderate alpha hemolytic *Streptococcus;* Few *Trueperella pyogenes*

Qualitative fecal analysis:
Moderate # coccidial oocysts; Many trichostrongyle type eggs; Few *Trichuris* eggs

Histopathologic Description:

Sections of the cranioventral lung consists of severe fibrinosuppurative bronchopneu-monia characterized by diffuse ne-crosis, complete loss of alveolar detail, di-ffuse filling of alveolar spaces with fibrin, and infiltration of large numbers of viable and degenerate neutrophils, and moderate numbers of multinucleated giant cells. Bronchioles are lined by moderately hyperplastic epithelium and are filled with degenerate neutrophils and myriad bacterial organisms. Thick mats of fibrin admixed with large numbers of neutrophils covered the visceral pleura. Multifocally, there are large lymphoid cuffs surrounding airways and blood vessels.

Sections of the caudodorsal lung (not provided) consist of moderate lymphocytic interstitial pneumonia characterized by diffuse thickening of the alveolar septae by lymphocytes, plasma cells, and mac-rophages. The inflammatory cells also form large lymphoid nodules, which were frequently seen surrounding blood vessels and airways. Mild type II pneumocyte hyperplasia is also present.

Contributor's Morphologic Diagnosis:
Lung: Severe necrotizing and fibri-nosuppurative bronchopneumonia, with intralesional bacterial bacilli; Severe, fibrinous pleuritis; Moderate, lymphocytic interstitial pneumonia (caudodorsal lung, not provided).
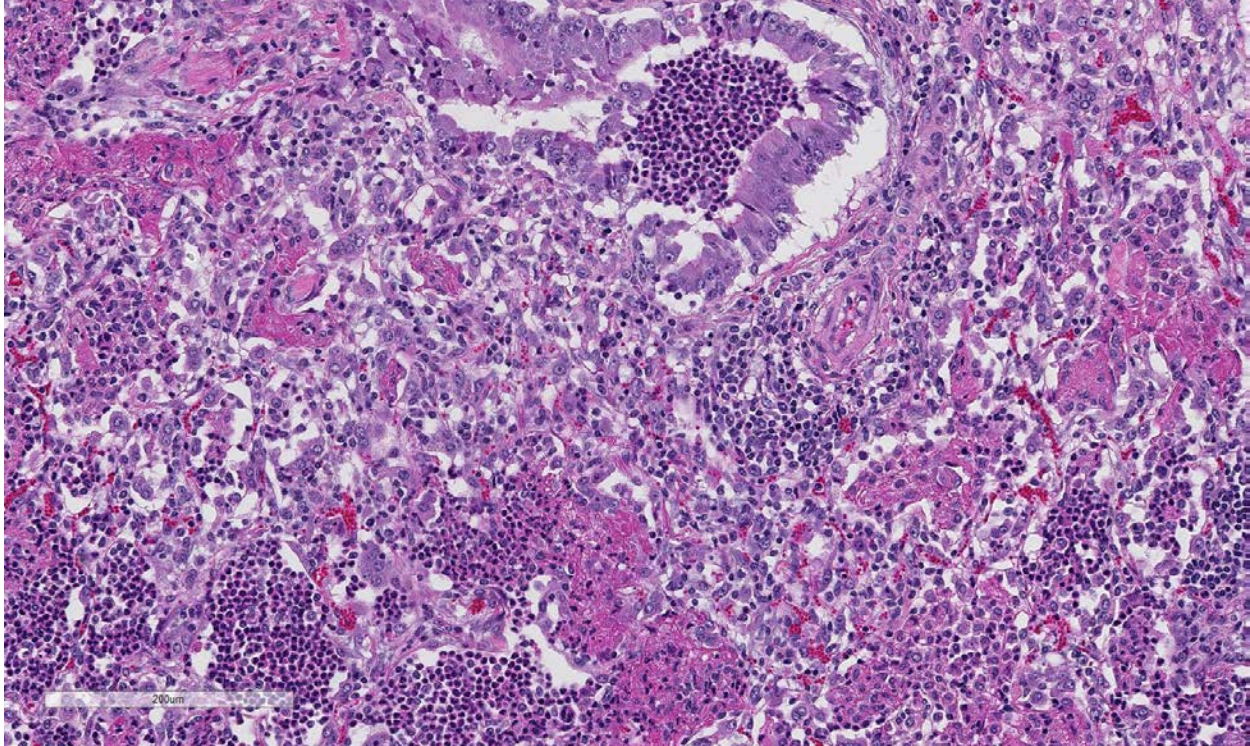
Lung, sheep. Neutrophils fill airways (top right) as well as alveoli, where they are admixed with variable amounts of polymerized fibrin. There is diffuse type II pneumocyte hyperplasia. (HE, 140X)

Contributor's Comment: The lesions in the (cranioventral) lung are characteristic for an infection with *Mannheimia haemolytica,* which was confirmed by bacterial culture. The isolated *Trueperella pyogenes* most likely represents an opportunistic bacterium.

Mannheimia haemolytica (formerly *Pasteurella haemolytica*, biotype A), a gram-negative coccobacillus, is a common cause of fibrinous and necrotizing pneumonia and pleuropneumonia in cattle, sheep, and goats. Although pneumonic mannheimiosis has been most extensively investigated in bovines, pathologic lesions in sheep are essentially similar to those described for cattle, with few differences. In sheep, the disease may be acute or chronic. In the acute form, there may be a hemorrhagic or fibrinonecrotic lobar pneumonia and fibrinous pleuritis, whereas in the chronic form, a fibrinopurulent bronchopneumonia with secondary abscessation

and fibrinous adhesions to the thoracic wall are common. Additionally, serous to serofibrinous fluid may also be seen in the pericardial sac and pleural and peritoneal cavities. [5]

Mannheimia haemolytica is an opportunistic pathogen; it is a normal inhabitant of the nasopharynx and tonsils of cattle and sheep. [3,7] Although the exact mechanisms are not known, stress or concurrent viral infections may alter the local innate and adaptive immune responses, contributing to the development of disease. The organism also possesses several virulence factors, including leukotoxin (LKT), lipopolysaccharide (LPS), adhesins, capsule, outer membrane proteins, and various proteases which allow promote colonization of the lung and evasion of the host immune response. [3,5,7]

The roles of LKT and LPS in the pathogenesis of pulmonary mannheimiosis

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are most well known in bovines with similar effects in small ruminants. LKT is a 102- to 105-kDa protein that is produced by all serotypes during the logarithmic phase of bacterial growth. [3,5,7] This pore-forming cytolysin is a member of the RTX (repeats in ToXin) family of toxins. These toxins are genetically related, sharing a highly conserved motif consisting of a series of glycine-aspartic acid nonapeptide repeats in the carboxy terminal third of the LKT protein molecule. [7] This motif serves two critical functions; it is involved in calcium binding which induces leukocyte toxicity and contains a recognition site required for transport of LKT across biological membranes in bacteria. [7] Although members of the RTX family of toxins are genetically similar, they differ in the target cell specificity. Other members of the RTX family and their toxins include: *Actinobacillus actinomycetemcomitans* (LtxA), *Actinobacillus pleuropneumoniae* cytotoxins (ApxI, ApxII, ApxIII, ApxIV), *Escherichia coli* alpha hemolysin, *Actinobacillus suis* subs *haemolyticus* toxin (Aqx), *Fusobacterium necrophorum* LKT, *Bibersteinia trehalosi¸* and *Bordetella pertussis* hemolysin. [7]

Pneumonia due to *Mannheimia haemolytica* occurs only in ruminants, in part due to LKT-induced effects which are specific for ruminant macrophages, lymphocytes, neutrophils, and platelets.[3,5,7] The response to LKT is species-specific and dose-dependent. Species specificity is due to the selective interaction of LKT with β2 integrin LFA-1 (lymphocyte function-associated antigen 1; CD11a/CD18 on target host cells). [7] At low levels, LKT activates neutrophils and macrophages to stimulate respiratory burst and degranulation, release of proinflammatory cytokines TNFα, IL-1, and IL-8 and histamine from mast cells, and inhibit lymphoid proliferation.[7] At higher concentrations, leukocytes undergo ap-optosis, whereas at highest concentrations, LKT causes transmembrane pore formation, cell swelling, and oncotic cell death.[3,5,7] Pulmonary damage occurs subsequent to the leakage of oxygen free radicals, superoxide anions, lysosomal enzymes, and arachidonic acid metabolites into pulmonary parenchyma.[7]

Pulmonary lesions also occur due to LPS, a molecule composed of polysaccharide side chain (O antigen), lipid A, and inner and outer cores of oligosaccharides. Lipid A is responsible for eliciting endotoxic effects, such as fever and hypotensive shock. [1] It is a potent vasodilator, is directly toxic to pulmonary endothelium, and can recruit neutrophils.[3,5] LPS stimulates alveolar macrophages to produce proinflammatory cytokines, reactive oxygen and nitrogen intermediates, and other mediators that participate in the inflammatory process, including IL-1β, IL_8, leukotriene 4, prostaglandin E2, and TNFα from leukocytes.[7] An influx of neutrophils occurs secondary to these proinflammatory cytokines and chemotactic mediators. LPS also enhances the effects of LKT. [7]

Although the sections of caudodorsal lung were not submitted, the lesions are worth mentioning as they were most suggestive of ovine lentiviral pneumonia, or maedi-visna (ovine progressive pneumonia). Lentiviruses (Retroviridae) cause slowly progressive inflammation in a variety of tissues, with the lung, CNS, mammary gland, and joint most commonly affected.[1] Syndromes may occur independently or concurrently in any combination. The primary pathologic change is infiltration of lymphocytes into the affected tissues, with occasional formation of lymphoid follicles similar to those seen in lymph nodes.[1] The respiratory form in sheep, *maedi* ("dyspnea in Icelandic) is the most common form in sheep usually greater than 3 years of age. Encephalitis, or *visna* ("fading away" in Icelandic), may present as
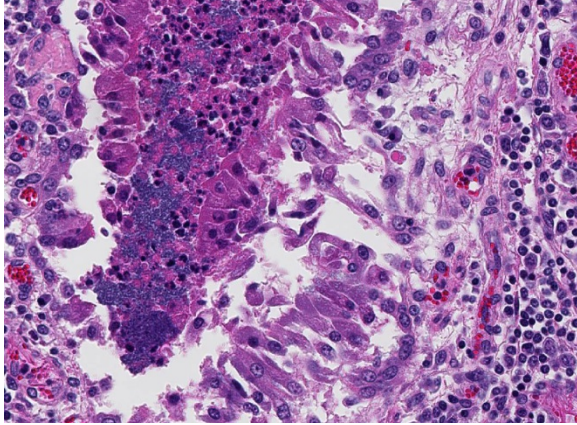
Lung, sheep. Large colonies of bacilli are admixed within necrotic debris within airways. (HE, 140X)

ataxia, trembling, and significant weight loss. Mastitis and arthritis also occur, but with little frequency. Small ruminant lentiviruses are primarily transmitted through colostrum or milk, although inhalation of respiratory secretions can occur.[1] In this case, despite the history of neurologic signs, lesions associated with lentiviral infection were not detected in the brain. A commercially available test to confirm this infection is not routinely available, however, given the clinical history and gross and histopathological findings in the lung, lentiviral infection remains a likely differential in this case.

JPC Diagnosis:

1. Lung: Bronchopneumonia, fibrinosuppurative, chronic-active, diffuse, severe with fibrinous pleuritis, multinucleate giant cells, and colonies of bacilli.

2. Lung: Interstitial pneumonia, lymphohistiocytic, multifocal, mild.

3. Heart: Essentially normal tissue.

Conference Comment: Ovine pneumonic manheimiosis is a common and economically important disease of sheep;

pulmonary infection is often facilitated by various stressors, including concurrent viral infection. Severe fibrinous and/or suppurative bronchopneumonia is characteristic and fibrinous pleural adhesions may be seen in some severe cases.[4] Grossly, pulmonary lesions due to *M. haemolytica, H. somni, B. trehalosi, Mycoplasma mycoides mycoides* (small colony variant

)and *P. multocida* may be indistinguishable, as all include a cranioventral, lobular distribution with or without the presence of fibrinous pleuritis, although well-demarcated foci of necrosis will distinguish both *M. haemolytica* and *H. somni*. Albeit characteristically attributed to *M. haemolytica* infection, leukocyte necrosis may also be seen in cases of *H. somni* infection. The presence of suppurative phlebitis with fibrinoid necrosis is more common in cases of *H. somni* infection.[2] *M. hemolytica* is also a common cause of mastitis in sheep, which can be a problem in both dairy and non-dairy flocks. In addition to *M. hemolytica*, *M. glucosida* may also cause pneumonia and mastitis in sheep. Horizontal transmission is common, with nursing being suggested as the major method of transmission, where the organism is passed to the mammary gland from the nasopharynx of the lamb.[6]

Besides the obvious fibrinosuppurative bronchopneumonia in the submitted sections, there was extensive discussion conference participants also identified a mild interstitial pneumonia with infiltration and expansion of alveolarsepta. The mild interstitial component along with occasional albeit prominent cuffing of vessels and small airways by mononuclear inflammatory cells (with a majority of lymphocytes) generated discussion of a concomitant lentiviral infection Lentiviral pneumonia, as discussed above, was postulated to be present in the

caudal-dorsal sections of lung which were not submitted. Although not conclusive, viral infection may have, or perhaps likely, resulted in a secondary bronchopneumonia in this animal. Ovine progressive pneumonia (maedi-visna) may cause severe interstitial pneumonia with BALT hyperplasia, thickened alveolar septa and peribronchial lymphocytic infiltrates. Al-veolar fibrosis and smooth muscle hyperplasia, the latter of which was described in this case, may also be seen.[4] The presence of multinucleated giant cells within alveoli was perplexing to some conference participants. However, ma-crophages and multinucleated histiocytes may be seen in more chronic cases of bacterial bronchopneumonia, particularly in areas of abundant fibrin exudation as they
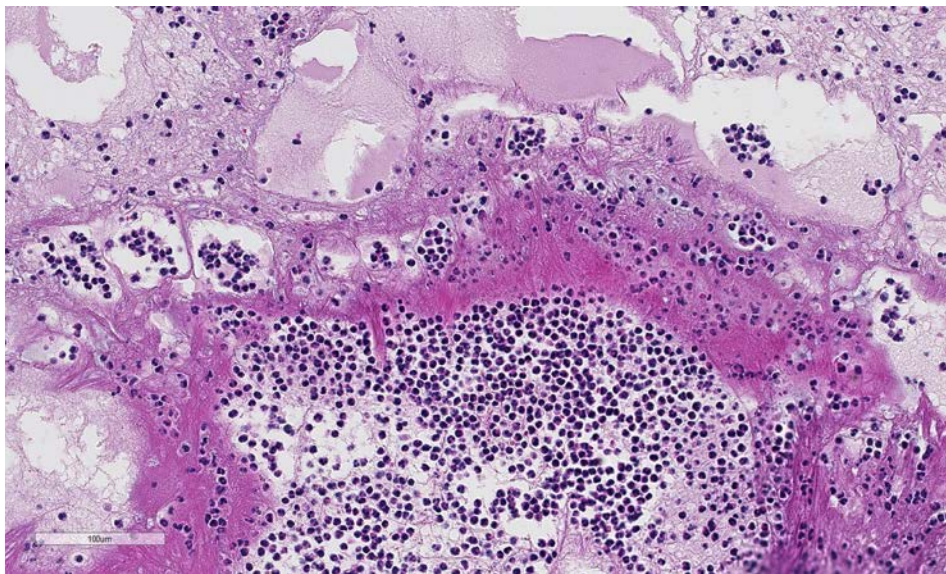
Lung, sheep. The pleura are covered with a 5mm-thick mat of fibrin throughout which are scattered aggregates of neutrophils. (HE, 116X)

help facilitate removal of fibrin.[2] Many conference participants supposed an etiologic diagnosis of *Pasteurella multocida* in this case due to absence of characteristic leukocyte necrosis with the linear streaming pattern, termed "oat cells," typically seen in *Mannheimia* infection, as well as the lack of significant epithelial necrosis in airways

(described as a feature of P. multocida infections.)

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CASE III: 2014#2 (JPC 4066678).

**Signalment:** 5 year-old, castrated male, bovine (*Bos taurus*)

History: The head was deformed by a hard swelling of the right maxilla of several months of evolution that resulted in difficult breathing and weight loss. Due to poor prognosis, the practitioner advised the owner to send the steer to the slaughterhouse.

Gross Pathology: A 25x12cm hard mass extended from the base of the right ear to around the right eye. In the rostral end of the mass there was a 7x4 cm cavity filled with dark, friable necrotic tissue. At the cut surface of a section in middle part of the bridge of the nose, the nasal cavity was deformed by a mass consisting of bony proliferation. Within the mass, there were focally extensive semisolid (caseous) yellow areas of irregular contour. These areas were surrounded by a dark green halo (necrotic tissue). Extensive firm white fibrotic tissue was also noticed at the cut surface of the lesion. The lesion destroyed the nasal turbinates and markedly displaced the nasal septum to the left.

Laboratory Results: NA

Histopathologic Description: Lesions consist of multiple pyogranulomas surrounded by well-defined and well differentiated osseous trabeculae. In the center of these pyogranulomas there are structures co-nsisting of an inner mass of filamentous bacilli that stained blue (gram-positive) in the Gram stain. This center is su-rrounded by strongly eosinophilic radial clubs (Splendore Hoeppli [SH] ph-enomenon) which are, in turn, surrounded by an inner layer of neutrophils and an outer layer of epithelioid macrophages and
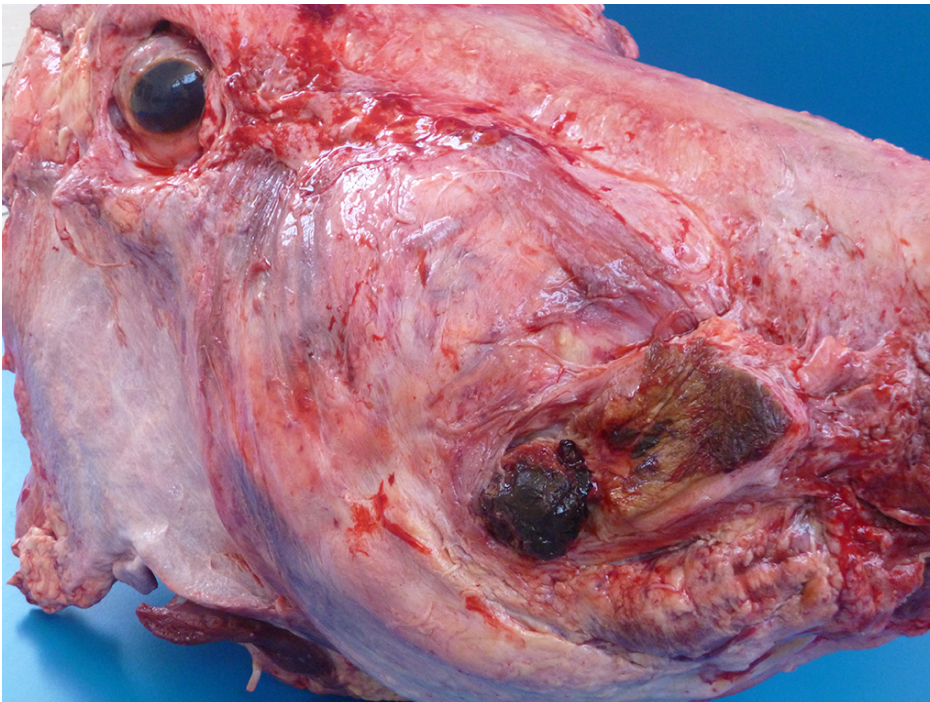
Maxilla, ox. A 25x12 cm hard mass extended from the base of the right ear to around the right eye. In the rostral end of the mass there was a 7x4 cm cavity filled with dark, friable necrotic tissue (Photo courtesy of: Setor de Patologia Veterinária, UFRGS, http://www.ufrgs.br/patologia/)

occasionally multinucleated giant cells. Occasionally, these SH structures are mineralized. Extensive fibrous tissue sprinkled with neutrophils, lymphocytes, and plasma cells surrounds the inflammatory reaction and extend into adjacent soft tissues. In some HE-stained sections, weakly basophilic bacilli are seen in the cytoplasm of epithelioid cells, multinucleated giant cells, and in the center of the SH phenomenon; fragments of these structures were also phagocytosed by multinucleated giant cells.

Contributor's Morphologic Diagnosis:
Maxillary pyogranulomatous osteomyelitis, castrated male, mixed breed, bovine.
Etiologic diagnosis: Bacterial osteomyelitis.

Etiology: *Actinomyces bovis*
Name of the condition: Actinomycosis.

Contributor's Comment: Actinomycosis is a pyogranulomatous osteomyelitis that primarily affects cattle and is caused by the gram-positive bacterium *Actinomyces bovis*.[9] Occasionally other species such as pigs, deer, sheep, goats and horses are affected.[9] A similar species of bacterium, *A. israeli*, is responsible for the disease in human beings.[6] The affected bone is thickened from multiple coalescing py-ogranulomas that impart to the bone tissue a honeycomb appearance.[5] The inoculation of *A. bovis* – a commensal organism of the oral cavity – into the oral mucosa of animals can


Maxilla, ox. The maxilla and nasal cavity were markedly deformed by a fibrous mass containing multiple foci of yellow caseous necrotic tissue surrounded by a green halo. (Photo courtesy of: Setor de Patologia Veterinária, UFRGS,
http://www.ufrgs.br/patologia/)

be facilitated by small wounds from hard straw present in the feed, foreign bodies and dental eruption. [9]

Although actinomycosis has been described in cattle at unusual sites such as the penis and in the maxilla, as in the case presented here, the classical presentation in cattle is in the mandible and rarely in the maxilla.[9,12] It is likely that the reason the mandible is the preferred site is because the direction of vegetal fibers being chewed is downwards forcing the fiber between the teeth and the gums, providing an entrance for the bacterium and resulting initially in a dental alveolitis.

Lesions of actinomycosis grow slowly over time[5,12] and the involvement of the bone and muscle tissue becomes so marked that it interferes with feeding,[9] which would explain the emaciation of the ox of this report. In osteomyelitis caused by actinomycosis, initially there is development of suppurative sinus tracts in the medullary spaces of the bone leading to multiple foci of both bone tissue resorption and proliferation. Bone sequestration does not occur; even if the cortical bone is invaded, probably due to the progressive course of
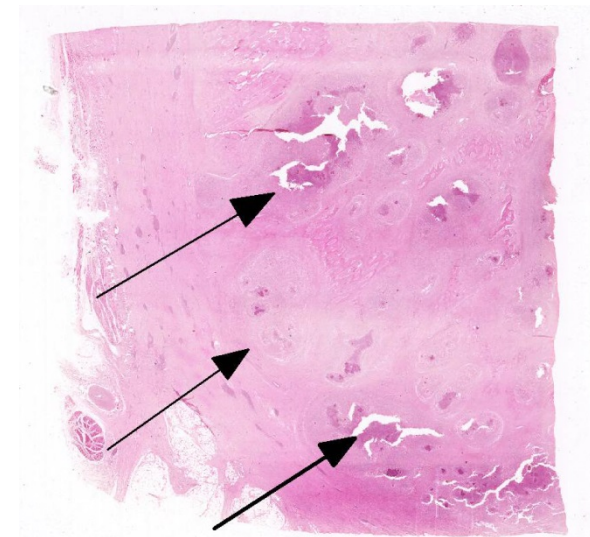
Maxilla, ox. In this decalcified section of the maxillary mass, abundant collagen surrounds areas of necrosis and pyogranulomatous infiltrate (arrows). (HE, 4X)

the disease.[9] The small granules – known colloquially as sulfur granules – observed grossly at the center of the caseous nodules[1] represents the bacterial colonies and the associated SH phenomenon and are typical of actinomycosis, although can be seen in other pyogranulomatous diseases of cattle such as actinobacillosis and sta-phyloccocosis.[8]

Little is known about the virulence factors of *A. bovis*. The interaction between ligand-
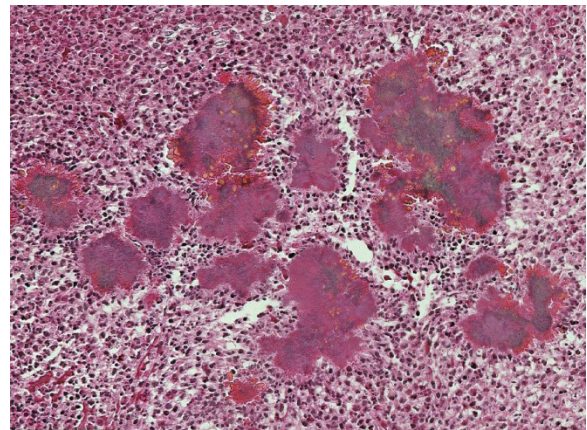
Maxilla ox. Centrally within necrotic/pyogranulomatous areas, there are colonies of small bacilli surrounded by club-shaped brightly eosinophilic Splendore-Hoeppli material. (HE, 256X)

receptor in the target cells, toxins, molecules in the bacterial capsule that compromise phagocytosis, and other factors are probably involved in the pathogenicity of *A. bovis*. It is also probable that *A. bovis* is capable of escaping destruction by neutrophils and macrophages and is capable of colonizing the tissue abscesses. This peculiar resistance of the agent to phagocytosis leads to the formation – around itself – of proteinaceous eosinophilic aggregates consisting of immunoglobulins, which are observed histologically as the SH reaction.[13]Other bacteria induce similar tissue reactions, particularly *Actinobacillus lig-nieresii*. However *A. bovis* colonies are larger and the radiating clubs in the SH reaction are shorter and less marked that in actinobacillosis, and

generally localized at the periphery of the colonies.[4,8,10] Ad-ditionally, actinobacillosis is a disease of soft tissues and the microorganisms of *A. lignieresii*, in contrast to those of *A. bovis*, are gram-negative.[5,8] *Fusobacterium nec-rophorum* and other bacteria can cause osteomyelitis by direct extension of per-iodontitis; however lesions induced by *F. necrophorum* are usually more destructive and less proliferative.[9]

Grossly, a lesion such as the one described here can be mistaken for - and the lesion was initially interpreted as - a squamous cell carcinoma at the slaughterhouse. Due to great extension of the lesions and the invasive characteristics of the mass reported here, an intranasal squamous cell carcinoma should be in the top of the list as a differential diagnosis at gross examination since this is one of the tumors more frequently observed in the nasal cavity or ruminants.[11] At cursory gross examination, the keratin commonly formed in these tumors may resemble the sulfur granules of actinomycosis.

JPC Diagnosis: Bone: Osteomyelitis, pyogranulomatous, chronic, diffuse, severe, with bone resorption, Splendore-Hoeppli material, and numerous bacterial colonies.

Conference Comment: "Lumpy jaw" not only occurs in domestic species, but also occurs in many wild ungulates, where it can be particularly problematic due to ch-allenges associated with treating an ag-gressive infection in fractious animals. *Ac-tinomyces bovis* is the most commonly associated agent resulting in py-ogranulomatous mandibular (or rarely maxillary) osteomyelitis but bony ma-lformations of the jaw may also occur secondary to periodontal infection. Man-dibular osteomyelitis involving the dentition is an important casue of morbidity in older exotic hoofstock, where it has also been referred to as chronic alveolar os-teomyelitis.[3] Inciting lesions may include a periodontal or tooth root abscess and/or a chronic pulpitis secondary to enamel or dentin abnormalities. Anaerobic bacteria can result in persistent dental infections which are challenging to treat successfully resulting in a chronic condition.[3] Organisms such as *Fusobacterium necrophorum* and other non-specific
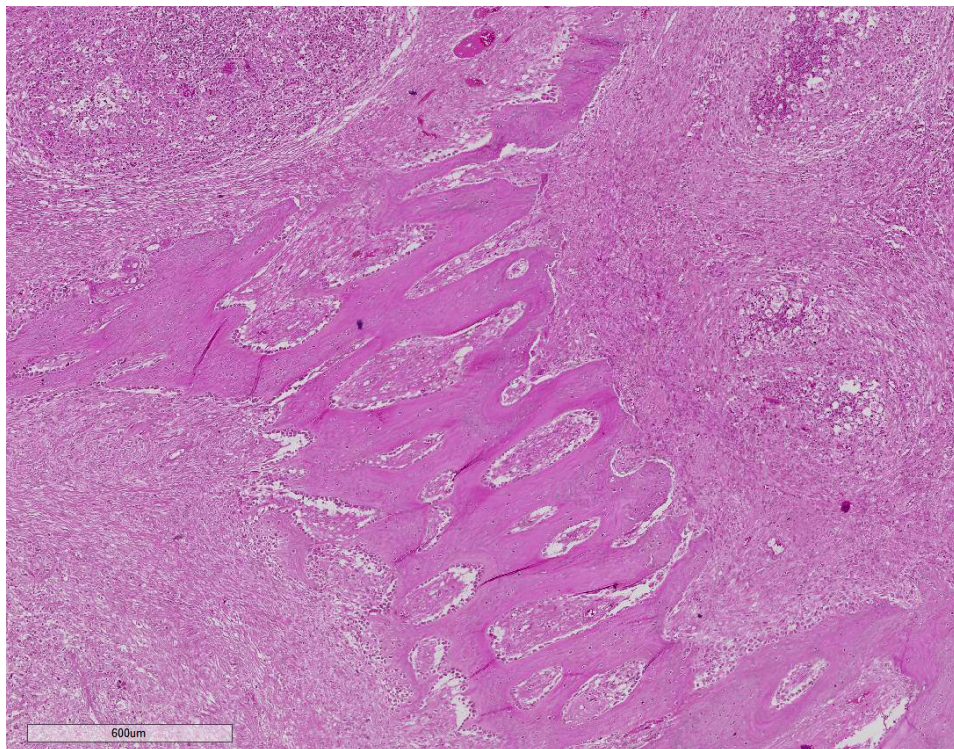
Maxilla, ox. Few trabecular remnants of periosteal new bone are entrapped within the inflammatory lesion. (HE, 35X)

bacteria that result in osteomyelitis as an extension from periodontal infection are generally more de-structive and less proliferative than lesions caused by *A. bovis* but lesions retain some similarities with actinomycosis in location and gross appearance.[2]

"Lumpy jaw" has also been described in a domestic cat and resembles the condition described in cattle. The cat in that case report did not have a history of trauma and the infection was due to *Nocardia* sp. The mass demonstrated progressive enlargement but was non-painful and initially did not interfere with eating or drinking. The proliferative lesion revealed new bone formation, osteolysis and clusters of filamentous bacteria surrounded by eosinophilic amorphous material and mixed inflammatory cells, predominantly neutrophils. The patient in that report demonstrated poor response to treatment and was humanely euthanized. On sectioning, the enlarged mandible had numerous brown nodules, contained a brownish granular material and had a "honeycomb" appearance,[7] which is also often used to describe the affected mandible in cases of bovine lumpy jaw. The causative agent was identified as *Nocardia cyriacigeorgica*, which is a relatively new species of *Nocardia* and is apparently a common *Norcardia* sp. pathogen in people. Traumatic gingival introduction was postulated as the source of infection in that case.[7]

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CASE IV: N14-17 (JPC 4066085).

Signalment: 2 year old Thoroughbred colt (*Equus caballus*)

History: The horse was normal and trained as usual the previous day, but did not consume all of his feed the night prior. The referring veterinarian examined the horse at 11 AM; the temperature was 104.8°F, the heart rate was elevated, and he was reluctant to move. He was treated with Banamine and Bactrim. The horse died spontaneously at 3:30 PM, at which time bloody froth was expelled from both nostrils.

Gross Pathology: The kidneys are bilaterally markedly enlarged. The right kidney weighs 1.85 kg and measures 20 x 19 x 8.5 cm, and the left kidney weighs 1.86 kg and measures 24.5 x 16 x 7 cm (each kidney is approximately 0.42% of body weight; 0.25% is normal). Bilaterally, the perirenal adipose tissue is mildly edematous. There are widespread multifocal, slightly raised, 1 to 10 mm diameter, tan-yellow foci on the subcapsular surface of both kidneys which occasionally bulge from the capsular surface. The discoloration frequently ex-


Kidney, foal: At low magnification, there are multifocal areas of cellular infiltration within the renal cortex. (HE, 5X)

tends into the renal capsule and occasionally into the perirenal adipose tissue. The capsules are friable and difficult to peel. Both kidneys moderately bulge on cut surface, and the cortices contain myriad 1mm diameter to 9 x 2 mm yellow-tan foci which occasionally have a cavitated center.

Laboratory Results: Microbiology: Aerobic culture of the kidney yielded heavy growth of *Actinobacillus equuli* ssp. *equuli.*

Histopathologic Description: Predominantly in the cortex, there are multifocal to coalescing, nodular inflammatory infiltrates predominantly of degenerate neutrophils, fewer macrophages, and cellular debris. This infiltrate is often centered on glomeruli, extending into and sometimes effacing the adjacent interstitium and renal tubules. Renal tubules in inflamed areas are sometimes degenerate or necrotic. Larger blood vessels sometimes contain luminal aggregates of fibrin, degenerate and viable neutrophils, cellular debris and erythrocytes; inflammation often extends into the vessel wall (vasculitis). Many foci of inflammation contain aggregates of gram-negative bacilli.

Contributor's Morphologic Diagnosis:
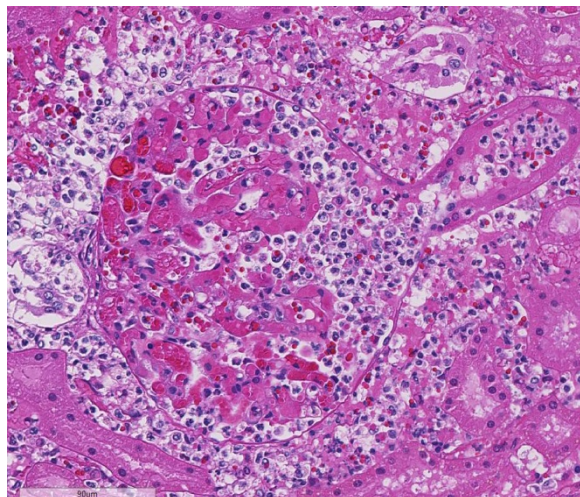
Kidney, foal: Suppurative inflammation is centered on glomeruli throughout the cortex.   (HE, 220X)

Embolic nephritis, suppurative, acute, multifocal to coalescing, severe, with intralesional gram-negative bacilli and vasculitis.

Contributor's Comment: The most frequently isolated agent in equine cases of embolic nephritis is *Actinobacillus equuli*, most often secondary to septicemia of foals.[4] *A. equuli* is a gram-negative bacillus that can be normal flora of the oral cavity, reproductive tract and intestinal tract of horses.[2,5] It is especially known for its tendency to create microabscesses in the kidneys and other organs.[4] In foals, *A. equuli* typically causes septicemia, also known as sleepy foal disease.[2] Septicemic lesions typically include embolic lesions in the kidney, lungs and liver, with lesions also reported in the umbilicus, adrenal gland, and joints.[2] In adult horses, *A. equuli* has been reported to cause sepsis, nephritis, endocarditis, pericarditis, peritonitis, en-teritis, pleuropneumonia, arthritis, pe-riorchitis, and abortion.[2,3,5] *A. equuli* infections in adult horses are thought to be predisposed by stress or other infections, such as respiratory viruses or salmonellosis.[2]

JPC Diagnosis: Kidney: Nephritis, embolic, suppurative, multifocal, marked with necrotizing vasculitis and colonies of bacilli.

Conference Comment: Actinobacillosis in adult horses is uncommon and most frequently associated with an underlying disease. There are two subspecies of *A. equuli*, a hemolytic subspecies termed *A. equuli* subsp. *haemolyticus* which is isolated from the normal oral cavity and respiratory tract, and a non-hemolytic form termed *A. equuli* subsp. *equuli*, which also resides in the oral cavity as well as the gastrointestinal tract of adult horses and is the agent of
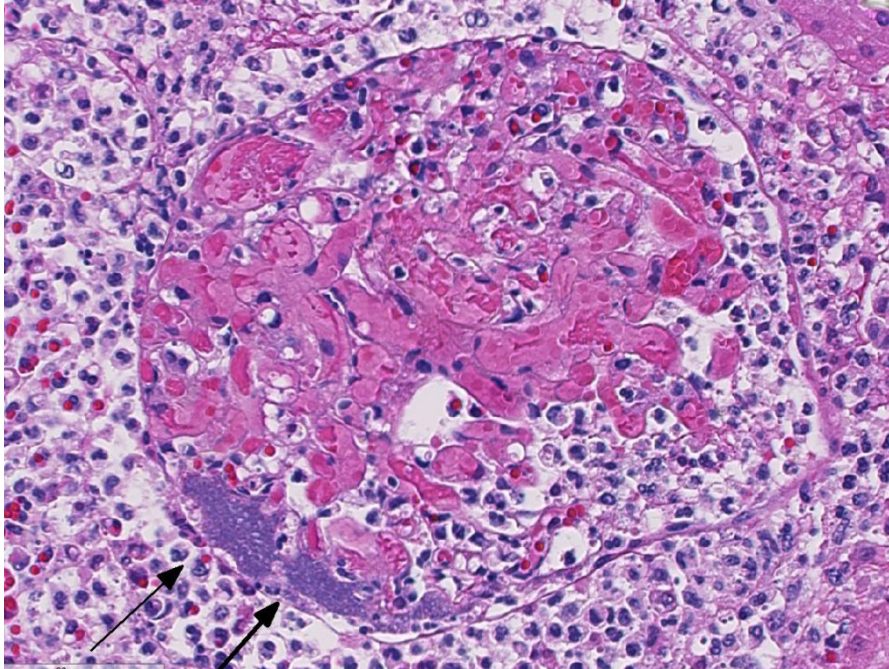
Kidney, foal: Glomerular capillaries contain fibrin thrombi, and rare, colonies of bacilli within capillary loops or free in Bowman's space (arrows)   (HE, 324 X)

In cases of embolic nephritis, bacteria become lodged in glomerular capillaries resulting in the presence of suppurative lesions or abscesses and, in some cases, when emboli are large enough, may occlude arteries resulting in septic infarcts. In septicemic foals that survive for a period of time, microabscesses will be seen in multiple organs and polyarthritis will be present. *A. equuli* may also cause diarrhea and hemorrhagic enteritis in foals.

septicemia in foals.[1] The hemolytic form has been associated with various infections including peritonitis, reproductive failure and respiratory disease. The bacterium possesses an RTX exotoxin known as Aqx that is cytotoxic to equine red blood cells. The non-hemolytic subspecies is more commonly present in cases of septicemia and may also be associated with respiratory disease, embolic nephritis and infection in other organs as well.[1] In septicemic cases, *Actinobacillus* can act as the primary agent or be a secondary infection following other viral or bacterial infections. The presence of endotoxin likely plays a role in the endothelial damage, vasculitis and bacterial emboli which are classically seen in septicemic cases. Although most often associated with foals in conjunction with events such as failure of passive transfer, septicemic lesions such as embolic nephritis, as seen in this case, as well as pneumonia, may be seen in adult horses.[1]

A common agent of embolic nephritis in swine, perhaps the most common, is *Erysipelothrix rhusiopathiae*; in cattle the common agent is *Trueperella pyogenes*; and in sheep and goats *Corynebacterium pseudotuberculosis* may be associated with embolic infections.[1] Although uncommon, *A. equuli* infection can be associated with endocarditis or myocarditis in adult horses[6] and has also been associated with reproductive losses.

Conference participants described the prolific inflammatory infiltrates as being centered on vessels as well as effacing glomeruli, with adjacent tubules being secondarily affected with varying severity. Tubules are multifocally ectatic and variably contain necrotic, sloughed tubular epithelial cells admixed with fibrin, hemorrhage and proteinaceous fluid. Fibrin thrombi with colonies of coccobacilli are occasionally seen in glomerular tufts. Some conference participants interpreted the most severely affected area of the cortex as coagulative

necrosis secondary to infarction due to fibrin thrombi; others attributed the tinctorial change as more consistent with some degree of autolysis. [7]The differential diagnosis discussed by participants in the case of this horse included *Escherichia coli*, *Klebsiella* sp., *Streptococcus* sp., *Rhodococcus equi* and *Salmonella* species.

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